Laboratory and field studies of *Macrocyclops albidus* (Crustacea: Copepoda) for biological control of mosquitoes in artificial containers in a subtropical environment

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ABSTRACT: The cyclopoid copepod *Macrocyclops albidus* (Jurine) was tested as a potential biological control agent of mosquitoes in laboratory microcosms, in controlled field conditions, and in a 22-mo field experiment using discarded tires. The predator was highly efficient in controlling mosquitoes in all three settings, reaching close to 90% reduction in larval survival under field conditions and exceeding the recommended predation rates for effective mosquito control in laboratory experiments. The predator was most effective on 1-4-d-old larvae. Alternate food and habitat structure significantly influenced the predation rates on mosquito larvae. Once established, the copepod was able to maintain reproducing populations in the field for the duration of the experiments. However, the predator failed to establish populations at four of the experimental field sites. Two of the failures can be attributed to characteristics of the individual tires, such as leaching chemicals, whereas the other two were probably due to site-specific factors. This copepod species is a promising candidate for control of mosquito larvae because it is a widespread and highly effective predator that is capable of establishing and maintaining populations under a wide variety of field conditions. Additionally, *M. albidus* is relatively easy to culture, maintain, and deliver to the target areas. ***Journal of Vector Ecology* 29 (1): 124-134. 2004.**

Keyword Index: Mosquito, biological control, containers, copepod, Florida.

INTRODUCTION

Mosquitoes are important pests that affect human health and well-being throughout the world. In many parts of the world, diseases transmitted by mosquitoes such as malaria, dengue, and yellow fever are serious threats to human health. The mobility of modern human populations and the globalization of commerce have greatly increased the probability that diseases could be exported to areas where they are not currently endemic. The current spread of West Nile virus in the United States is a good example of how quickly these diseases can spread given the right conditions. Mosquitoes can also be serious nuisances with significant implications for the economy and quality of life.

Biological control is an important option for the future of mosquito control, particularly in view of current restrictions on pesticide use and habitat management, and continuing problems with chemical resistance. Biological control techniques are usually relatively inexpensive, making them particularly attractive to developing countries, where human resources are usually more available than funds for expensive control alternatives. Although a variety of biological control agents ranging from viruses to fish have been tried for mosquito control, we still do not have consistent evidence of acceptable mosquito population reduction brought about by biological control agents (Lounibos and Frank 1994).

Several species of copepods, particularly those in the genus *Mesocyclops,* have shown promise as biological control agents for container-breeding mosquitoes. Past studies report varying results depending upon the copepod and mosquito species (Marten 2000), the weather (Marten et al. 1994), and upon the physical and biological milieu of the containers (Suárez et al. 1984, Riviere et al. 1987, Marten 1990a, b, Marten et al. 1994, Tietze et al. 1994, Schreiber et al.

1996). A notable success in the use of copepods for reduction of disease vectors is that of Vu Sinh Nam and co-workers, who report effective control of *Aedes* vectors of dengue in Vietnam with copepods of the genus *Mesocyclops* (Kay et al. 2002a).

Macrocyclops albidus (Jurine) is a common copepod species with worldwide distribution that inhabits natural bodies of water and is often found in artificial containers such as discarded tires (Marten 1989). It is a promising species for biological control of mosquitoes due to its relatively large size (1.0-1.5 mm adult body length excluding caudal setae) and aggressive behavior towards potential prey that include mosquitoes, protozoa, rotifers, phytoplankton, and others (Marten 2000). However, Marten (1989) reported that in tropical climates this species tends to maintain lower population sizes in containers (less than 100 individuals per container), and is less tolerant of high water temperatures than other predaceous Cyclops species such as *Mesocyclops longisetus* (Thiebaud) and *Mesocyclops aspericornis* (Daday). On the other hand, this species is more tolerant of cold temperatures than the above species (it can survive at 0°C for many months; G. Marten, personal communication), and may be well-suited for subtropical areas, where regular cold spells below 0°C are common.

This paper reports on the results of long-term laboratory and field studies of *M. albidus* as a predator of container-inhabiting mosquitoes in a subtropical environment. Laboratory experiments were conducted to examine the predation efficiency of this species on mosquito larvae of different ages (predation efficiency of copepods has been reported to drop off considerably with older mosquito larvae (Tietze et al. 1994)), and the effects of habitat structure and alternative prey on predation. Further experiments were carried out in a large outdoor enclosure (see below) that simulated field conditions but allowed for control of wild mosquito oviposition and copepod densities. Finally, field experiments were conducted in a natural subtropical hammock in east-central Florida, U.S.A. (27.58761°N, 80.36956°W).

MATERIALS AND METHODS

Experimental animals

Adult *M. albidus* colonies were established in plastic pools containing approximately 80 l of water with aeration provided by standard aquarium pumps. Initially, 1000 ml of *Paramecium caudatum* culture and a small amount of wheat grain (approximately 100 grains) were added to each pool. Thereafter, 500 ml of *P. caudatum* were added to each pool per week. Old wheat grain was filtered out and replaced every three wk. Additional backup cultures were maintained at separate locations in rectangular (25x27x5cm) plastic trays. Seed stock for the cultures were obtained from the Public Health Entomology Research and Education Center, Florida A&M University, Panama City, FL. Other details of copepod rearing can be found in Suárez et al. (1992), Marten (1990a), Riviere et al. (1987), and Marten and Thompson (1997). For all the experiments, only non-parous adults were used.

Aedes aegypti (Linnaeus) and *Aedes albopictus* (Skuse) larvae were obtained from eggs laid on wet paper towels by females from colonies maintained at the Florida Medical Entomology Laboratory (FMEL) in Vero Beach, FL, U.S.A. using standard methods (Gerberg et al. 1994). Eggs were hatched by immersion in fresh water approximately 24 h before needed. When large numbers of synchronous larvae were needed for experiments, hatching was induced by using deoxygenated water.

Laboratory experiments

High density and low density laboratory predation experiments were conducted in circular plastic containers 9.5 cm in diameter and 3.5 cm tall, and in plastic containers 14x14x9 cm, respectively. Low density treatments consisted of various combinations of numbers of adult copepods (1, 5, 10), numbers of *Ae. aegypti* larvae (5, 10), and age of larvae (newly hatched (age = 0), 1, 2, 3, 4 and 5 d; grown at room temperature without additional food). A total of 740 replicates was run between January 2000 and August 2001. For each replicate, the appropriate combination was introduced into the containers with 50 ml of distilled water and allowed to interact for 24 h. After 24 h, the number of copepods and larvae remaining were counted under a binocular microscope and the data were tabulated. The high density treatments consisted of 200 newly-hatched *Ae. aegypti* larvae and five adult copepods in 500 ml of distilled water and were otherwise conducted as above.

Additional laboratory experiments were conducted to test the effects of alternative prey and of habitat structure on predation of mosquito larvae by *M. albidus*. For these experiments, we introduced one *M. albidus* and 25 newly-hatched *Ae. aegypti* larvae to the plastic containers with 50 ml distilled water and added either 1 g (dw) leaf litter, 50 ml of *P. caudatum* culture, or 1.0 - 1.1 g of plastic aquarium plants (10 replicates of each). Numbers of larvae remaining in each were tabulated as before. For each treatment (litter, *P. caudatum*, plastic plants), 10 additional replicates were run which consisted of the same experimental set up minus the predator.

Roundhouse experiments

The experiments were carried out in the FMEL "Roundhouse," a roofed circular outdoor cage measuring 15 m in diameter and 25 m in height (Bidlingmayer 1977). The walls of the structure consisted of mosquito screening with double entrance doors. This arrangement allowed experiments to be conducted under near ambient conditions but excluded wild mosquitoes from the experimental area.

Experiments were conducted in discarded golf cart tires (40.6 cm diameter x 19.1 cm wide) during November, 1999. Prior to use, the tires were immersed in scalding hot water, scrubbed with a brush, dipped in dilute bleach solution, and rinsed thoroughly. Two combinations of predator densities (10 and 100 *M. albidus*) and three combinations of prey densities (300 *Ae. aegypti* : *0 Ae. albopictus*; 150 *Ae. aegypti* : 150 *Ae. albopictus*; and 0 *Ae. aegypti* : 300 *Ae. albopictus*) were replicated five times each for a total of 30 replicates. Each replicate was assigned randomly to one golf cart tire placed along the inner walls of the Roundhouse. Each tire received one l of water suctioned from automobile tires that contained mosquitoes that were maintained outdoors at the FMEL grounds. The water was sieved to remove detritus and macrobiota and frozen at -20 °C to kill remaining organisms. Oak (*Quercus virginiana* Mill.) leaves were collected from a tropical hardwood hammock on the FMEL property, dried at 80°C for 48 h and added to each tire (3 g each). Separate controls containing 300 *Ae. aegypti* and no copepods (5 replicates) and 300 *Ae. albopictus* and no copepods (5 replicates) were also run in similar golf cart tires.

Adult *M. albidus* were counted under stereoscopes and separated into labeled containers and left overnight in the Roundhouse without food. The appropriate number of newly-hatched mosquito larvae of each species were counted and placed in separate labeled containers and both predators and prey were then introduced into the experimental tires.

Prey and predators were allowed to interact without disturbance for 10 d, at which time the surviving larvae were close to pupation. At that time, all contents of each tire, including the leaf litter, were removed and placed in separate containers. In the laboratory, the contents were decanted into plastic trays. The remaining litter was washed repeatedly over a series of screens (80µm to 200µm mesh size), until no mosquito larvae or copepods could be found by inspection under a stereoscope, to recover any animals that may have been lodged in the litter. The screens were then washed again into the trays, and all larvae and copepods found were identified and counted.

Field experiments

The field experiments were run from December 2000 to October 2002. Twenty golf cart tires similar to the ones used in the Roundhouse experiments were haphazardly deployed in pairs in the FMEL hammock. Tires were placed on the ground, with members of each pair resting side by side under a combination canopy of sabal palms (*Sabal palmetto*) and live oaks (*Quercus virginiana*). All tires were placed in (qualitatively) similarly shaded situations, but quantitative radiation measurements were not taken.

Each tire received one l of distilled water, and 3 g (dw) of leaf litter. After 24 h, one tire in each pair received 150 copepods (treatment). Each tire was checked for mosquito larvae and copepods approximately every 4 wk. The contents of each tire were collected using a turkey baster, and a plastic scoop to collect the litter. The contents were immediately surveyed as described for the Roundhouse experiments and quickly returned to their tire. Number, species, and instar of mosquito larvae, number of copepods and number of egg-bearing females as well as air and water temperature were recorded.

All tires were checked approximately twice per wk for spiders, eggs of *Toxorhynchites* spp., and other potential mosquito larval predators. These were manually removed from the tires as soon as they were discovered. *Toxorhynchites* spp. were removed primarily in the egg stage although some eggs were missed and some young larvae were also removed from the tires.

When larvae were found, all the tires were checked and eggs and the larvae were immediately removed. In addition to *Toxorhynchites*, 11 spiders, 1 springtail, and 5 *Corethrella apendiculata*, were found and removed during the experiments. Several water striders were also removed on two occasions. During these checks, and after heavy rainfall events, water levels in the tires were also checked. If tires were close to overflowing, levels were brought back to normal by removing water with a plastic cup, passing it through a 200 µm sieve, and washing the sieve contents back into the tire. A total of seven overflow events in one or more tires were detected, all during the summer months.

At the conclusion of the experiments, all of the tire contents were removed. Concentrations of NH_4, NO_2, NO_3, and PO_4 in the liquid from each tire were determined using the indophenol, sulphanilic acid, diazo, and ammonium molybdate methods, respectively, and a YSI Model 9000 Photometer. Total litter content (dw) from each tire was also measured. Weather data were compiled from the University of Florida, Institute of Food and Agricultural Sciences (IFAS) weather station at Ft. Pierce, FL, approximately 15 km S of the study

area.

Data analysis

All analyses were performed using Statistica (Stat Soft, Inc, Tulsa Oklahoma). ANOVA assumptions were tested using Levene's Test for homogeneity of variances, Kolmogorov-Smirnov Test for normality, and plots of means vs. variances. Analyses were performed on raw data except for fractional data which were subjected to angular transformations before analysis. When appropriate, Tukey's HSD test was used for post-hoc comparison of factors.

Data from the laboratory experiments were analyzed using the General Linear Model procedures of Statistica. Because of missing cells in the design, Type V sums of squares were utilized for significance testing (Hocking 1996). This procedure produces a reduced model with the appropriate degrees of freedom for each term but may not allow significance tests for certain interaction effects. The alternative food-habitat complexity experiments were analyzed using a nested ANOVA (presence-absence of copepods within treatment).

Results from the Roundhouse experiments were subjected to separate analyses of variance for the overall effect of treatment upon larval survival and for the effects of copepod density, species mix, and species upon survival. The field tire data were analyzed via repeated measures ANOVA. A simple linear regression analysis was also conducted to explore the relationship between the average number of copepods in each tire (copepod treatment only) vs. the total number of larvae collected from each tire throughout the experiment. Single-linkage Euclidean classification trees of the tires based upon the nutrient and litter data collected at the end of the experiment were constructed using the cluster analysis program of Statistica.

RESULTS

Laboratory predation

Mean larval survival was 92.2% in controls (no copepods) and 29.8% in experimental runs (1, 5, or 10 copepods). Average survival of experimental treatments ranged from greater than 80% for 5-d-old larvae to 0% for newly-hatched larvae (Figure 1). ANOVA results indicate significant effects of initial number of copepods, initial number of mosquito larvae, and age of mosquito larvae upon larval survival, with significant interactions between larval age and numbers of copepods and initial numbers of larvae (Table 1). Post-hoc tests of main effects show that, as expected, larval survival was greatest when no copepods were present, followed by one copepod per dish and then 5 and 10 copepods (no difference between the last two). Overall survival was greater for 10 larvae than for 5, and greater for 4-d-old larvae than for 3-day and 5-day-old, which in turn had greater survival than newly hatched, 1-d and 2-d old larvae (Table 1).

Examination of the data reveals that 4-d-old larvae had higher survival than 5-d-old larvae without predators or with only one copepod, but that the situation was reversed when five or more copepods were present (Figure 1); hence the significant interaction effects of number of predators with prey age. The interaction with initial numbers of larvae is more difficult to understand because age-specific survival varied differently with the five predator densities and some of the marginal cells for the three-way effects (age x copepod density x larval density) were empty.

In the high density experiments, an average of 195.35 (1.62 S.E.) mosquito larvae survived after 24 h in the controls, whereas only 39.2 (5.32 S.E.) survived in the treatments with five copepods. This results in average kill rate of 32.16 larvae per copepod in 24 h. The difference between the two treatments is highly

Table 1. Results of analysis of variance for larval survival in the laboratory predation experiments. Type V sums of squares could not be computed for the C x L and the C x L x A interactions due to missing cells. HSD denotes significant differences in larval survival determined via Tukey's HSD post hoc test.

FACTOR	df	F	*P*≤	HSD
Copepods (C)	$^{1}/_{739}$	127.39	0.001	0 > 1 > 5 = 10
Larvae (L)	$^{3}/_{739}$	253.62	0.001	10 > 5
Age (A)	$^{5}/_{739}$	7.64	0.006	4 > 5 = 3 > 0 = 1 = 2
C x L	-	-	-	-
C x A	$^{15}/_{739}$	25.972	0.001	-
L x A	$^{5}/_{739}$	3.622	0.003	-
C x L x A	-	-	-	-

Table 2. Results of nested analysis of variance for larval survival in the food/structure experiments. HSD denotes significant differences in larval survival determined via Tukey's HSD post hoc test (un-nested factors). Each replicate consisted of the treatment (PC = *Paramecium caudatum*, P = plastic plant, L = litter), 25 mosquito larvae, and 1 *M. albidus*.

FACTOR	df	F	*P*≤	HSD
Treatment	$^{2}/_{54}$	22.923	0.001	PC > P = L
Copepods (Treat)	$^{3}/_{54}$	109.239	0.001	0 > 1

Table 3. Results of analyses of variance for larval survival in the Roundhouse experiments.

FACTOR	df	F	*P*≤
OVERALL			
Treatment	$^{7}/_{32}$	73.887	0.001
DENSITY/MIX			
Copepod Density	$^{1}/_{26}$	92.950	0.001
Mix (single spp./mixed spp.)	$^{1}/_{26}$	0.0938	0.757
Density x Mix	$^{1}/_{26}$	1.028	0.321
SPECIES			
Species	$^{2}/_{37}$	1.873	0.168

Table 4. Results of *a posteriori* comparison of the proportion of larvae surviving the different treatments. Numbers indicate probabilities (£) that the given pairs are significantly different by Tukey's HSD Test. Letters indicate the initial *Macrocyclops* density (C=0, L=10, H=100) and numbers indicate the initial *A. aegypti-A.albopictus* densities). Numbers in parentheses are the larval survivorship for the group.

	L300-0 (0.255)	**H300-0** (0.005)	**L150-150** (0.281)	**H150-150** (0.0001)	**L0-300** (0.248)	**H0-300** (0.049)	**C300-0** (0.876)	**C0-300** (0.895)
L300-0	-	0.001	0.990	0.001	0.997	0.001	0.001	0.001
H300-0		-	0.001	0.997	0.001	0.981	0.001	0.001
L150-150			-	0.001	0.998	0.001	0.001	0.001
H150-150				-	0.001	0.898	0.001	0.001
L0-300					-	0.001	0.001	0.001
H0-300						-	0.001	0.001
C300-0							-	0.990
C0-300								-

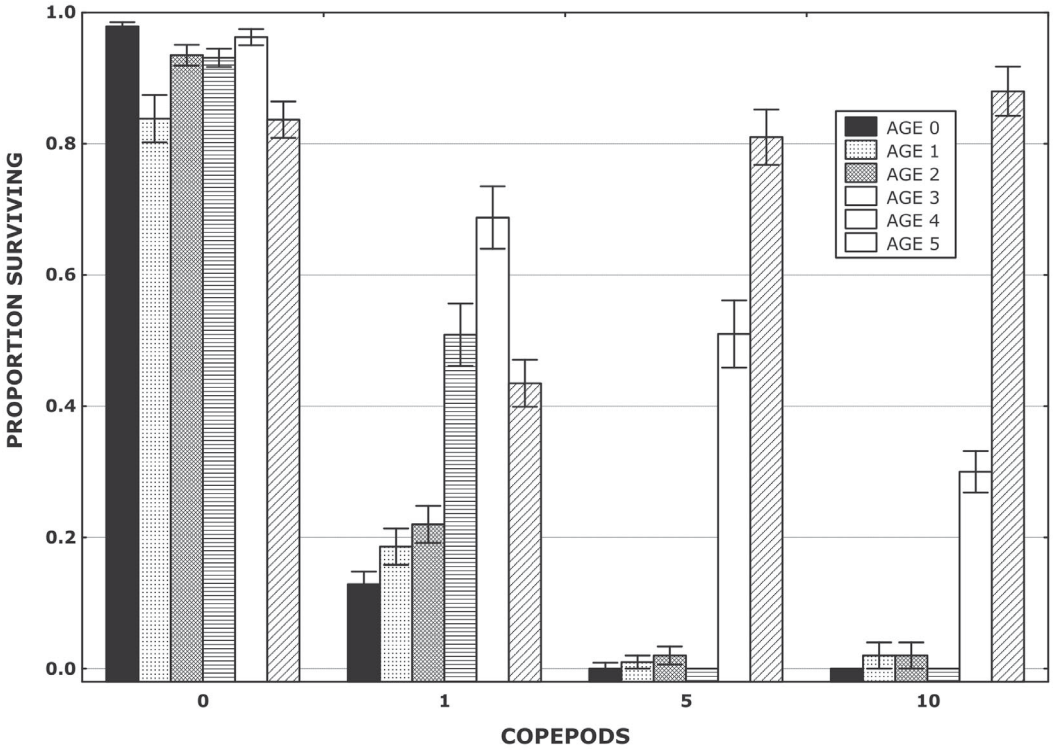

Figure 1. Mean (± S.E.) *Aedes aegypti* larvae survival after 24 h in laboratory microcosms. Larval ages in days (age 0 = newly-hatched).

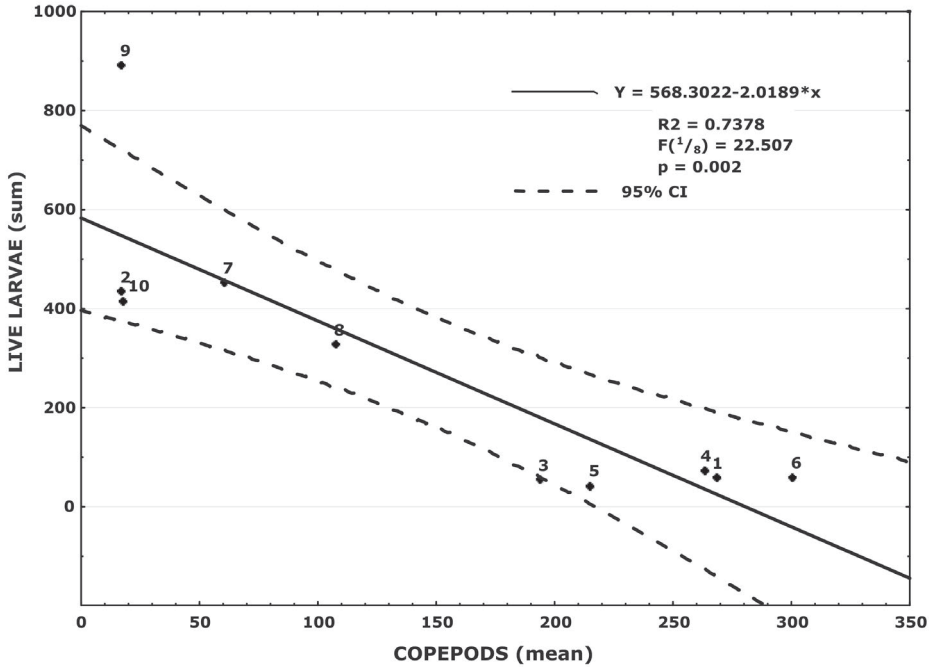

Figure 2. Linear regression of mean number of copepods vs. total number of mosquito larvae in the experimental field tires.

Table 5. Number of live and gravid copepods and water temperature in the experimental tires.

Month/Year	Live Copepods	Gravid Copepods	Water Temp. (°C)
December-2000	1,500	0	20.0
January-2001	421	23	12.7
February-2001	363	30	18.5
March-2001	407	31	19.4
April-2001	2,362	102	21.1
May-2001	978	57	22.6
June-2001	1,969	120	24.8
July-2001	1,380	150	25.3
August-2001	2,551	262	26.3
September-2001	2,172	537	23.7
October-2001	2,326	740	22.9
November-2001	2,242	450	20.8
December-2001	1,899	160	18.7
January-2022	1,638	294	18.4
February-2002	1,889	356	15.8
March-2002	2,272	422	21.2
April-2002	1,754	251	21.8
May-2002	1,677	300	23.4
June-2002	1,123	259	24.9
July-2002	1,258	87	25.3

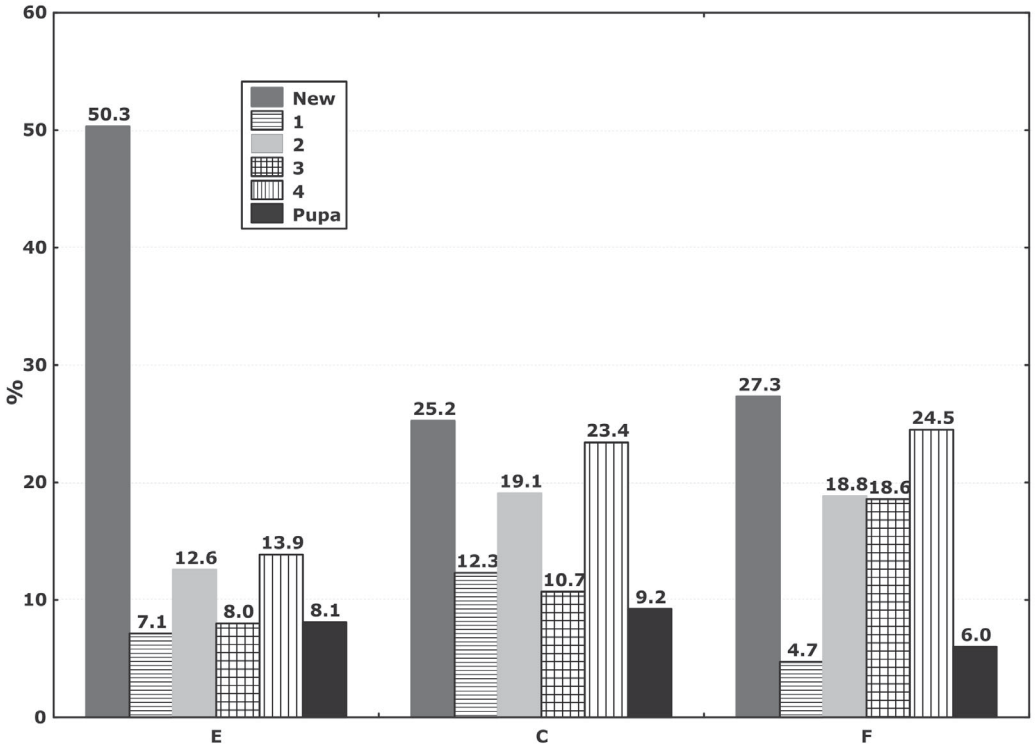

Figure 3. Percent of mosquito immatures remaining in the field tires by instar. E = experimental, C = control, F= failed experimental.

Table 6. Results of repeated measures ANOVA for the effects of sampling date and presence/absence of copepods (Cops) upon numbers of mosquito larvae in the field tires.

FACTOR	df	F	*P*≤
Date	$^{19}/_{171}$	4.532	0.001
Cops	$^{1}/_{9}$	8.166	0.018
Date X Cops	$^{19}/_{171}$	1.3007	0.188

significant (ANOVA, F = 786.96, $^{1}/_{38}$ df, $P > 0.0001$).

Both presence/absence of predators and structure/food treatment had highly significant effects upon mosquito larval survival in the food/structure experiments (Table 2). In all treatments, larvae had significantly higher survival in the absence of *M. albidus*, than when the predator was present and higher survival in the *P. caudatum* treatments than in the leaf litter and plastic aquarium plant treatments. Mosquito larval survival was greater than 96% in all treatments without predators. In the treatments with 25 larvae and one predator, larval survival was reduced after 24 h to 33% with leaf litter and 30% with plastic aquarium plants. In the *P. caudatum* treatments, survival was reduced to 86%.

Roundhouse experiments

There was a significant overall effect of treatment (copepod density and larval density) upon larval survival in the tires (Table 3). The initial copepod density effect upon larval survival was highly significant. Treatments with high initial copepod density had lower survival than those with low densities, which in turn had lower survival than controls (Table 4). There were no significant differences between treatments with mixed mosquito species vs. treatments with single species, nor between the two mosquito species in single species treatments. Average survival of mosquito larvae was 88.6% in tires with no copepods, 22.0% in tires with 10 copepods, and 1% in tires with 100 copepods. *M. albidus* was able to survive and reproduce in the experimental tires, with average recovery at the end of the experiments of 1.9 times the number of copepods originally introduced in the low density treatments (range 1.5 - 2.6), and 1.7 times the number introduced in the high density treatments (range 1.1 - 1.8).

Field experiments

Copepods survived and reproduced well in six of the ten field tires. After the initial introduction of 1,500 *M. albidus* (10 tires, 150 per tire) in December of 2000, there was a decline in numbers, and then a sharp increase to a peak of close to 2,600 in August of 2001. Gravid females were present in at least some of the tires throughout the course of the experiments reaching a peak of more than 700 gravid females in October 2001 and a minimum of 23 in January 2001, less than one month after the initial introduction (Table 5). Survival was poor in tires 2, 7, 9, and 10. In these tires, establishment of copepod populations was not successful even after repeated introductions. Cluster analysis based upon the nutrient and litter data from the tires indicate that tires 2, 9, and 10 cluster close together. Examination of the individual variables reveals that the only consistent pattern among these tires is that they had the lowest litter content of all the experimental tires. No relation between the nutrient variables measured and copepod abundance in the experimental tires was evident.

An additional tire with copepods was added at station 2 in November, 2001 and at stations 7, 9, and 10 in May, 2002 (B-series). Copepod survival was good in the new tires at stations 2B and 7B with an average of 129.5 and 96.8 copepods collected per sampling, respectively, and poor at stations 9B and 10B (48.2 and 41.0 copepods per sampling, respectively). After the initial introduction, egg-bearing females were found in tires 2B and 7B during every sampling (mean = 12.4 and 25.0 gravid females per tire per sampling, respectively), but none in tires 9B and 10B. On December 2, 2002 the original tires at stations 9 and 10 were moved to the former location of tire 4, where copepod survival had been good during the original experiments, and additional 150-copepod complements were added to each. Copepod survival at the new locations was good. On December 23, 2002, numerous egg-bearing females were observed in the tires. Because of an uncharacteristically prolonged cold snap during January of 2003, tires 9 and 10 were moved indoors on January 13, 2003. On February 18, 2003, we counted 541 copepods in tire 9 and 18 copepods in tire 10.

Effects of treatment (copepods/no copepods) on mosquito larvae survival was highly significant overall (Table 6). Mean number of live larvae collected per tire was 27.5 from control tires, 14.2 from all experimental tires, and 5.2 from tires where copepod establishment was successful (tires 2, 7, 9, and 10 excluded). There was a significant negative regression of mean number of copepods per tire with total number of live mosquito larvae collected from each treated tire (Figure 2). Over 50% of the larvae recovered from the tires with copepods were new hatches, whereas only 25.2% and 27.3% were new hatches in the controls and in the failed experimental tires, respectively (Figure 3).

DISCUSSION

The laboratory predation rates observed in these experiments compare favorably with those observed by Marten (1990b), who reported single-copepod predation rates of 90% on first instar mosquito larvae after 24 h. A significant difference between the two experiments, however, is that Marten's copepods were starved for 24 h prior to prey exposure whereas ours were not. Williamson (1980) showed that attack and consumption rates by the copepod *Mesocyclops edax* on various prey increased after starvation for periods as short as 24 h. The predation rates observed in the high density laboratory experiments (>32 larvae per copepod in 24 h) exceed those considered "excellent" for biological control of mosquitoes (30 first instar larvae per 24 h; Marten and Thompson 1997).

One of the characteristics that make some cyclopoid copepods species good candidates for biological control of mosquitoes is their broad prey base. This may allow some of these copepod species to survive in containers even when mosquito larvae are absent and may facilitate population establishment in new containers (Jennings et al. 1994). The experiments where *P. caudatum* was also offered as food indicate that *M. albidus* will readily consume alternate prey when present. However, this characteristic does not appear to negate its effectiveness as a mosquito larval predator, as demonstrated by the results of this and other studies (Marten 1989, 1990a, b). Once copepod populations build up in a container, they quickly reduce other available food to very low levels (Marten 1989).

These experiments also show that habitat structure will influence predation rates at least in the short term (24 h), presumably by providing hiding spaces for potential prey. This may not be very important for biocontrol efforts in discarded tires and other artificial containers that have little internal structure except, perhaps, when heavy accumulation of litter and other debris provide such structure. However, effective control in the experimental field tires was achieved through a relatively large range of litter content (from 3 g at the start of the experiments to a mean of 12.2 g 22 mo later (range 5.4 - 24.2 g for all tires) and natural accumulation of debris.

M. albidus was a more efficient predator of younger than of older larvae. Predation dropped considerably for 4-d and older larvae, which is consistent with previous observations for *Mesocyclops longisetus* (Tietze et al. 1994). However, even at 4 d, there was close to 50% reduction in larval survival after 24 h with 5 copepods, and more than 70% reduction with 10 copepods (Figure 1). These results also illustrate that *M. albidus* prefers to prey on younger larvae, but that it will increasingly attack older larvae as greater predator densities reduce the supply of younger ones. The fact that there were no significant differences in survival between mosquito species and between single versus mixed species treatments also reflects the broad preference in prey selection by *M. albidus*, but only to a limited extent, because *Ae. aegypti* and *Ae. albopictus* larvae have very similar foraging behavior. Swimming behavior, as well as size, has been shown to influence predation success in copepods (Kerfoot 1978, Dieng et al. 2002).

Other investigators have reported that it took several weeks for copepod populations to show significant growth in tires (Marten 1990b, Tietze et al. 1994), a result also observed during the field portion of this study. However, in the Roundhouse experiments, *M. albidus* was able to almost double in numbers in the tires even though the experiments were of relatively short duration (10 d). It is possible that, in this case, population maintenance and growth was fostered by the relatively large number of prey (newly-hatched mosquito larvae) introduced into the containers simultaneously.

Once established, *M. albidus* was able to maintain viable reproducing populations in the field during the 22-mo duration of the experiments. Marten (1990b) recorded *M. albidus* population sizes in discarded tires in New Orleans of 30 to 150 individuals per tire. In contrast, we observed mean population sizes of 225 individuals and as high as 651 individuals per tire in the tires where establishment was successful (mean = 144 if the failed tires are included). Differences in food supplies may be responsible for the higher population sizes at our sites. Milder winter temperatures may have also contributed to the observed differences as the 2001 and 2002 Florida winters were mild and no hard freezes were experienced in the area where the experiments were conducted.

We cannot offer a clear explanation for the failure of *M. albidus* to establish at stations 2, 7, 9, and 10. Results of the B-tire experiments indicate that at two of these sites (2B and 7B) the failure was probably due to some characteristic of the original tires and not the sites, as copepod population growth and reproduction was good at those sites in the replacement tires. Conversely, it appears that site-specific effects were at work at station 9 because population increase and reproduction by *M. albidus* were clearly evident when the tire was moved to a different location. Although copepod numbers were low at tire 10 in the new location, copepods and gravid females were still present 2.5 mo after it was moved.

Marten (1990b) attributed poor copepod survival in some of the tires in similar experiments to high water temperatures close to the tolerance limits of most

copepod species (up to 40°C) caused by direct sun exposure and low food content. Because all of our tires were in a tropical hammock with plenty of shade, water temperatures above 30°C were never recorded in any tire. At the end of the experiments, litter content at three of the four failed sites (2, 9, and 10) was the lowest of all the experimental tires, but it was still higher than when the experiments were started, and copepod populations thrived at the other sites under these conditions.

Mosquito control in the tires with copepods was excellent, and the levels of reduction of mosquito larvae suggest that this species is capable of affecting satisfactory control of mosquito larvae in discarded tires and possibly other types of containers. Furthermore, over half of the immature mosquitoes recovered from the tires with copepods were newly hatched and thus still likely to be preyed upon by the copepods. Only about 22% of the immature mosquitoes remaining in these tires were 4th instar or pupae. Marten considers that other species, such as *Mesocyclops longisetus* and *Mesocyclops aspericornis,* may be better suited for biological control in tropical areas because they maintain larger population sizes than *M. albidus* (Marten 1990b and personal communication). However, in this study, population sizes of *M. albidus* in field tires were much higher than previously reported from more temperate (Marten 1990b) and tropical (Marten et al. 1994) areas. Also, because of its cold-hardiness, *M. albidus* should be better able to maintain year-round populations in subtropical areas than species recommended for the tropics and may be able to survive even under extreme conditions (e.g. record cold weather) which, in the subtropics, are usually of short duration. Further tests are needed to determine the effectiveness of this predator in larger bodies of water such as ditches and swales, as available data are scant and inconsistent (e.g., Marten 1990c, Kay et al. 2002b, de Roa et al. 2002).

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