EVALUATION OF CYCLOPOID COPEPODS FOR *AEDES ALBOPICTUS* CONTROL IN TIRES

GERALD G. MARTEN[1]

Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, CO 80522 and New Orleans Mosquito Control Board, New Orleans, LA 70126

ABSTRACT. Six species of cyclopoid copepods in New Orleans were tested for biological control of *Aedes albopictus* larvae in discarded tires. Six to 8 weeks after introduction, *Diacyclops navus*, *Acanthocyclops vernalis*, *Mesocyclops ruttneri* and *Mesocyclops edax* reduced the number of *Ae. albopictus* larvae by 83, 90, 95 and 96%, respectively. *Macrocyclops albidus* and *Mesocyclops longisetus* were the most effective species. Six to 8 weeks after introduction, *Macrocyclops albidus* reduced *Ae. albopictus* larvae by 99%. Three months after introduction *Macrocyclops albidus* reduced *Ae. albopictus* larvae by 100%, and *Mesocyclops longisetus* reduced *Ae. albopictus* larvae by 99.8%. *Macrocyclops albidus* and *Mesocyclops longisetus* were equally effective at eliminating *Ae. aegypti* and *Ae. triseriatus* larvae.

INTRODUCTION

Cyclopoid copepods are an exceptionally promising new form of biological control for container-breeding *Aedes* larvae (Marten 1990a). The Centers for Disease Control and the New Orleans Mosquito Control Board recently undertook a joint project aimed at developing an operational capacity for using these copepods to control *Aedes albopictus* (Skuse). The project provided an opportunity to assess species of cyclopoids that had never before been examined for biological control of mosquito larvae. Only one species of cyclopoid—*Mesocyclops aspericornis* (Daday)—had previously been investigated for mosquito control (Marten 1984, Suárez et al. 1984, Rivière et al. 1987).

Seven larvivorous species of cyclopoids that occur naturally in New Orleans were identified and cultured (Marten 1989): *Acanthocyclops vernalis* (Fischer), *Diacyclops navus* (Herrick), *Macrocyclops albidus* (Jurine), *Megacyclops* sp. (*M. viridis* species group), *Mesocyclops edax* (Forbes), *Mesocyclops longisetus* (Thiébaud) and *Mesocyclops ruttneri* Kiefer. *Mesocyclops bernardi* Petkovsky was also collected in New Orleans; although it is probably larvivorous, not enough specimens were collected to establish a culture or conduct tests.

Laboratory and field trials were conducted on *Acanthocyclops vernalis*, *Diacyclops navus*, *Macrocyclops albidus*, *Mesocyclops edax*, *Mesocyclops longisetus* and *Mesocyclops ruttneri*. Only laboratory tests were conducted on *Megacyclops* sp. Results from New Orleans are of general relevance to much of North America because *Acanthocyclops vernalis*, *Diacyclops navus*, *Macrocyclops albidus* and *Mesocyclops edax* have broad geographic distributions on the continent. *Mesocyclops ruttneri* is the same species that Marten (1989) called *Mesocyclops* sp.; it belongs to the *Mesocyclops leuckarti* species group, which is widely distributed in North America. *Mesocyclops longisetus* is a neotropical species with the northernmost part of its range in southeastern United States.

MATERIALS AND METHODS

Laboratory trials: The purpose of the first laboratory trials was to determine how many first instar *Ae. albopictus* larvae each species of cyclopoid will kill in 1 day. Individual adult female cyclopoids were placed in small dishes containing 10 ml of pond water that was previously heated to 80°C to kill all organisms. After the cyclopoids were in the dishes for 24 h without food, 50 newly hatched *Ae. albopictus* larvae were placed in each dish. The number of living larvae was counted 24 h later. The water temperature was 23°C. The cyclopoids in this experiment, as well as all other cyclopoids used in this study, came from laboratory colonies that were less than 3 months old.

The objectives of the second laboratory trials were to determine: 1) how many *Ae. albopictus* larvae are killed by cyclopoid populations under more natural conditions, 2) the long-term effectiveness of each cyclopoid species as a larval predator, and 3) how the effectiveness is affected by food conditions in a container. Plastic food containers (1 liter capacity) were half-filled with water collected from discarded tires that contained no natural populations of cyclopoids. Twenty g (drained wet weight) of decomposing leaves collected from the same tires was placed in half of the containers to provide "high food" conditions; 12 ml of fine detritus from the tires (primarily feces of aquatic animals such as mosquito larvae) was placed in the other half of the containers for "low food" conditions. The temperature varied from 21 to 25°C.

[1] Current address: New Orleans Mosquito Control Board, 6601 South Shore Harbor Drive, New Orleans, LA 70126.

Reprinted from Journal of the American Mosquito Control Association
Vol 6 December 1990 Number 4

Acanthocyclops vernalis, Diacyclops navus, Macrocyclops albidus, Mesocyclops edax and *Mesocyclops ruttneri* were each introduced to 10 containers with high food and 10 with low food, a single species in each container. Each introduction consisted of 10 adult females. Mixtures of *Macrocyclops albidus* with *Mesocyclops ruttneri* (5 adult females of each species) were introduced to the same number of containers, and the same was done with mixtures of *Macrocyclops albidus* and *Diacyclops navus.*

One hundred first instar *Ae. albopictus* larvae were placed in each container 3 days after introducing the cyclopoids. All surviving larvae were removed after 3 days and counted. Two months later, 100 first instar larvae were placed in the containers, and surviving larvae were removed and counted at the end of 3 days. Copepodids and adult cyclopoids in each container were also counted. Subsequently, 500 first instar larvae were introduced into containers where predation with 100 larvae was 95% or more, and again the larvae were removed and counted after 3 days. Two thousand larvae were introduced to containers with *M. albidus*, following the same procedure.

Field trials: Ten adult females of *Acanthocyclops vernalis, Diacyclops navus, Macrocyclops albidus, Mesocyclops edax* or *Mesocyclops ruttneri*, as well as mixtures of *Macrocyclops albidus* and *Mesocyclops ruttneri*, were introduced to 600 discarded automobile tires from May 20 to June 10, 1989. *Mesocyclops longisetus* was introduced to 50 tires in May 1990. The introduction procedure was to count cyclopoids into vials in the laboratory and empty the vials into tires in the field that were previously verified to contain no natural cyclopoid populations.

The tires were at 4 locations, the first 3 in eastern New Orleans and the fourth in the inner city: 1) a pile of approximately 50,000 tires in an opening in a wooded area, 2) piles of several hundred tires at the edge of a dirt road in a wooded area, 3) scattered piles of about 20 tires each, in an area of scattered trees, and 4) a pile of about 500 tires in an industrial area.

Tires at the first 3 locations were ecologically similar because they contained leaves that fell from nearby trees. The most common mosquito larvae in these tires were *Ae. albopictus*, but there were also larvae of *Ae. triseriatus* (Say), *Culex salinarius* Coq., *Orthopodomyia signifera* (Coq.) and *Toxorhynchites rutilus* (Coq.). The water in shaded tires was usually clear and brownish, whereas the water in tires fully exposed to the sun was usually green with phytoplankton.

There were no trees or bushes at the fourth location; tires were exposed to sun most of the day, were often dry and did not contain leaves. The mosquito larvae were *Ae. aegypti* (Linn.) and *Ae. albopictus.* The water in some of the tires at the fourth location was green with phytoplankton, but the water in many of the tires was clear, suggesting that little food was available for animals such as cyclopoids or mosquito larvae.

Adult *Ae. albopictus* populations were large at all 4 locations during the study period; therefore, the natural input of *Ae. albopictus* eggs to the tires was high. Cyclopoids were introduced to only a small fraction of the tires at each location. Tires that contained no cyclopoids served as controls and were interspersed among the tires to which cyclopoids were introduced.

Approximately 500 of the tires to which cyclopoids were introduced, plus 150 control tires, were examined 6–14 weeks after the introductions. Many of the tires were examined twice—at the beginning and end of this period.

When a tire was examined, all water and other materials, including leaves and detritus, were removed from the tire and taken to the laboratory so that mosquito larvae and cyclopoids (copepodids and adults) could be counted. Each sample was placed in a glass dish (38 × 28 × 6 cm) on a table that illuminated the dish from the top, bottom and sides. Intense illumination was necessary so that all cyclopoids and mosquito larvae could be seen in water that was turbid with phytoplankton or sediment. Cyclopoids were counted live. Mosquito larvae and pupae were removed and placed in a vial of alcohol for later identification and counting under a stereomicroscope. Each sample, including cyclopoids, was then returned to its tire.

Toxorhynchites rutilus was found in 49% of the sampled tires at the first 3 locations and could have affected the results by preying on *Aedes* larvae. Numbers of cyclopoids and mosquito larvae were therefore tabulated separately with regard to the presence or absence of *Toxorhynchites.*

RESULTS

Laboratory trials: In the first laboratory trials, the number of larvae (of 50) killed by single cyclopoids in 24 h ranged from an average of 9.5 killed by *Diacyclops navus* to 45.0 killed by *Macrocyclops albidus* (Table 1). Although *Macrocyclops albidus* killed the most larvae, *Acanthocyclops vernalis* and the 3 species of *Mesocyclops* also killed large numbers. Most of the larvae were only partially eaten. When large numbers of larvae were killed, some were killed without being eaten at all.

In the second laboratory trials, when 100 *Ae.*

Table 1. Number of newly hatched *Aedes albopictus* larvae killed in 24 h by individual cyclopoids. 50 larvae were available to each cyclopoid.

Species	Larvae killed		No. of replicates
	x̄ ± SE	Range	
Macrocyclops albidus[a]	45.0 ± 1.1	39–50	35
Macrocyclops albidus[b]	43.6 ± 0.8	35–50	37
Mesocyclops ruttneri[c]	40.6 ± 1.2	29–46	18
Mesocyclops longisetus[c]	38.4 ± 1.9	18–48	19
Mesocyclops edax[c]	34.0 ± 2.2	9–48	28
Acanthocyclops vernalis[b]	33.3 ± 2.7	12–50	27
Megacyclops sp.[b,d]	18.3 ± 2.7	8–49	20
Diacyclops navus[b]	9.5 ± 1.6	1–20	17
Controls	0.1 ± 0.1	0–1	13

[a] Cyclopoids from natural populations in tires.
[b] Cyclopoids from ground pools.
[c] Cyclopoids from a freshwater lagoon.
[d] *Megacyclops viridis* species group.

albopictus larvae were placed in plastic containers 3 days after cyclopoids were introduced, larval mortality was near 100% only in the low-food containers, in particular those with *Macrocyclops albidus, Mesocyclops ruttneri,* or *Mesocyclops edax* (Table 2). Predation rates with high food were lower, apparently due to an abundance of protozoa and rotifers that competed with mosquito larvae as food for the cyclopoids. The mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri* killed the most larvae under high-food conditions.

Substantial numbers of cyclopoids were observed in all the high-food containers within a month of introduction. Visual observations indicated that food organisms such as protozoa and rotifers, which might compete with mosquito larvae for the cyclopoids' attention, were more or less depleted by this time. All containers in which 2 species were introduced still had both species. *Diacyclops navus* were more numerous than *Macrocyclops albidus* in mixtures of those 2 species; the numbers in mixtures of *Macrocyclops albidus* and *Mesocyclops ruttneri* were more even.

When 100 first instar larvae were placed in high-food containers 2 months after the introduction of cyclopoids, *Acanthocyclops vernalis* and *Diacyclops navus* killed 85–90% of the larvae on average (Table 3). *Macrocyclops albidus, Mesocyclops ruttneri* and *Mesocyclops edax* killed 99% or more of the larvae. *Macrocyclops albidus* and *Mesocyclops ruttneri* killed 99% or more of the larvae when 500 were placed in the container, and *Macrocyclops albidus* killed more than 99% of the larvae when 2,000 larvae were placed in the container at the same time.

Few cyclopoids remained in the low-food containers after 2 months, generally not enough to eliminate a high percentage of introduced *Ae. albopictus* larvae (Table 3). *Diacyclops navus* had the largest populations in the low-food containers, but larval mortality was highest with *Macrocyclops albidus.*

Table 2. Mortality of 100 first instar *Aedes albopictus* larvae that were placed in laboratory containers 3 days after introducing 10 adult female cyclopoids to each container.

Species introduced	Percent mortality[a]	
	Low food	High food
Macrocyclops albidus	100 ± 0.0	83 ± 4.9
Mesocyclops ruttneri	99 ± 0.3	89 ± 1.5
Macrocyclops/M. ruttneri[b]	98 ± 0.6	94 ± 1.9
Mesocyclops edax	97 ± 0.5	86 ± 1.4
Macrocyclops/Diacyclops[c]	77 ± 2.2	85 ± 3.5
Acanthocyclops vernalis	75 ± 2.7	30 ± 3.2
Diacyclops navus	55 ± 2.0	16 ± 2.6
Controls[d]	10 ± 1.1	4 ± 1.0

[a] Average mortality ± SE during 3 days, based on 10 replicates for each cyclopoid species (or species mixture).
[b] Mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri.*
[c] Mixture of *Macrocyclops albidus* and *Diacyclops navus.*
[d] No cyclopoids introduced to containers.

Field trials: All 6 cyclopoid species in the field trials rapidly established populations when they were introduced to tires at the first 3 locations. Because results from the first 3 locations were very similar, they were combined for presentation.

The percentage of successful introductions at the first 3 field locations, evaluated 6–8 weeks after introducing the cyclopoids, was close to 100% for species that occur naturally in tires, i.e., *Macrocyclops albidus, Acanthocyclops vernalis* and *Diacyclops navus* (Table 4). Introduc-

Table 3. Mortality of first instar *Aedes albopictus* larvae that were placed in laboratory containers 2 months after introducing cyclopoids.

Species introduced	No. of cyclopoids[a]		Percent mortality[b] (100 larvae)		Percent mortality[b] (500 larvae)
	Low food	High food	Low food	High food	High food
Macrocyclops albidus	1.8 ± 0.3	21 ± 2.4	95 ± 2.3	100 ± 0.3	99 ± 0.8
Mesocyclops ruttneri	1.0 ± 0.2	43 ± 5.0	80 ± 3.9	99 ± 0.7	100 ± 0.2
Macrocyclops/M. ruttneri[c]	3.3 ± 0.6	19 ± 2.2	83 ± 5.2	100 ± 0.0	95 ± 2.7
Mesocyclops edax	0.5 ± 0.3	25 ± 1.4	78 ± 9.8	99 ± 0.8	92 ± 3.1
Macrocyclops/Diacyclops[d]	4.3 ± 0.8	27 ± 1.7	94 ± 1.3	100 ± 0.2	100 ± 0.3
Acanthocyclops vernalis	1.2 ± 0.3	20 ± 2.3	57 ± 4.0	85 ± 1.2	ND
Diacyclops navus	10.0 ± 2.2	126 ± 12	57 ± 2.1	90 ± 0.8	ND
Controls[e]	0	0	42 ± 2.9	8 ± 1.9	15 ± 2.5

[a] Average number of cyclopoids per container ± SE, based on 10 experimental containers.
[b] Average mortality during 3 days ± SE.
[c] Mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri.*
[d] Mixture of *Macrocyclops albidus* and *Diacyclops navus.*
[e] No cyclopoids introduced to containers.
ND = no data.

Table 4. Percentage of tires at the first 3 field locations which contained cyclopoids at specified periods after cyclopoid introduction.

Species introduced	Percent positive[a] Weeks[c]		Sample size[b] Weeks[c]	
	6–8	12–16	6–8	12–16
Macrocyclops albidus	96	91	67	44
Mesocyclops longisetus	ND	91	ND	46
Mesocyclops ruttneri	89	79	62	53
Macrocyclops/M. ruttneri[d]	100	100	49	42
Mesocyclops edax	83	62	42	29
Acanthocyclops vernalis	96	83	39	18
Diacyclops navus	96	94	38	18

[a] Percentage of tires that still contained the cyclopoid species introduced.
[b] Number of tires examined.
[c] Number of weeks after cyclopoid introduction.
[d] Mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri.*
ND = no data.

tion success of *Mesocyclops longisetus*, which has not been found in tires in New Orleans, was also very high. The success of introductions was only 80–90% for *Mesocyclops ruttneri* and *M. edax*, which have been found in New Orleans only in permanent canals and lagoons.

Some of the cyclopoid populations disappeared from the tires by the time they were examined again 3 months after introduction (Table 4). Survival of *Mesocyclops ruttneri*, *Mesocyclops edax* and *Acanthocyclops vernalis* was lowest. In contrast, survival of the mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri* was 100%; and nearly 100% of the single-species populations of *Diacyclops navus*, *Macrocyclops albidus* and *Mesocyclops longisetus* that were in tires 6 weeks after introduction were still there 3 months after introduction.

Diacyclops navus had the largest populations in the tires, often more than 1,000 individuals (Table 5). *Acanthocyclops vernalis* also had large numbers in the tires. As a rule, few individuals in the exceptionally large populations of *Diacyclops* or *Acanthocyclops* were large enough to prey on mosquito larvae.

Mesocyclops ruttneri and *Mesocyclops longisetus* typically numbered in the hundreds. Populations of *Macrocyclops albidus* and *Mesocyclops edax* were smaller (Table 5). *Macrocyclops albidus* populations were almost always between 30 and 150 individuals; *Mesocyclops edax* populations were sometimes smaller. *Macrocyclops albidus* and *Mesocyclops edax* never showed signs of overcrowding. Once established, their populations consisted almost entirely of adults and copepodids large enough to prey on mosquito larvae.

The combined numbers in mixtures of *Macrocyclops albidus* and *Mesocyclops ruttneri* were about the same as with *Mesocyclops ruttneri* alone. Both species were always represented in the mixtures, but the species composition varied from an even mix of the 2 species to nearly complete predominance of *Macrocyclops albidus* or *Mesocyclops ruttneri.*

Toxorhynchites were observed to prey on cyclopoids in the laboratory when larger food was not available. However, the presence of *Toxorhynchites* in tires had no deleterious effect on cyclopoid populations (Table 5).

Six to 8 weeks after introduction, *Mesocyclops ruttneri* and *Mesocyclops edax* in tires without *Toxorhynchites* reduced *Ae. albopictus* larvae and pupae by about 95% on average compared with controls (Table 6). *Acanthocyclops vernalis*

Table 5. Number of cyclopoids per tire at the first 3 field locations.

Time after introduction and species introduced	Range	x̄ ± SE[a] *Toxorhynchites* Absent	x̄ ± SE[a] *Toxorhynchites* Present	Sample size[b] *Toxorhynchites* Absent	Sample size[b] *Toxorhynchites* Present
6–8 weeks after introduction					
Macrocyclops albidus	3–100	45 ± 8	59 ± 6	31	34
Mesocyclops ruttneri	5–400	87 ± 15	119 ± 19	28	28
Macrocyclops/M. ruttneri[c]	5–400	136 ± 30	112 ± 19	20	29
Mesocyclops edax	10–250	72 ± 15	141 ± 39	18	17
Acanthocyclops vernalis	10–1,000	358 ± 80	255 ± 60	15	22
Diacyclops navus	10–2,000	664 ± 195	572 ± 135	19	17
3 months after introduction					
Macrocyclops albidus	20–200	81 ± 10	117 ± 22	32	10
Mesocyclops longisetus	10–1,000	218 ± 43	189 ± 35	25	17
Mesocyclops ruttneri	1–750	154 ± 42	158 ± 26	25	17

[a] Average number of cyclopoids per tire, based only on tires that were positive for the species indicated.
[b] Number of tires.
[c] Mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri.*

Table 6. *Aedes albopictus* larvae and pupae in tires with and without *Toxorhynchites* at the first 3 field locations 6–8 weeks after introducing cyclopoids.[a]

Species introduced	Larvae Number[b]	Larvae Percent reduction[c]	Pupae Number[b]	Pupae Percent reduction[c]
No *Toxorhynchites*				
Macrocyclops albidus	0.7 ± 0.3	99	0.03 ± 0.03	99
Mesocyclops ruttneri	3.3 ± 1.1	95	0.8 ± 0.4	80
Macrocyclops/M. ruttneri[d]	1.2 ± 0.7	98	0.05 ± 0.05	98
Mesocyclops edax	2.6 ± 0.4	96	0.4 ± 0.1	90
Acanthocyclops vernalis	6.4 ± 1.2	90	2.0 ± 0.9	51
Diacyclops navus	11.2 ± 2.4	83	3.3 ± 1.0	20
Controls[e]	65.4 ± 12.0	—	4.1 ± 1.3	—
Toxorhynchites present				
Macrocyclops albidus	0.22 ± 0.09	>99	0	100
Mesocyclops ruttneri	0.70 ± 0.24	99	0.07 ± 0.05	98
Macrocyclops/M. ruttneri[d]	0.21 ± 0.14	>99	0	100
Mesocyclops edax	1.0 ± 0.3	98	0.3 ± 0.1	93
Acanthocyclops vernalis	2.8 ± 1.9	96	0.2 ± 0.1	97
Diacyclops navus	3.0 ± 1.0	95	0.5 ± 0.3	88
No cyclopoids[f]	16.6 ± 3.0	74	1.1 ± 0.4	73

[a] Sample sizes (i.e., number of tires) are the same as in Table 5.
[b] Average number of larvae or pupae ± SE per tire.
[c] Reduction in number of larvae or pupae compared with controls.
[d] Mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri.*
[e] Tires containing neither cyclopoids nor *Toxorhynchites* (sample size = 36 tires).
[f] Sample size = 48 tires.

and *Diacyclops navus* were significantly less effective. The largest numbers of *Ae. albopictus* larvae were observed in tires with exceptionally large populations of *Diacyclops navus.* Larvae appeared to escape predation because the crowded and stunted *Diacyclops* were too small to prey on them.

Macrocyclops albidus was the most effective predator. The number of *Ae. albopictus* larvae in tires with *Macrocyclops albidus* 6–8 weeks after introduction was only 1% of the number in control tires (Table 6). The same was true for pupae. The mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri* was nearly as effective as *Macrocyclops albidus* alone.

No *Ae. albopictus* larvae or pupae were found in any of the tires with *Macrocyclops albidus* when they were sampled 3 months after introduction (Table 7). The same was true for mixtures of *Macrocyclops albidus* and *Mesocyclops ruttneri.* A total of 4 *Ae. albopictus* larvae were found in all the tires with *Mesocyclops longisetus.* More than 1,000 *Ae. albopictus* larvae were collected in the same number of control tires at that time.

Aedes albopictus larvae were reduced most

Table 7. *Aedes albopictus* larvae and pupae in tires without *Toxorhynchites* at the first field location 3 months after introducing cyclopoids.

Species introduced	Larvae Number[a]	Larvae Percent reduction[b]	Pupae Number[a]	Pupae Percent reduction[b]	Sample size[c]
Macrocyclops albidus	0	100	0	100	32
Mesocyclops longisetus[d]	0.1 ± 0.1	>99	0	100	25
Mesocyclops ruttneri	2.9 ± 1.8	90	1.6 ± 1.1	73	25
Macrocyclops/M. ruttneri[e]	0	100	0	100	23
Controls[f]	28.0 ± 9.3	—	6.0 ± 1.3	—	27

[a] Average number of larvae or pupae ± SE per tire.
[b] Reduction in number of larvae or pupae compared with controls.
[c] Number of tires.
[d] *Mesocyclops longisetus* field trials were conducted a year later than other cyclopoid species. Average number of larvae in control tires was 84 ± 22; average number of pupae was 1.0 ± 0.6.
[e] Mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri.*
[f] Tires containing neither cyclopoids nor *Toxorhynchites.*

when *Toxorhynchites* were present in tires with the cyclopoids (Table 6). Pupae were reduced as much as larvae. Although *Tx. rutilus* reduced *Ae. albopictus* larvae and pupae only 74% when by itself, even the weaker cyclopoid predators reduced both larvae and pupae nearly 100% when *Toxorhynchites* were present in the tires.

Cyclopoids eliminated *Ae. triseriatus* larvae almost as effectively as *Ae. albopictus* larvae. Seven weeks after cyclopoid introduction, no *Ae. triseriatus* larvae were found in tires with a mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri* (Table 8). Tires containing a single species of cyclopoid averaged about one *Ae. triseriatus* larva/tire. (Control tires averaged 5.7 *Ae. triseriatus* larvae/tire.) When the tires were examined 3 months after cyclopoid introduction, no *Ae. triseriatus* larvae were found in tires with *Macrocyclops albidus, Mesocyclops ruttneri* or a mixture of the two species. Only 2 *Ae. triseriatus* larvae were found in all the tires with *Mesocyclops longisetus.*

Culex salinarius and *Or. signifera* larvae were not reduced significantly by any of the cyclopoid species.

Cyclopoid survival was poor in the 85 tires examined at the fourth location 10 weeks after introducing cyclopoids. *Diacyclops navus* were present in 50% of the tires to which they had been introduced, but other species were found in only 10–20% of the tires to which they had been introduced. There were not enough tires with cyclopoids at the fourth location to provide precise information about their impact on mosquito larvae, but none of the tires with *Macrocyclops albidus* (or a mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri*) contained *Aedes* larvae. A few tires with *Mesocyclops ruttneri* or *Diacyclops navus* contained a small number of larvae. Every tire without cyclopoids contained *Ae. albopictus* or *Ae. aegypti* larvae, averaging 15 larvae/tire.

Table 8. *Aedes triseriatus* larvae in tires after cyclopoid introductions at the first field location.

Time after introduction and species introduced	No. of larvae[a]	Percent reduction[b]	Sample size[c]
7 weeks after introduction			
Macrocyclops albidus	1.2 ± 0.9	79	23
Mesocyclops ruttneri	1.3 ± 0.9	77	14
Macrocyclops/M. ruttneri[d]	0	100	16
Controls[e]	5.7 ± 2.6	—	31
3 months after introduction			
Macrocyclops albidus	0	100	40
Mesocyclops longisetus[f]	0.1 ± 0.1	99	42
Mesocyclops ruttneri	0	100	15
Macrocyclops/M. ruttneri[d]	0	100	15
Controls[e]	4.9 ± 1.5	—	32

[a] Average number of larvae per tire ± SE.
[b] Reduction in number of larvae compared to controls.
[c] Number of tires.
[d] Mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri.*
[e] Tires without cyclopoids.
[f] *Mesocyclops longisetus* field trials were conducted a year later than other cyclopoid species. Average number of larvae in control tires was 6.8 ± 3.1.

DISCUSSION

Macrocyclops albidus was the most effective predator; it was the only species that consistently eliminated all *Aedes* larvae. *Mesocyclops longisetus* was also highly effective; predation by *Mesocyclops longisetus* was nearly 100%. *Macrocyclops albidus* and *Mesocyclops longisetus* were also among the species that survived best in tires (Table 4).

Mesocyclops ruttneri and *Mesocyclops edax* were reasonably effective as predators, but their survival in the tires was inferior. *Acanthocyclops vernalis* was not particularly effective with regard to predation or survival. *Diacyclops navus* survived the best of all the species, but it was the weakest predator.

The mixtures of *Macrocyclops albidus* and *Mesocyclops ruttneri* performed more or less as well as *Macrocyclops albidus* alone, i.e., nearly perfect predation. This was much better than the natural mixture of larvivorous cyclopoids that occurs most commonly in tires in New Orleans—*Macrocyclops albidus* and *Diacyclops navus*—which was observed to reduce *Ae. albopictus* larvae only 95% (Marten 1989). A particular advantage of the mixture of *Macrocyclops albidus* and *Mesocyclops ruttneri* was that success of introduction and subsequent survival in tires were better with the mixture than with *Macrocyclops albidus* alone. At the first 3 field locations all tires to which *Macrocyclops albidus* and *Mesocyclops ruttneri* were introduced together still had the cyclopoids (and complete control of *Aedes* larvae) after 3 months (Table 4, Table 7).

The number of pupae in a tire is a measure of the production of adult mosquitoes. The weaker cyclopoid predators did not reduce pupae as much as they reduced larvae (Table 6, Table 7). For example, *Acanthocyclops vernalis* reduced larvae by 90% but reduced pupae by only 51% (Table 6). The explanation appears to be that the input of larvae to many of the tires was well in excess of the capacity of food resources in the tires to produce adult mosquitoes. Laboratory studies with *Ae. albopictus* under simulated tire conditions have shown that the production of adult mosquitoes does not increase in proportion to the number of larvae when there is crowding (G. G. Marten, unpublished data). Incomplete predation thins out overcrowded larvae without correspondingly reducing the production of adult mosquitoes (Service 1985).

The mutual reinforcement of predation by cyclopoids and *Toxorhynchites* is worth noting. Because *Toxorhynchites* are effective predators of third and fourth instar larvae, they complement first instar predation by cyclopoids. *Toxorhynchites* larvae are helpful when combined with species of cyclopoids that are not strong enough predators to eliminate all the mosquito larvae by themselves.

Toxorhynchites also accelerate the elimination of larvae by highly effective cyclopoid species. For example, 6–8 weeks after *Macrocyclops albidus* introduction there were fewer larvae and pupae in tires that also contained *Tx. rutilus* (Table 6), even though *Macrocyclops albidus* eventually caused the disappearance of all larvae regardless of *Toxorhynchites.*

Our information is limited on the survival of cyclopoids in tires for periods longer than this study (Marten 1990b), but there is reason for optimism. Nearly all the tires to which *Macrocyclops albidus* and *Mesocyclops longisetus* were successfully introduced in the field trials still had populations 3 months later (Table 4). Moreover, there are natural *Macrocyclops albidus* populations in New Orleans that appear to have been in tires for years, even though some of the tires dry out from time to time (Marten 1989).

However, survival of all species was poor at the fourth field location, where there was no vegetation around the tires, the food supply in the tires was poor, and the tires were fully exposed to the sun. Water temperatures sometimes reached 40°C, the highest temperature that many cyclopoid species can tolerate (G. G. Marten, unpublished data). The fourth site was also poor for mosquito production; although mosquito larvae were numerous, pupae were seldom observed. In general, it appears that any tire with enough food to support significant mosquito production has enough food to sustain a cyclopoid population.

Judging from their performance in this study, *Macrocyclops albidus* and *Mesocyclops longisetus* merit serious consideration for operational use to control *Aedes* larvae in tires. *Macrocyclops albidus* and some species of *Mesocyclops* could also prove useful for a variety of mosquito breeding habitats in addition to tires. A particularly promising prospect is water storage containers in the tropics that are breeding *Ae. aegypti* where dengue fever is a problem. There is also evidence that natural populations of cyclopoids eliminate *Aedes* and *Anopheles* larvae from ground water habitats (Marten et al. 1989, Marten 1990c).

ACKNOWLEDGMENTS

I wish to thank Raymond Bailey, Donald Eliason, Bruce Francy, Jerome Freier, Thomas Monath and Chester Moore of the Division of Vector-Borne Infectious Diseases, Centers for Disease Control, for their suggestions, encouragement and support. I am highly grateful to Edgar Bordes, Michael Carroll, Edward Freytag, Jimmy Loupe, Jack Leonard, Martin Rauch, Stephen Sackett and others on the staff of the New Orleans Mosquito Control Board for their numerous contributions to the study. Mieu Nguyen assisted in all aspects of the study. Earl Anthony, Jr., Mary Klinger, Willie McKinney, Giai Ngo and Peter Omonde also provided laboratory and field assistance. Special thanks are due to Janet Reid, National Museum of Natural History (Smithsonian Institution), who provided authoritative species identifications of cyclopoid copepods as well as information on their biology. The author was supported by a National Research Council senior research associateship.

REFERENCES CITED

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

Marten, G. G. 1989. A survey of cyclopoid copepods for control of *Aedes albopictus* larvae. Bull. Soc. Vector Ecol. 14:232–236.

Marten, G. G. 1990a. Issues in the development of cyclops for mosquito control, pp. 159–164. *In:* M. F. Uren, J. Blok, L. H. Manderson (eds.), Proc. Fifth Symp. Arbovirus Research in Australia. CSIRO and Queensland Inst. Med. Res., Australia.

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

Marten G. G. 1990c. Biological control (copepods). New Orleans Mosquito Control Board Monthly Report, March 1990, p. 2–3.

Marten, G. G., R. Astaiza, M. F. Suárez, C. Monje and J. W. Reid. 1989. Natural control of larval *Anopheles albimanus* (Diptera: Culicidae) by the predator *Mesocyclops* (Copepoda: Cyclopoida). J. Med. Entomol. 26:624–627.

Rivière, F., B. H. Kay, J. M. Klein and Y. Sechan. 1987. *Mesocyclops asperincornis* (Copepoda) and *Bacillus thuringiensis* var. *israelensis* for biological control of *Aedes* and *Culex* vectors (Diptera: Culicidae) breeding in crab holes, tree holes and artificial containers. J. Med. Entomol. 24:425–430.

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

Service, M. W. 1985. Population dynamics and mortalities of mosquito preadults, pp. 185–201. *In:* L. P. Lounibos, J. R. Rey and J. H. Frank (eds.), Ecology of mosquitoes. Florida Medical Entomology Laboratory, Vero Beach, FL.