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PREY AND SIZE PREFERENCE OF *MESOCYCLOPS LONGISETUS* (COPEPODA) FOR *AEDES ALBOPICTUS* AND *CULEX QUINQUEFASCIATUS* LARVAE[1]

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ABSTRACT. Laboratory studies investigated prey choice of the adult copepod *Mesocyclops longisetus* for *Aedes albopictus* and *Culex quinquefasciatus* larvae. Prey size preference by this predator was tested within and between instar classes at 10 and 30°C. Single copepod adults preferred to prey on 1st and 2nd instars regardless of whether either species was alone or combined. Generally, *M. longisetus* preyed more on *Ae. albopictus* than on *Cx. quinquefasciatus* when similar larval stages were present. Also more prey of both species were consumed at 30°C compared with 10°C.

KEY WORDS Predator, copepod, predation preference, container-inhabiting mosquitoes

INTRODUCTION

Crustaceans in the subclass Copepoda are almost universally distributed in aquatic habitats. Many copepods are free living, whereas others can be parasitic on fish (Pennak 1989). Of the free-living copepods, members of *Macrocyclops, Megalocyclops,* and *Mesocyclops* have been reported as predators of mosquito larvae with promising potential as biological control agents against larval *Aedes, Anopheles,* and *Culex* (Nasci et al. 1987; Marten 1989, 1990a, 1990b; Marten et al. 1989; Calliari et al. 2003; Dieng et al. 2003). *Mesocyclops longisetus* (Thiebaud) has been reported to effectively reduce or eliminate larval populations of *Anopheles albimanus* Wiedemann in roadside ditches and cattle watering ponds (Marten et al. 1989), as well as *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in container habitats (Schreiber et al. 1993, Marten et al. 1994, Manrique-Saide et al. 1998). However, not all copepod species prey equally on all mosquito larvae. Riviere et al. (1987) and Rawlins et al. (1997) found that *Mesocyclops aspercornis* (Daday) and *M. longisetus* effectively reduced *Ae. aegypti* larvae in tires but were relatively ineffective against some *Culex* species. Also Dieng et al. (2003) reported that *Macrocyclops distinctus* (Richard), *Megalocyclops viridis* (Jurine), and *Mesocyclops pehpeiensis* Hu exhibited limited predation on *Culex tritaeniorhynchus* Giles compared with *Ae. albopictus* larvae. Prey preference, therefore, might have a profound influence on the effectiveness of a predator to regulate pest populations.

Globally, container-inhabiting mosquitoes remain an important source of public health concern with regard to disease transmission. The pathogens that cause yellow fever, dengue, and, more recently, West Nile in the Western Hemisphere can be transmitted via mosquito species that use artificial containers as their larval developmental site. One such species, *Culex quinquefasciatus* Say, is a vector of St. Louis encephalitis (SLE) and more recently has been found to be infected with West Nile virus in several areas of the United States (Sardelis et al. 2001). Moreover, *Ae. albopictus* has been reported to be a potential vector of SLE, dengue, and West Nile virus under laboratory conditions (Harwood and James 1979, Rai 1991, Turell et al. 2001). Given the medical importance of container-inhabiting mosquitoes, we investigated *M. longisetus* for its potential as a predator on instars of *Ae. albopictus* and *Cx. quinquefasciatus* under laboratory conditions.

MATERIALS AND METHODS

Mesocyclops longisetus was obtained from a colony maintained at the John A. Mulrennan Sr. Public Health Entomology Research and Education Center (PHEREC; Florida A&M University, Panama City, FL). This colony originated from the New Orleans Mosquito Control Board (New Orleans, LA). Copepods were reared at room temperature (25°C) in an 18.9-liter plastic container and maintained on *Paramecium caudatum* as a source of prey following the procedures of Riviere et al. (1987). Prey were colonized in similar types and sizes of containers as *M. longisetus.*

Larval *Ae. albopictus* (obtained as eggs from a colony maintained at the U.S. Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL) and *Cx. quinquefasciatus* (obtained as eggs from a colony maintained at PHEREC) were used as prey in all predator preference tests. Each mosquito species was reared separately at room temperature and fed

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Table 1. Mean ± SE mortality (24 h) of *Aedes albopictus* and *Culex quinquefasciatus* 1st- through 4th-stage larvae (I–IV) in the presence (treatment) or absence (control) of a single adult *Mesocyclops longisetus* in laboratory studies conducted at 10 and 30°C. (Mean of 8 replications of 40 individuals per instar for each mosquito species.)

Temperature	Species	Instar	Mean number dead[1]	
			Treatment	Control
10°C	*Ae. albopictus*	I	16.1 ± 1.1 a	6.1 ± 0.6
		II	10.6 ± 1.0 b	4.9 ± 0.7
		III	4.3 ± 1.0 c	2.1 ± 0.4
		IV	2.3 ± 0.4 c	2.0 ± 0.4
	Cx. quinquefasciatus	I	13.6 ± 1.0 a	4.6 ± 0.4
		II	11.8 ± 1.4 a	3.9 ± 0.7
		III	3.8 ± 0.8 b	2.0 ± 0.4
		IV	2.0 ± 0.6 b	1.6 ± 0.4
30°C	*Ae. albopictus*	I	30.0 ± 4.9 a	3.3 ± 0.7
		II	23.0 ± 2.5 b	2.4 ± 1.5
		III	4.9 ± 2.1 c	1.6 ± 0.5
		IV	3.9 ± 1.2 c	1.5 ± 0.5
	Cx. quinquefasciatus	I	26.8 ± 1.8 a	3.0 ± 0.5
		II	23.0 ± 2.5 a	2.4 ± 1.5
		III	4.6 ± 0.8 b	1.4 ± 0.5
		IV	2.9 ± 1.1 b	0.9 ± 0.3

[1] Treatment means within a data block (I–IV) for each species and temperature followed by a different letter are significantly different ($P < 0.05$) by the Student-Newman-Keuls mean separation test.

an aqueous 5% mixture of liver powder and brewer's yeast following the methods of Munstermann and Wasmuth (1985) and Jones and Schreiber (1997).

Separation of mosquito larvae into instars (1st through 4th) used size as the criterion (Soumare 2002). Because predator size can influence prey selection, adult *M. longisetus* were measured before each study. They averaged 3.80 ± 0.10 mm (dorsal edge of carapace to beginning of caudal filaments) by 1.60 ± 0.12 mm (midway dorsal width of carapace). *Mesocyclops longisetus* were held for 24 h without food before studies commenced.

Individual instar and species: Single adult copepods were placed with 40 larval cohorts per instar of each species in separate tissue culture plate wells (35 mm diameter and 18 mm deep) that contained 10 ml of dechlorinated tap water. Culture plates with larvae and copepods were then incubated in climate-controlled chambers at either 10 or 30°C. Controls consisted of 40 larvae without a copepod and were handled similarly.

Mixed instar–species interaction: To determine the relative capacity of *M. longisetus* to consume mixed larval instars of both mosquito species, 40 larvae (i.e., 20 *Aedes* and 20 *Culex*) were placed together in a tissue culture plate well with a single unfed copepod. Culture plates were then incubated at either 10 or 30°C. Controls were handled similarly.

All studies were conducted at both temperatures on the same day and replicated 8 times. After 24 h, larval mortality (i.e., dead and consumed) was calculated by subtracting the number of surviving larvae from the total number of initial larvae of each species and instar. Percent larval predation from *M. longisetus* was calculated as follows by the modified formula of Abbott (1925).

$$\frac{\text{no. surviving larvae without predator} - \text{no. surviving larvae with predator}}{\text{no. surviving larvae without predator}} \times 100$$

Statistical analyses: Mean larval mortality per instar, within each species and instar combination at 10 and 30°C, were separately subjected to ANOVA (Proc GLM, SAS Institute 1990) after $\sqrt{(x + 1)}$ transformation. Student-Newman-Keuls multiple comparison test was performed separately on the mean larval mortality for each species that resulted from *M. longisetus* predation at each temperature (Cochran and Cox 1957). Paired *t*-tests were performed separately on mean mortality from copepod predation within and between instar cohorts for each species at each temperature (Cochran and Cox 1957). All differences were considered significant at $P < 0.05$. All data reported in tables and figures are nontransformed.

RESULTS

At 10 and 30°C, mortality of 1st-stage *Ae. albopictus* larvae from *M. longisetus* predation was significantly greater when compared with mortality of the other instars of this species (Table 1). Also, mortality of 2nd instars was significantly greater compared with 3rd and 4th instars. At both temperatures, no significant difference in larval mortality was observed between 3rd and 4th instars.

At either temperature, mortality of 1st- and 2nd-stage *Cx. quinquefasciatus* larvae was significantly greater, as a result of *M. longisetus* predation, when compared with the other instars of this species (Ta-

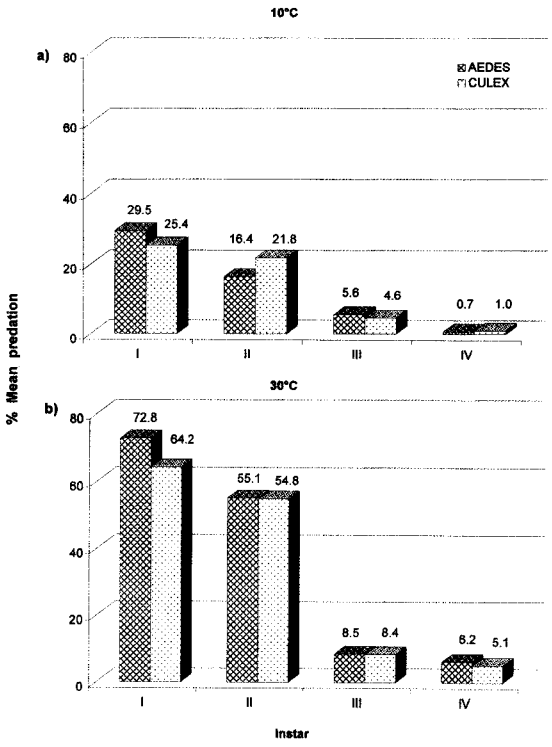

Fig. 1. Mean percent predation of *Mesocyclops longisetus* on 1st- through 4th-stage *Aedes albopictus* and *Culex quinquefasciatus* larvae (instars I–IV) exposed separately to 10 and 30°C.

ble 1). However, no significant difference in mortality was observed between 1st and 2nd instars or between 3rd and 4th instars at either temperature.

At 10°C, mean larval predation was similar for both species, ranging from 0.7 to 29.5% for *Ae. albopictus* and 1.0 to 25.4% for *Cx. quinquefasciatus* (Fig. 1). However, mean predation of both prey species was greater at 30°C, ranging from 6.2 to 72.8% for *Ae. albopictus* and 5.1 to 64.2% for *Cx. quinquefasciatus*. At both temperatures, greater predation was observed on 1st and 2nd instars.

Prey instar–species interaction: At 10°C, *M. longisetus* predation caused significantly greater mortality of *Ae. albopictus* 1st instars when in the presence of 2nd through 4th instars of *Cx. quinquefasciatus* (Table 2). Percent predation preference for *Ae. albopictus* was greatest with the 1st-stage *Aedes*–4th-stage *Culex* larval combination (Fig. 2a).

Mortality caused by copepod predation of 2nd-stage *Ae. albopictus* larvae was significantly lower when in the presence of 1st-stage *Cx. quinquefasciatus* larvae (Table 2). There was no difference in mortality when 2nd instars of both species were placed together with the predator. Conversely, more *Ae. albopictus* 2nd instars were preyed on by *M. longisetus* when in the presence of 3rd or 4th instars of *Cx. quinquefasciatus* (Table 2). The greatest predator preference for 2nd-stage *Ae. albopictus* larvae occurred with the 2nd-stage *Aedes*–3rd-stage *Culex* larval combination (Fig. 2b).

In the presence of 3rd-stage *Ae. albopictus* larvae, significantly more 1st- and 2nd-stage *Cx. quinquefasciatus* larvae were preyed on by copepods (Table 2). No difference in mortality was observed when 3rd instars of either species were placed to-

Table 2. Mean ± SE mortality (24 h) of various cohort combinations of *Aedes albopictus* and *Culex quinquefasciatus* 1st- though 4th-stage larvae (I–IV) caused by a single adult *Mesocyclops longisetus* in laboratory studies conducted at 10°C. (Mean of 8 replications of 20 individuals per instar for each mosquito species.)

Instar combination		Mean number dead[1]			
		Treatment		Control	
Ae. albopictus	*Cx. quinquefasciatus*	*Ae. albopictus*	*Cx. quinquefasciatus*	*Ae. albopictus*	*Cx. quinquefasciatus*
I	I	8.6 ± 1.6	6.8 ± 1.6	4.1 ± 1.1	3.5 ± 1.3
I	II	10.6 ± 0.8	5.2 ± 2.0*	4.0 ± 0.9	2.6 ± 1.1
I	III	11.4 ± 1.9	2.5 ± 2.0*	3.6 ± 1.1	2.3 ± 0.9
I	IV	12.5 ± 2.1	1.6 ± 0.8*	3.8 ± 1.1	1.5 ± 0.5
II	I	3.4 ± 1.9	8.6 ± 1.8*	3.1 ± 0.9	3.6 ± 0.8
II	II	8.1 ± 2.2	5.6 ± 1.6	3.3 ± 0.8	2.5 ± 1.1
II	III	10.0 ± 1.7	1.9 ± 1.4*	2.8 ± 0.9	1.6 ± 0.8
II	IV	9.2 ± 1.7	1.5 ± 1.1*	3.4 ± 1.5	1.4 ± 1.4
III	I	2.1 ± 1.8	9.2 ± 2.1*	1.5 ± 0.5	3.5 ± 0.8
III	II	2.1 ± 1.8	10.9 ± 2.6*	2.3 ± 1.3	2.1 ± 0.8
III	III	4.5 ± 1.9	2.5 ± 1.5	2.6 ± 1.0	2.3 ± 1.4
III	IV	3.6 ± 1.0	1.8 ± 0.9*	2.6 ± 1.0	1.6 ± 0.8
IV	I	1.4 ± 0.9	9.9 ± 1.3*	1.3 ± 0.8	3.4 ± 1.1
IV	II	1.0 ± 1.2	8.9 ± 2.7*	0.9 ± 1.2	2.6 ± 1.0
IV	III	2.4 ± 1.1	2.6 ± 0.9	1.6 ± 0.8	1.8 ± 0.7
IV	IV	3.1 ± 3.9	1.4 ± 1.0	1.4 ± 0.5	1.3 ± 0.6

[1] Paired treatment means with asterisks (between columns and within rows) are significantly different ($P < 0.05$, t-test).

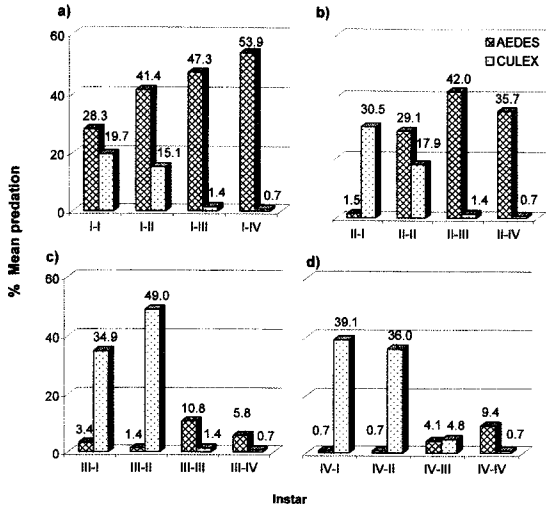

Fig. 2. Mean percent predation of *Mesocyclops longisetus* on 1st- through 4th-stage *Aedes albopictus* and *Culex quinquefasciatus* larval combinations (instars I–IV) at 10°C.

gether with a predator. Significantly greater mortality by *M. longisetus* predation occurred when 3rd-stage *Ae. albopictus* larvae were in the presence of 4th-stage *Cx. quinquefasciatus* larvae. Predation preference was greatest with the 3rd-stage *Aedes*–2nd-stage *Culex* larval combination (Fig. 2c).

In the presence of 4th-stage *Ae. albopictus* larvae, significantly more *Cx. quinquefasciatus* 1st and 2nd instars were preyed on by copepods (Table 2). No difference in mortality, as caused by *M. longisetus* predation, was observed when 3rd- or 4th-stage *Cx. quinquefasciatus* larvae were placed together with 4th-stage *Ae. albopictus* larvae. Predation preference was greatest with the 4th-stage *Aedes*–1st-stage *Culex* larval combination (Fig. 2d).

At 30°C, mortality of 1st-stage *Ae. albopictus* larvae from *M. longisetus* predation was significantly greater compared with any of the *Cx. quinquefasciatus* instars (Table 3). Percent predation preference for *Ae. albopictus* was highest with the 1st-stage *Aedes*–4th-stage *Culex* larval combination (Fig. 3a).

Mortality by the predator was greatest on *Ae. albopictus* 2nd instars when placed with *Cx. quinquefasciatus* 3rd or 4th instars, but mortality of *Cx. quinquefasciatus* 1st instars was greatest when in the presence of *Ae. albopictus* 2nd instars (Table 3). There was no difference in larval mortality when 2nd instars of each species were placed together with the predator. The greatest percent predation occurred with the 2nd-stage *Aedes*–1st-stage *Culex* larval combination (Fig. 3b).

Mortality caused by the predator was greater on *Cx. quinquefasciatus* 1st and 2nd instars in combination with 3rd-stage *Ae. albopictus* larvae (Table 3). Conversely, mortality of 3rd-stage *Ae. albopictus* larvae was greater in the presence of 3rd- or 4th-stage *Cx. quinquefasciatus* larvae. The greatest predation on mosquito larvae occurred in the 3rd-stage *Aedes*–1st-stage *Culex* larval combination (Fig. 3c).

In the presence of 4th-stage *Ae. albopictus* larvae, *M. longisetus* mortality was greatest against *Cx. quinquefasciatus* 1st through 3rd instars (Table 3). However, when 4th instars of both species were

Table 3. Mean ± SE mortality (24 h) of various cohort combinations of *Aedes albopictus* and *Culex quinquefasciatus* 1st- through 4th-stage larvae (I–IV) caused by a single adult *Mesocyclops longisetus* in laboratory studies conducted at 30°C. (Mean of 8 replications of 20 individuals per instar for each mosquito species.)

Instar combination		Mean number dead[1]			
		Treatment		Control	
Ae. albopictus	*Cx. quinquefasciatus*	*Ae. albopictus*	*Cx. quinquefasciatus*	*Ae. albopictus*	*Cx. quinquefasciatus*
I	I	15.8 ± 0.8	11.9 ± 1.0*	3.8 ± 0.9	3.8 ± 1.2
I	II	17.1 ± 0.7	10.4 ± 1.4*	3.4 ± 1.7	2.4 ± 0.8
I	III	18.1 ± 0.8	3.8 ± 1.3*	3.4 ± 1.0	1.4 ± 0.5
I	IV	18.5 ± 0.8	1.5 ± 0.4*	3.2 ± 1.1	0.9 ± 0.9
II	I	9.5 ± 2.6	16.5 ± 2.7*	2.0 ± 0.9	2.8 ± 1.2
II	II	14.5 ± 3.0	13.4 ± 4.1	2.5 ± 0.5	1.8 ± 1.0
II	III	14.9 ± 3.4	3.1 ± 1.1*	2.9 ± 0.7	3.0 ± 1.1
II	IV	14.9 ± 3.5	2.0 ± 1.6*	2.6 ± 1.0	1.4 ± 0.8
III	I	4.0 ± 2.4	17.5 ± 2.2*	1.0 ± 1.1	3.0 ± 1.2
III	II	3.4 ± 1.8	9.5 ± 2.3*	1.5 ± 0.9	2.5 ± 1.0
III	III	6.5 ± 1.3	4.8 ± 2.4*	1.9 ± 0.9	1.1 ± 1.1
III	IV	6.0 ± 1.8	1.6 ± 1.3*	2.0 ± 0.7	1.5 ± 1.4
IV	I	2.4 ± 1.8	19.1 ± 1.3*	1.1 ± 1.1	2.8 ± 0.8
IV	II	1.3 ± 1.1	12.0 ± 3.5*	0.9 ± 0.8	1.9 ± 0.6
IV	III	2.1 ± 1.1	5.6 ± 0.8*	1.0 ± 0.7	1.1 ± 1.0
IV	IV	3.4 ± 1.8	1.1 ± 0.8*	1.0 ± 0.9	0.5 ± 0.5

[1] Paired treatment means with asterisks (between columns and within rows) are significantly different ($P < 0.05$, *t*-test).

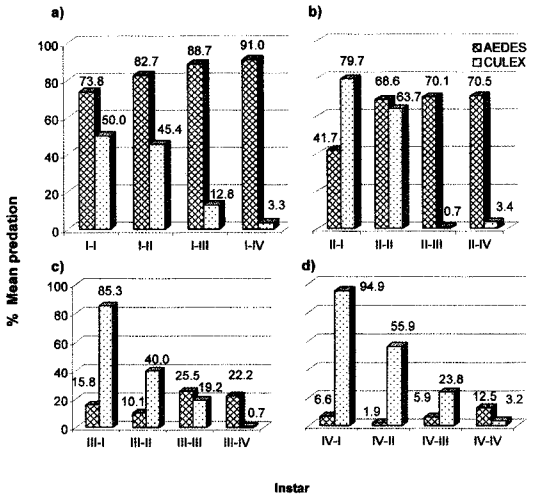

Fig. 3. Mean percent predation of *Mesocyclops longisetus* on 1st- through 4th-stage *Aedes albopictus* and *Culex quinquefasciatus* larval combinations (instars I–IV) at 30°C.

together, *Ae. albopictus* mortality was greater that of *Cx. quinquefasciatus*. Predation preference was greatest with the 4th-stage *Aedes*–1st-stage *Culex* larval combination (Fig. 3d).

DISCUSSION

We found that *M. longisetus* generally consumed more 1st and 2nd instars of *Ae. albopictus* and *Cx. quinquefasciatus* compared with 3rd and 4th instars. These results agree with those of Marten et al. (1989, 1994), Schreiber et al. (1996), and Manrique-Saide et al. (1998), who reported that predation by *M. longisetus* was generally greatest on earlier instars compared with the latter stages. Indeed, Allan et al. (1987) has stated that small predators exhibit greater capture success on smaller prey than larger prey. In our study, the average body length of 1st and 2nd instars ranged from 0.8 to 3.3 mm, which was less than the predator average body length of 3.8 mm. Average body length of 3rd and 4th instars ranged from 1.1- to 1.5-fold greater than that of the predator.

Mesocyclops longisetus preyed more on *Ae. albopictus* than on *Cx. quinquefasciatus* when similar larval stages were present (Figs. 2 and 3). Dieng et al. (2003) suggested that such differences might be due to the greater mobility of *Aedes*. Natchigalli (1965) observed that *Aedes* spp. were very active. Moreover, Dieng et al. (2003) stated that copepods could judge prey speed and attack only moving prey. Larval *Aedes* swimming behavior might increase the water disturbance, thereby attracting *M. longisetus*. Conversely, Marten et al. (1994) found that *Mesocyclops* were not very successful at killing *Culex* larvae and argued that even though copepod attacks were frequent, *Culex* often escaped by deflecting predator attacks with their "numerous long bristles." Dieng et al. (2003) also cited studies in which *Culex* spp. minimized detection by reducing their movement in the presence of predators or escaped predation by generally being less motile in their environment.

Generally, more prey of both species were consumed at 30 than at 10°C. Indeed, predation on 1st and 2nd instars for both species dropped >40% at the lower temperature. These results agree with those of Schreiber et al. (1993, 1996) and Calliari et al. (2003) that *M. longisetus* was not as effective a larval predator in cooler water temperatures. They postulated that such an effect might be because of lower metabolic activity of the copepod.

Mobility, prey size, and temperature all have been reported to affect the relative numbers of mosquito larvae that *M. longisetus* can consume. Whether or not any, or all, of these factors are of major importance in limiting the potential of this predator for the biological control of container-inhabiting mosquito larvae in field situations remains to be addressed.

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