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EVALUATION OF *MESOCYCLOPS ASPERICORNIS* (CYCLOPOIDA: CYCLOPIDAE) AND *TOXORHYNCHITES SPECIOSUS* AS INTEGRATED PREDATORS OF MOSQUITOES IN TIRE HABITATS IN QUEENSLAND

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ABSTRACT. This study addressed biological control of peridomestic *Aedes notoscriptus,* known to be a highly effective colonizer of tire habitats and a possible vector of Ross River virus. A laboratory trial of the compatibility of the predators *Mesocyclops aspericornis* and *Toxorhynchites speciosus* in small container habitats showed that 4th-instar *Tx. speciosus* did not significantly affect *M. aspericornis* mortality. Introduced *M. aspericornis* and naturally occuring *Tx. speciosus* were found to form a compatible predator pair for reduction of larval *Ae. notoscriptus* and *Culex quinquefasciatus* populations in tire habitats. Over 22 months of field survey, 97% of tires without predators contained mosquito larvae, at a median density of 43 larvae/liter. By comparison, 51% of tires containing both predator species held mosquito larvae at a median density of 4 larvae/liter. Predation by *Tx. speciosus* persisted for the duration of the study. The inability of the Lake Kurwongbah strain of *M. aspericornis* to tolerate temperatures of ≤10°C, which are prevalent in Brisbane during winter, resulted in a failure to deliver persistent reduction of mosquitoes in tires. The temperature-dependent population characteristics of *M. aspericornis* emphasize the long-recognized importance of matching a biological control candidate's physiological requirements to the environment in which control is sought.

INTRODUCTION

In Australia, as in much of the world, discarded tires are a threat to public health. Exotic vectors of disease such as *Aedes aegypti* (Linn.) (Frank 1981, Barker-Hudson et al. 1988), and *Aedes albopictus* (Skuse) (Hawley 1988) are becoming increasingly prevalent in tires despite warnings from public health authorities.

Much research has been aimed at utilizing the inherent predatory behavior of cyclopoid copepods and *Toxorhynchites* mosquitoes for the biological control of insect vectors inhabiting container habitats (Laird 1988). In the field, various species of cyclopoids have produced a high level of control of *Aedes* mosquitoes, in a variety of natural and artificial containers (Riviere et al. 1987; Marten 1990a, 1990b). In New Orleans, *Macrocyclops albidus* (Jurine) and *Mesocyclops longisetus* (Thiebaud) were the most effective predators of *Ae. albopictus* in discarded tires over a 1-year period (Marten 1990a).

In a study of habitats in which mosquitoes and copepods naturally coexist, *Toxorhynchites rutilus septentrionalis* (Dyar and Knab) was found in association with a number of cyclopoid species in tires (Nasci et al. 1987). The cohabitation and compatibility of *Toxorhynchites* with cyclopoid copepods in tires has also been noted by Riviere (1985), Riviere et al. (1986), and Marten (1990a). These observations are encouraging, as in field practice, the degree of control achieved by *Toxorhynchites* spp. alone has been disappointingly low (Mogi et al. 1985, Pillai 1985, Annis et al. 1989).

A population of *Mesocyclops aspericornis* (Daday) from Lake Kurwongbah (27°15′S, 152°58′E) in northeastern Australia was found to be more effective than 6 other species of native *Mesocyclops* for killing *Ae. aegypti* and *Culex quinquefasciatus* Say in artificial containers (Brown et al. 1991). It was also the most fecund *Mesocyclops* species evaluated, and survived at water temperatures similar to a subtropical Australian winter. Therefore, this copepod warranted investigation in subtropical northeastern Australia as a biological control agent for mosquitoes breeding and developing in tire habitats.

In Brisbane (27°28′S, 153°01′E), *Aedes notoscriptus* (Skuse) (a suspected vector of Ross River virus causing epidemic polyarthritis [Watson, Ritchie, and Kay, unpublished data]) and *Cx. quinquefasciatus* (a proven filaria vector [Boreham and Marks 1986]), commonly inhabit discarded tires. Larvae of the large larvivorous and nonhematophagous *Toxorhynchites speciosus* (Skuse) are also not uncommon in this habitat type (Marks 1982). This naturally occurring assemblage of mosquito species provided an ideal opportunity to study both the efficiency with which *M. aspericornis* reduces *Aedes* and *Culex* larvae in tires, and the compatibility of the co-

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pepod with *Toxorhynchites* larvae as integrated control agents.

Accordingly, a small-scale trial was initiated in which populations of each species were monitored over 22 months, and the control efficacy of the 2 predators was gauged according to season. The goal of this study was the delivery of a long-term, self-sustaining, inexpensive method of control. In addition to field assessments, a laboratory compatibility trial of *M. aspericornis* and 4th-instar *Tx. speciosus* in small container habitats was conducted.

MATERIALS AND METHODS

Study site: The study was conducted in a wooded undisturbed section of the Queensland Institute of Medical Research grounds, Brisbane. Nine standard car tires, selected for inoculation with *M. aspericornis,* were arranged into 3 groups of 3. Two groups of 3 tires, to be used as untreated controls, were placed approximately 5 m apart, and 10 m from the treated tire group. Four liters of tap water and a sediment of leaves and twigs were introduced into each tire. The tires were left undisturbed for 2 wk prior to inoculation, thus allowing time for colonization by the indigenous mosquito fauna and the breakdown of chlorine to levels nontoxic to *M. aspericornis* (Brown et al. 1994).

Inoculation and sampling: In January 1990, 5 gravid *M. aspericornis* were introduced into each tire via 20-ml plastic tubes to give a dosage of 1.25 copepods/liter. The inoculum was collected from a 2,000-liter culture tank. Oviposition in the tires by naturally occurring *Tx. speciosus* made artificial introduction and manipulation of this predator unnecessary. Every 14 days, copepods and mosquitoes in each tire were sampled, and water temperature recorded for 22 months. A 1-liter sample was taken from the bottom of each tire by sweeping a 5 × 10-cm-aperture net of 100-μm mesh a distance of approximately 20 cm. The contents of each sample were emptied into a white tray for counting and mosquitoes identified according to Marks (1982). Once counted, the sample was returned to the tire, and distilled water added to return the volume to 4 liters. Copepod identity was periodically verified according to Van de Velde (1984). All sampling was completed between 0800 and 1200 h.

Analyses of data: The probability distributions of all species sampled were J-shaped (an index of homogeneity or relative diversity [Pielou 1966]). In light of these distributions, 2 approaches to analyses were used:

1) Analysis of binary variables consisting of 2 classes (absent [count = 0]; present [count > 0]) from which combinations of these categories for prey (*Ae. notoscriptus* and *Cx. quinquefasciatus*) and predators (*M. aspericornis* and *Tx. speciosus*) were derived. Chi-square tests of Fisher's exact test (in the case of small expected frequencies) were applied to cross-classification tables involving these combinations and any 2-group factors of interest. The primary categories of interest in the hypotheses were tires where there were no mosquitoes (none) compared with tires where mosquitoes were present; and tires where there were no predators (none) compared with tires where predators were present.

2) For those tires where mosquitoes were present, mosquito prey counts were analyzed with respect to any 2-group factors of interest, using the Wilcoxon–Mann–Whitney *U*-test. This test is independent of the fact that the distributions are J-shaped and only assumes that types of probability distributions are similar. The test statistic involves rank transformation of counts and evaluates the probability distributions of the groups and whether the 2-group factors have similar locations (H_0) or different locations (H_1). As the distribution was J-shaped, median counts were used in place of averages.

The data were divided into 3 summer and 2 winter seasons and analyzed on the basis of water temperature groupings as follows: summer was defined as having water temperatures >15°C, and winter, temperatures ≤15°C. Analysis of copepod predation efficacy above and below 15°C was of interest as this is the temperature at which Brown et al. (1991) found *M. aspericornis* to exhibit reduced activity. Monthly averages were expressed as means (range) and seasonal averages were expressed as medians. Statistical Analysis System software (SAS Institute 1988) was used for all computations.

Laboratory trial of M. aspericornis *and* Tx. speciosus *compatibility:* The compatibility of large predacious 4th-instar *Toxorhynchites* larvae and *M. aspericornis* at 2 and 10 individuals per liter, respectively, was studied in three 3-liter tin cans. Three cans containing 10 copepods/liter without *Tx. speciosus* were used as controls. The trial was run for 72 h, with the numbers of surviving copepods and mosquitoes counted every 12 h.

In order to approximate field conditions where alternative food sources are available, 400 ml of *Chlorella* suspension and 200 ml of protozoan culture were provided in each can. The protozoan culture consisted of a mixed *Euglena, Paramecium,* and rotifer fauna. Harper's powdered dog food (5 μg/can) was added as larval mosquito food. Distilled water was added to maintain the volume at 2 liters. A lid was fitted to cover 80% of the top to simulate an enclosed container habitat, and the trial was conducted at 25°C, a common summer temperature of container habitats in southeastern Queensland (Jennings et al. 1994).

Differences in survival of *M. aspericornis* in the *Toxorhynchites* and *Mesocyclops* containers, and the *Mesocyclops* only (control) containers, were compared using Kaplan–Meir survival curves and the log-rank test statistic (Kalbfleisch and Prentice 1980).

RESULTS

Predator population density characteristics: 1) *Mesocyclops aspericornis:* During the first 12 months (1990), the number of copepods present in the treated tires was closely related to temperature (Fig. 1). Following introduction into the tires, the numbers of copepods increased exponentially, reaching a mean density of 24 (range 8–76) copepods/liter after 4 wk. From February to May, when temperatures were above 15°C, an equilibrium density of approximately 18 (range 6–35) copepods/liter was maintained. At temperatures below 15°C (May–August), a copepod density of approximately 2 (range 0–3) copepods/liter was maintained. With increased temperature from August 1990 to March 1991, copepod densities varied between 23 (range 18–28) and 10 (range 3–28) copepods/liter, respectively.

The median densities were 9, 2, and 23 copepods/liter during the first 3 seasons. The increase in density between the first and second summer seasons was significant ($P = 0.02$). The numbers then decreased with temperature to 0 copepods/liter by May 1991. From May to November, no copepods were recovered. At the conclusion of the study, 2 of these tires contained *M. aspericornis* at densities of 1 and 6 copepods/liter.

2) *Toxorhynchites speciosus:* Maximum densities of 3 (range 1–5) and 4 (range 2–6) *Tx. speciosus* larvae/liter were recorded in May and February 1990, for treated and untreated tires, respectively (Fig. 1). From August to November in both 1990 and 1991, numbers were reduced. The percentage of tires containing only *Toxorhynchites* larvae reached a maximum at 42% in the second winter, by which time *M. aspericornis* had disappeared. The percentage of tires containing only *Tx. speciosus* differed significantly over the 5 seasons ($P < 0.04$). Using a chi-square goodness-of-fit test, and adjusting for the number of tires and their location, total *Toxorhynchites* larval counts also varied significantly over the seasons ($P = 0.02$), with a mean density of 2.5 *Toxorhynchites*/liter in the first summer, and a mean density of 1.5 larvae/liter in the second winter.

Prey mosquito population density characteristics: 1) *Aedes notoscriptus:* This was the first of the prey species to colonize the tires. Two weeks after the tires had been filled with water, and on the day *M. aspericornis* was introduced into the selected tires, *Ae. notoscriptus* numbers had reached a density of 4 (range 0–9) and 31 (range 7–270) larvae/liter in the treatment and control tires, respectively (Fig. 1). From February to May 1990 in the treated tires, larval densities varied between 2 (range 1–3) and 2 (range 0–4) larvae/liter, and the median density in this first summer season was 2 larvae/liter. During this period, densities varied between 47 (range 12–190) and 5 (range 1–14) larvae/liter in the untreated tires, with a median of 30 larvae/liter. The 2 medians were significantly different ($P < 0.0001$). In July, at a temperature of 14°C, when copepod numbers were reduced to a mean density of 2 (range 0–4) copepods/liter, 13 (range 5–33) larvae/liter was recorded in the treated tires. After 12 months of study, densities of 3 (range 0–9) and 19 (8–60) larvae/liter were recorded in the treated and untreated tires, respectively. At the conclusion of the study, 7 (range 1–13) and 79 (range 35–100) larvae/liter were sampled from the treated and untreated tires, respectively (Fig. 1). Consistently, the median densities (seasonal averages expressed as medians) in the treated tires were significantly lower than in the control tires ($P < 0.005$), with overall medians of 9.5 larvae/liter in the treated tires and 31 larvae/liter in the control tires.

2) *Culex quinquefasciatus: Culex quinquefasciatus* was first sampled 2.5 months after the study was initiated, at a density of 1 (range 0–2) larva/liter. Larval numbers then oscillated independently of temperature in both the treated and untreated tires for the remainder of the study. A maximum density of 25 larvae/liter was recorded in August and September 1990 in the untreated tires, when treated densities were 6 (range 0–12) larvae/liter. For the last 2 months of the study, no *Culex* were sampled from either the treated or untreated tires. In terms of mosquito prey species composition in the tires during the 22 months of study, 50.9% of the samples contained only *Ae. notoscriptus*, 4.5% only *Cx. quinquefasciatus*, and 23.6% both species.

Analysis of control efficacy: During the first summer, 68% of the tires in which the only contained predator was *M. aspericornis* also contained mosquito larvae (Table 1). In these tires, the mosquito prey (both *Aedes* and *Culex* species) were sampled at a low median density of 3 larvae/liter. In tires in which the only predator was *Tx. speciosus,* 67% contained mosquito lar-

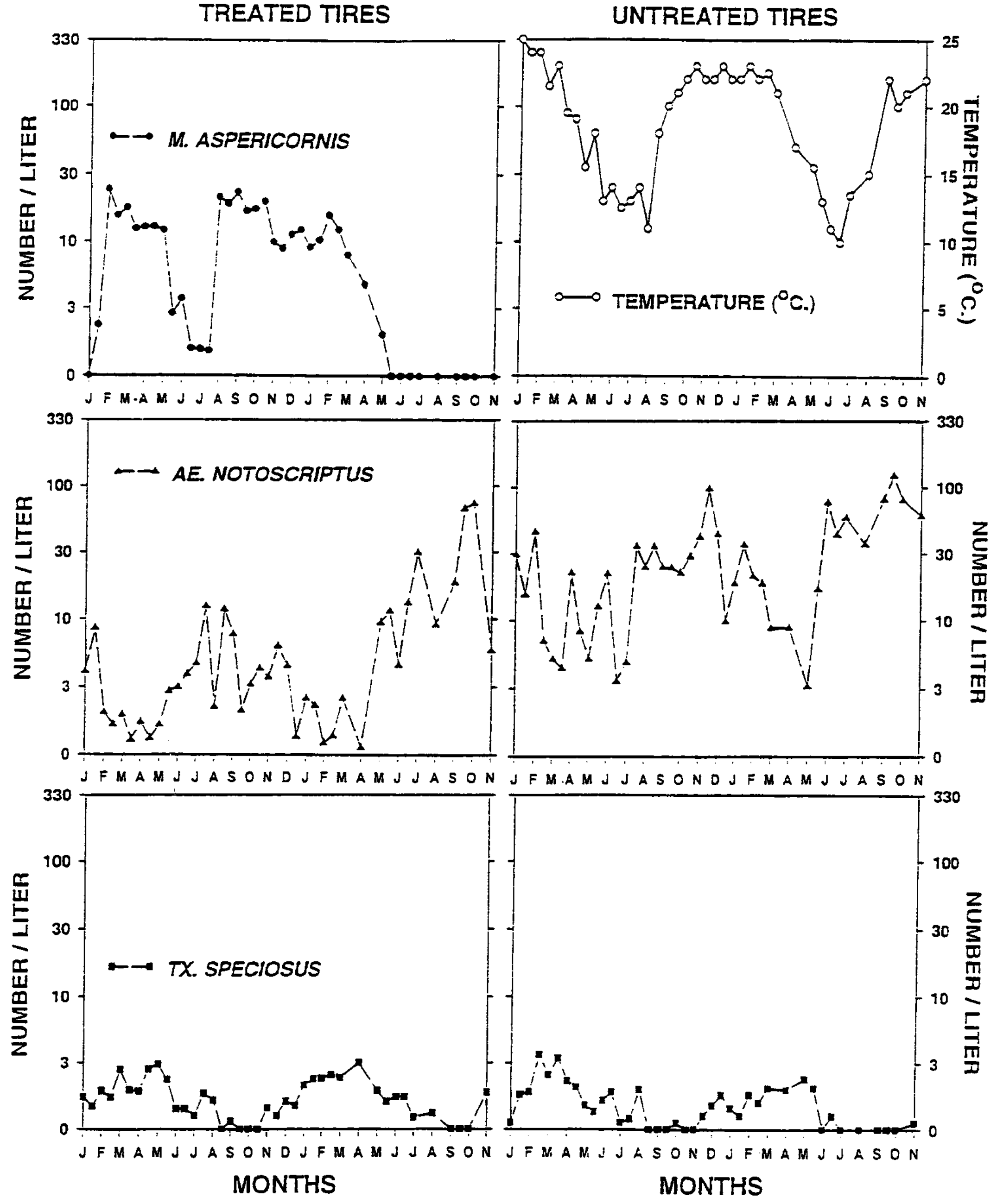

Fig. 1. Number of *Mesocyclops aspericornis* and larval mosquitoes in treated and untreated tires during 22 months of field study. Points for copepods and mosquitoes shown as mean of 9 and 6 tires for treated and untreated studies, respectively.

vae, and these occurred at a median density of 17.5 larvae/liter. In tires where both predators occurred, 49% contained mosquito larvae at a median density of 2 larvae/liter. In the absence of predators, 92% of tires contained mosquito larvae at a higher median density of 45 larvae/liter, which was significantly different from the above ($P < 0.05$).

The second summer of the study was the last period in which *M. aspericornis* exerted any mosquito control (Table 1). *Toxorhynchites speciosus* preyed on mosquito larvae (both *Aedes*

Table 1. Summary of the effectiveness of *Mesocyclops aspericornis* and *Toxorhynchites speciosus* control of mosquitoes in tires over a 22-month period. The presence of predators is compared with no predators in statistical tests.

	Percentage of tires with prey mosquitoes and median mosquito numbers (n)[1]		
	Jan.–May 1990	June–Aug. 1990	Sept. 1990–May 1991
No predators	92% & 45 (23)	89% & 31 (25)	98% & 37 (60)
Predators			
M. aspericornis	68%* & 3** (15)	75% & 14.5 (12)	84%** & 7** (3)
Tx. speciosus	67%* & 17.5** (26)	89% & 12.5 (16)	81%** & 13** (46)
Both predators	49%** & 2** (24)	94% & 7** (6)	31%** & 3.5** (12)

[1] Levels of significance denoted as follows: * $P < 0.05$; ** $P < 0.001$, blank is nonsignificant.

and *Culex*) for the duration of the study, and the median numbers ranged between 12.5 and 22.5 larvae/liter.

Of the tires treated with *M. aspericornis,* only 6% contained copepods after 22 months of study (Table 2). With reduced numbers of *M. aspericornis* over time, and the oscillating density of *Tx. speciosus,* the percentage of tires containing predators decreased from 98% in the first summer to 23% in the third summer (Table 2).

Analysis showed that where *M. aspericornis* was the only predator, 66% of tires had 100% elimination of *Ae. notoscriptus,* and this occurred at copepod densities of ≥4 *M. aspericornis*/liter (Fig. 2). In contrast, with *Tx. speciosus* only, 61% of tires displayed 100% elimination of the prey, and these contained at least 2 *Tx. speciosus*/liter.

Analysis of laboratory trial: There was no evidence to suggest that the survival rates of *M. aspericornis* were different in the presence or absence of 4th-instar *Tx. speciosus* ($\chi^2 = 0.0$, 1 df, $P = 0.99$) (Fig. 3).

DISCUSSION

The temperature-dependent population characteristics of *M. aspericornis* during this study emphasize the importance of matching the physiological requirements of biocontrol candidates with the environment in which control is sought. Although the *M. aspericornis* population was derived from a large south Queensland lake, it seems that water temperatures in tires are subject to greater extremes because of the smaller water volume. After an expected decline in copepod numbers during the second winter, the population failed to redevelop with increased summer temperatures (Fig. 1). In a study of *Mesocyclops* population growth in relation to temperature, Brown et al. (1991) found that *M. aspericornis* from both Lake Kurwongbah (Brisbane) and French Polynesia died at 10°C. The low morning temperatures recorded during the 1991 winter (12 ± 2°C) may have proved limiting.

Aedes notoscriptus was proven to be an opportunistic colonizer of tire habitats. The speed with which this mosquito species invaded the tires, and the high larval densities that were maintained throughout the summer and winter seasons, indicate why this mosquito has so successfully adapted to the peridomestic environment in southern Queensland and New South Wales (Russell et al. 1984). The *Tx. speciosus* larvae were if anything more common during the cooler winter months and were entirely dependent on *Ae. notoscriptus* and *Cx. quinquefasciatus* larval populations. As a consequence of their slow development time and greater food demands (May and Anderson 1979), they never exceeded a mean density of 4 larvae/liter.

This study indicated that tire habitats, rather than rainwater tanks (Lardeux 1992, Brown et al. 1994), are suitable for *M. aspericornis* colo-

Table 2. Percentage occurrence of predators in tires treated with *Mesocyclops aspericornis* over a 22-month period.

	Percentage of tires with predators for summers (S1–S3) and winters (W1 and W2)				
Predator	S1	W1	S2	W2	S3
M. aspericornis only	26	19	24	0	4
Toxorhynchites speciosus only	3	9	20	48	17
Both predators	69	25	27	6	2
Total percentage	98	53	71	54	23
No. of observations	83	46	119	54	45

Table 1. Extended.

Percentage of tires with prey mosquitoes and median mosquito numbers (*n*)[1]		
June–Sept. 1991	Sept.–Nov. 1991	All data
100% & 44 (49)	100% & 64 (60)	97% & 43 (217)
—	—	78%** & 7** (60)
68%** & 12.5** (26)	50%* & 22.5** (6)	73%** & 14** (120)
—	—	51%** & 4** (55)

nization. In contrast to the lack of persistence of copepods in rainwater tanks, *M. aspericornis* maintained median densities of 9 and 23 copepods/liter for 2 consecutive summers in tire habitats. The comparative levels of contained organic nutrients may provide an explanation for the contrasting results. Low food levels are a major factor limiting *Mesocyclops* population growth in rainwater tanks (Jennings et al. 1994). The thick substrate of leaves and organic matter present in the bottom of the tires during this study would have provided a readily available source of energy for primary (bacteria and fungi), secondary (protozoa, rotifers), and tertiary (dipterous larvae) food production (Riviere 1985). Although we added detritus to our tires artificially, the levels were realistic in view of field observations.

Although *M. aspericornis* and *Tx. speciosus* appear to be a compatible predator pair for use in tire habitats, their long-term performance, as evidenced in this study, could be considered a failure, as Ross River virus transmission is still possible even with low mosquito numbers (Kay and Aaskov 1989). When the results of this study are extrapolated theoretically to a large tire dump, with 51% of tires containing a median of 4 mosquito larvae (all data considered, Table 1), significant adult mosquito production will still occur. The continued abundance of *Ae. notoscriptus* through winter and the presence of Ross River transmission (Hargreaves and Hall 1992) in the absence of known major vectors suggest that *Ae. notoscriptus* could be involved in urban transmission. Furthermore, less than total predation may increase adult mosquito fitness as a consequence of decreased intraspecific competition for limited resources during the larval stages (Ho et al. 1989).

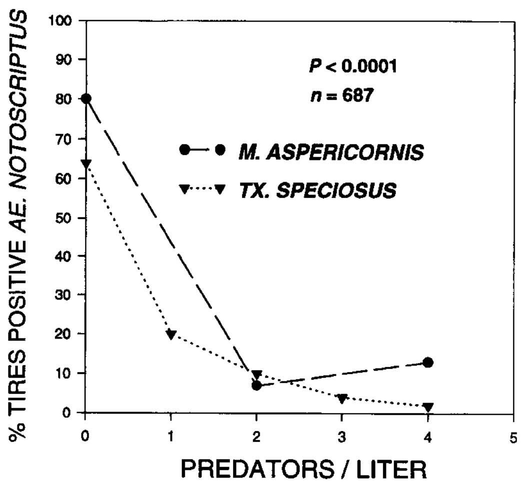

Fig. 2. Optimal densities of *Mesocyclops aspericornis* and *Toxorhynchites speciosus*/liter for elimination of *Aedes notoscriptus* in tire habitats.

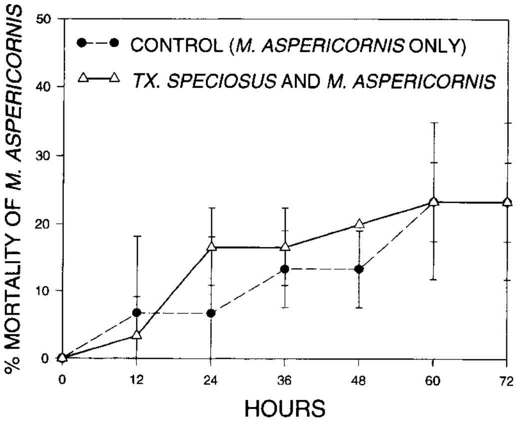

Fig. 3. Percentage mortality of *Mesocyclops aspericornis* from laboratory trials over 72 h, at *Toxorhynchites speciosus* and *Mesocyclops aspericornis* densities of 2 larvae and 10 copepods/liter, respectively. Points for copepod mortality are shown as mean ± SD of 3 replicates.

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