Laboratory Evaluation of *Mesocyclops annulatus* (Wierzejski, 1892) (Copepoda: Cyclopidea) as a Predator of Container-breeding Mosquitoes in Argentina

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In laboratory bioassays we tested the predatory capacity of the copepod Mesocyclops annulatus *on* Aedes aegypti *and* Culex pipiens *larvae. A single adult female of* M. annulatus *caused 51.6% and 52.3% mortality of 50 first instar larvae of* Ae. aegypti *and* Cx. pipiens *respectively, in a 72 h test period. When alternative food was added to the containers, mortality rates declined to 16% and 10.3% for* Ae. aegypti *and* Cx. pipiens *respectively. When 50 first instar larvae of each of the two mosquito species tested were placed together with a single adult female of* M. annulatus, *mortality rates were 75.5% for* Ae. aegypti *larvae and 23.5% for* Cx. pipiens *larvae in a three day test period. Different density of adult females of* M. annulatus *ranged from 5 to 25 females produced mortality rates of* Ae. aegypti *first instar larvae from 50% to 100% respectively. When a single adult female of* M. annulatus *was exposed to an increasing number of first-instar* Ae. aegypti *larvae ranging from 10 to 100, 100% mortality was recorded from 1 to 25 larvae, then mortality declined to 30% with 100 larvae. The average larvae killed per 24 h period by a single copepod were 29.*

Key words: copepod - *Mesocyclops annulatus* - *Aedes aegypti* - *Culex pipiens* - Argentina

Aedes aegypti L., eradicated from Argentina in 1963, has reinfested this country as far as south of Buenos Aires city and dengue is certainly emerging as an important disease in Argentina (Avilés et al. 1999). Vector control with insecticides may cause environmental damage and mosquito resistance (Breakley et al. 1984). Source reduction is appropriate in controlling *Ae. aegypti* larvae but some breeding sites are not amenable to this method. The use of biological control against *Ae. aegypti* is an alternative control measure that should be considered. *Culex pipiens* L. is also one of the major mosquito pest in Argentina and with *Ae. aegypti* are the two most common container-breeding mosquitoes.

Among all the natural enemies mentioned in the literature as potential candidates for mosquito control (Jenkins 1964, Roberts & Strand 1977, Roberts & Castillo 1980, Roberts et al. 1983), predacious copepods are promising candidates for biological control of container-breeding mosquitoes (Marten 1990). They are particularly effective predators of mosquito larvae. In addition, copepods do not depend on the supply of mosquito larvae for food. Copepods can feed on algae, protozoa, rotifers and others arthropod larvae. Various species of *Mesocyclops*, *Macrocyclops*, *Megacyclops* and *Acanthocyclops* have been tested in a variety of *Aedes*-breeding habitats (Riviere & Thirel 1981, Suarez et al. 1984, Riviere et al. 1987, Kay et al. 1992, Marten et al. 1994) with promising results.

Laboratory evaluation of these potential agents of biological control is an essential preliminary step toward selection of an appropriate candidate for field releases. In this study we reported the predatory behavior of a common temporary pool copepod, *M. annulatus* (Wierzejski, 1892). Laboratory trials were conducted to evaluate the effectiveness of this predaceous cyclopoid for control of *Ae. aegypti* and *Cx. pipiens* larvae in domestic containers in La Plata, Argentina.

MATERIALS AND METHODS

Collection and culture of M. annulatus - Copepods were collected using a net (100 μm mesh) through the margins of transient freshwater ponds near La Plata city, Buenos Aires province, Argentina (34° 51' 07" S, 58° 57' 30" W). Copepods were identified according to Reid (1985) and reconfirmed by Dr S Menú Marquez, University of Buenos Aires, Argentina. Individual gravid females of *M. annulatus* were placed in containers containing 200 ml of dechlorinated tap water, and added protozoan and algae as food. Copepod larvae were placed in containers with 2000 ml of dechlorinated water and incubated at 26 ± 2°C to adult stage.

Source of mosquito larvae - Larvae of *Ae. aegypti* and *Cx. pipiens* used in this study were obtained from colonies maintained at Centro de Estudios Parasitológicos y de Vectores, La Plata, Argentina. Larvae were kept in plastic pans (40 x 30 x 6 cm) with dechlorinated tap water until pupation and they were fed on powdered chicken food. Pupae were placed in screened cages for adult emergence. Adults of both species were fed on 10% sucrose solution and a restrained chicken was offered as a blood source twice a week. *Cx. pipiens* females were allowed to lay eggs in a container half filled with water. A strip of filter paper was added inside the perimeter of the container for oviposition of *Ae. aegypti* females.

Predation trials - Experiment 1: predatory capacity of *M. annulatus* on first-instar larvae of *Ae. aegypti* and *Cx. pipiens* was determined. A single *M. annulatus* adult female was placed with 50 first-instar mosquito larvae (24 h old) in a 250 ml plastic container (8 x 8 x 5 cm) containing 100 ml of dechlorinated tap water at 26°C. This experiment was conducted over 3 days, and number of surviving larvae on each day was recorded. To assess copepod feeding in the presence of an alternate food source, identical trials were conducted under similar conditions as described above with addition of 10 ml algal-protozoan suspension per container from an eutrophic pond. *Experiment 2*: this experiment was carried out for comparison of the effectiveness of *M. annulatus* as predators of *Ae. aegypti* and *Cx. pipiens* larvae living in the same container. Fifty larvae of each mosquito species and one adult female copepod were introduced in containers containing 100 ml of dechlorinated tap water. *Experiment 3*: it was designed to determine the number of adult copepods needed to consume a large number of first instar *Ae. aegypti* larvae. Five, 10, 15, 20 and 25 *M. annulatus* adult female were introduced in containers (11 x 11 x 12 cm) with 100 first instar larvae of *Ae. aegypti* and 700 ml of dechlorinated water. Surviving larvae were counted at 24 h. *Experiment 4*: predation capacity of individual *M. annulatus* female was determined in relation to different *Ae. aegypti* larval densities. Densities of first instar larvae of *Ae. aegypti* from 1 to 29 (increasing by 1) and from 30 to 100 (increasing by 10) were placed in 24 well plates (1.6 cm diam.) with 1 *M. annulatus* adult female and 3 ml of dechlorinated tap water per well. Larval mortality was determined at 24 h. Similar tests were conducted in 250 ml containers with 100 ml of water.

These experiments were conducted at 26 ± 1 °C under a photoperiod of 12:12 (L:D) h. Six replicates and two controls (containers with larvae without copepods) were conducted for each of the four experiments.

Statistical analysis - Mean number of killed larvae was compared between treatment and time by a two-way ANOVA. Multiple comparisons were tested by Tukey HSD test (Sokal & Rohlf 1995). In order to homogenize the variances, killed larvae numbers (n) were log (n + 1) transformed before to the analysis.

RESULTS

M. annulatus preyed on first and second instar larvae of both mosquito species, *Ae. aegypti* and *Cx. pipiens*. However, predation on *Ae. aegypti* larvae was affected significantly by presence of alternative food (F = 34.03, df = 3,36, P < 0.001). In the absence of alternative food, significant larval mortality was observed within 24 h and 48 h. Larval mortality was not significantly different between treatment and control at 72 h (Table I). By day 3, an average of 51.7% larval reduction was recorded in containers with copepods. In presence of alternative food, no significant larval mortality relative to controls was detected (Table I). Total mortality of *Ae. aegypti* larvae in containers with alternative food averaged 16%.

Predatory effectiveness of *M. annulatus* against first and second instar *Cx. pipiens* was affected significantly by presence of alternative food (F = 44.8, df = 3,36, P < 0.001). In absence of alternative food, daily larval mortality was significantly different from controls at 24, 48 and 72 h (Table II). Mortality was 52.3% during the 3 day test period. In presence of an alternate food source, no significant larval mortality relative to controls was detected. Total mortality was 10.3% for the 72 h test period.

When 50 first instar larvae of *Ae. aegypti* and 50 first instar larvae of *Cx. pipiens* were added in containers with 1 copepod, 53.5% of *Ae aegypti* and 11% of *Cx. pipiens*

TABLE I

Average daily mortality of first and second instar larvae of *Aedes aegypti* exposed to *Mesocyclops annulatus* in the presence and absence of an alternative food in 250 ml container

	Mortality (Mean ± Standard Deviation)			Accumulative mortality (%)
	24 h	48 h	72 h	
Aedes	16.66 ± 5.39b	7.16 ± 1.72b	2 ± 1.54a	51.7
Aedes + food	3.16 ± 0.75a	2.16 ± 1.72a	2.66 ± 0.51a	16
Control	0.5 ± 0.7 a	0 ± 0 a	1.5 ± 2.12a	4

Different letters denote significant differences (p < 0.05) between means.

TABLE II

Average daily mortality of first and second instar larvae of *Culex pipiens* exposed to *Mesocyclops annulatus* in the presence and absence of an alternative food in 250 ml container

	Mortality (Mean ± Standard Deviation)			Accumulative mortality (%)
	24 h	48 h	72 h	
Culex	13.3 ± 17.98b	6.56 ± 3.56b	6.33 ± 4.13 b	52.3
Culex + food	1.5 ± 1.76a	0.83 ± 1.16a	2.83 ± 3.06a	10.3
Control	0 ± 0a	0 ± 0a	1 ± 1.41a	2

Different letters denote significant differences (p < 0.05) between means.

larvae were killed at 24 h. Total larvae mortality was 23.5% in *Cx. pipiens* and 75.5% in *Ae. aegypti* larvae for the 72 h test period. This difference was significant (F = 23.55, df = 3,36, P < 0.001).

Densities of 5, 10, 20 and 25 adult females of *M. annulatus* placed in 700 ml containers with 100 first-instar larvae of *Ae. aegypti* produced 49.7%, 84%, 97% and 100% mortality respectively during 72 h test period.

When a single *M. annulatus* female was placed with larval densities of 1 to 29, 30, 40, 50, 60, 70, 80, 90 and 100 in 24 well plates with 3 ml per well, 100% mortality was obtained from 1 to 25 larvae. The average number of larvae killed by a single copepod female was 29 (26-35) (Figure). When a similar experiment was conducted in containers with 100 ml, at densities from 40 to 100 larvae per container no significant differences were observed on mortality rates. Mortality rates on densities of 10, 20 and 30 larvae per container were significantly different with densities larger than 30, 40 and 50 respectively (Table IV).

DISCUSSION

In this study, we demonstrated that adult female of *M. annulatus* preyed on larvae of *Ae. aegypti* and *Cx. pipiens* younger than 72 h (early second instar). In addition, presence of alternative food in containers with mosquito larvae and *M. annulatus* produced from 3 to 5 fold reduction on predation effectiveness. Our results suggest that *M. annulatus* did not have a feeding preference for mosquito larvae in containers if algae and protozoan were available. Reduction of predation rates of copepods on mosquito larvae in the presence of abundantly alternative food has been reported by previous authors (Rivière et al. 1987, Laird 1988, Andreadis & Gere 1992).
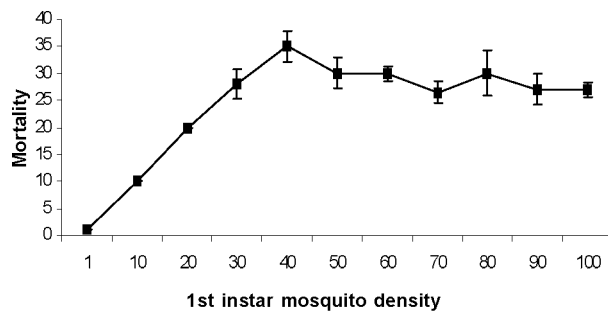
Average daily mortality (Mean ± SD) of first-instar larvae of *Aedes aegypti* exposed to one *Mesocyclops annulatus* female in different larval densities in 24 well plates.

TABLE IV

Predation capacity of individual *Mesocyclops annulatus* females upon different *Aedes aegypti* larvae densities in 250 ml containers

Density of mosquito larvae	Mortality	
	Mean ± SE	%
10	5.33 ± 1.52 a	53.3
20	10.66 ± 3.21 ab	53.3
30	13.66 ± 4.04 bc	45.5
40	17.33 ± 4.72 dc	43.3
50	30 ± 4.58 d	60
60	28 ± 2.82 d	46.7
70	25.33 ± 1.52 d	36.2
80	26.33 ± 7.63 d	32.9
90	26.67 ± 3.05 d	29.6
100	23 ± 4.35 d	23

Values followed by different letters differ significantly (p < 0.05, Tukey test).

Some species of *Mesocyclops* have been previously reported as non-efficient predators of *Culex* because of the larvae ability to escape capture by copepods (Rivière et al. 1987, Marten et al. 1989, Marten 1990). This study also reports reduced mortality on *Culex* compared to *Aedes* larvae.

In experiments carried out in 700-ml artificial containers, which simulated common breeding sites for *Ae. aegypti* and *Cx. pipiens* such as flower vases in cemeteries, we found that the addition of an average of 25 *M. annulatus* adult female produced 100% control on first instar *Ae. aegypti* larvae. There was no significant differences in mortality when *Ae. aegypti* and *Cx. pipiens* larvae were tested separately, in contrast when both culicid species were simultaneously offered to copepod a selective consumption of *Ae. aegypti* larvae was registered. Larvae of *Ae. aegypti* are benthic feeder while *Cx. pipiens* larvae are surface feeder (Laird 1988). Although most of the *Mesocyclops* in lakes are known as planktonic organisms (Laird 1988), our observations on feeding behavior of *M. annulatus* indicated that this copepod species remained at the bottom of the containers most of the time. Feeding behavior of these mosquito and copepod species may lend to increase encounter opportunities with *Ae. aegypti* larvae by *M. annulatus* resulting in the differential predation observed. Previous studies have shown

TABLE III

Average daily mortality of first and second instar larvae of *Aedes aegypti* and *Culex pipiens* breeding in the same container with one female *Mesocyclops annulatus* in 250 ml containers

	Mortality (Mean ± Standard Deviation)			Accumulative mortality (%)
	24 h	48 h	72 h	
Culex	5.5 ± 9.03	3.5 ± 3.51	2.75 ± 3.59	23.5
Aedes	16.75 ± 10.9	15.5 ± 6.95	5.5 ± 1.91	75.5
Aedes control	0.25 ± 0.43	0 ± 0	0.5 ± 0.87	1.5
Culex control	0 ± 0	0 ± 0	2 ± 2.12	4

the importance of morphological and behavioral characteristics other than prey size as defense mechanisms from predation by cyclopoids (Kerfoot 1978, Williamson 1983).

Prey density and container size were also important components in the predation effectiveness of *M. annulatus*. When a single copepod was placed in a small container, it killed on average of 29 larvae in 24 h, independent of the larvae density. When larger containers were used, similar numbers of larvae were killed only at densities larger than 50 larvae. We speculate that *M. annulatus* was not able to reach the daily consumption of 29 larvae averaged at densities lower than 50 larvae because of its lack of ability to locate the prey in larger containers.

These laboratory studies indicate that although *M. annulatus* seems promising as a biocontrol agent, similar trials should be done with other local species prior to choice of a candidate for field testing.

ACKNOWLEDGMENTS

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