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Copepod Predation on *Anopheles quadrimaculatus* Larvae in Rice Fields

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ABSTRACT: Cyclopoid copepods and mosquito larvae were surveyed in southwestern Louisiana rice fields. Almost every rice field had a natural population of *Mesocyclops ruttneri*, *Acanthocyclops vernalis*, or *Macrocyclops albidus*. Judging from the abundance of pupae, 29% of the fields were responsible for virtually all *Anopheles quadrimaculatus* production, apparently because larval mortality suppressed production in the other fields. *Mesocyclops ruttneri* had the strongest negative association of naturally occurring copepod populations with *An. quadrimaculatus* larvae, though a few fields with *M. ruttneri* had substantial *Anopheles* production. *Macrocyclops albidus*, *M. ruttneri*, *Mesocyclops edax*, and *Mesocyclops longisetus* were introduced to experimental rice field plots. It took two months for the introduced copepods to build up their numbers; *Anopheles* larvae then disappeared from all treated plots while larvae continued to be present in the adjacent control field. Copepods were observed to kill the following number of first instar *An. quadrimaculatus* larvae in the laboratory: *Mesocyclops ruttneri* (36 larvae/day), *Macrocyclops albidus* (23 larvae/day), *Mesocyclops longisetus* (24 larvae/day), and *Acanthocyclops vernalis* (15 larvae/ day). It is concluded that introducing select species of copepods and encouraging their populations offer possibilities for contributing to *Anopheles* control in rice fields.

Keyword Index: Copepod, mosquito larvae, mosquito control, biological control, *Anophleles*, rice field, malaria.

INTRODUCTION

Some of the larger species of cyclopoid copepods, as predators of first and second-instar mosquito larvae, are now in operational use to eliminate *Aedes* larvae from container habitats such as tires, water storage tanks, and wells (Marten et al. 1994a, Nam et al. 1998). Copepods may also offer possibilities for *Anopheles* control. *Mesocyclops longisetus* (Thiébaud) and *Mesocyclops aspericornis* (Daday) are known to kill large numbers of *Anopheles* larvae in the laboratory (Marten et al. 1989, Brown et al. 1991). Marten et al. (1989) observed *Anopheles albimanus* Wiedeman larvae to be scarce in ponds and other aquatic habitats in Colombia where *M. longisetus* was present.

Rice fields are a major breeding habitat for *Anopheles quadrimaculatus* Say in southeastern United States. We surveyed natural populations of cyclopoid copepods in rice fields of southwestern Louisiana and assessed the impact of natural and introduced populations of copepods on *Anopheles* larvae in the fields.

MATERIALS AND METHODS

Laboratory Predation Tests

Laboratory colonies of four species of larvivorous copepods—*Acanthocyclops vernalis* (Fischer), *Macrocyclops albidus* (Jurine), *Mesocyclops longisetus*, and *Mesocyclops ruttneri* Kiefer—were established from collections at a canal in New Orleans, Louisiana. *Megacyclops latipes* (Lowndes) were collected from a roadside drainage ditch in Slidell, Louisiana. Culture methods followed Marten et al. (1997), a system based on wheat seed, *Chilomonas*, and *Paramecium caudatum*. A laboratory colony of *An. quadrimaculatus* was established from eggs provided by the USDA Medical and Veterinary Entomology Research Laboratory in Gainesville, Florida.

The capacity of each copepod species to kill *An. quadrimaculatus* larvae was assessed by placing single adult female copepods in tissue culture plate wells (35 mm diameter, 18 mm deep) with 50 newly hatched first instar *An. quadrimaculatus* larvae from the laboratory colony. The number of surviving larvae was counted after 24 hours at a temperature of 24°-26°C.

Field Survey

Thirty-two rice fields in Jefferson Davis Parish, Louisiana, were sampled for mosquito larvae and copepods in late September 1991, about a month after the fields were flooded for the second rice crop of the year. Twenty-four liters of water (approximately 100 dips) were dipped from each field with a standard dipper for mosquito larvae and passed through a net (0.2 mm mesh) to capture mosquito larvae and copepods. All copepods and mosquito larvae were preserved in alcohol for subsequent identification and counting. Copepods were identified to species, and mosquito larvae were identified to genus. All *Anopheles* larvae appeared to be *An. quadrimaculatus*, though there may have been some *Anopheles crucians* Wiedemann that passed unnoticed. Mosquito pupae were held for identification as emerging adults.

Field Experiment

Four adjacent experimental plots were established in a rice field approximately 15 km NW of Jennings, Louisiana. The plots were 10 m on each side and were constructed by placing four parallel levees across one end of a rice field that was fallow the previous year. Approximately 500 adult female *Acanthocyclops vernalis*, 500 *Macrocyclops albidus*, 500 *Mesocyclops longisetus*, 500 *Mesocyclops ruttneri*, and 500 *Mesocyclops edax* (Forbes), were introduced to each plot in late April 1990, about two weeks after the field was flooded for the first rice crop. The irrigation water was pumped from underground. The introduced copepods came from the same laboratory cultures as copepods in the laboratory predation tests. (*M. edax* was originally collected from a New Orleans canal.)

Each plot was sampled for copepods and mosquito larvae in June and again in late July. The sampling procedure was as described for the field survey, except 40 liters of water were dipped from each plot. Samples from the same field outside the treatment plots served as controls.

The field was harvested in early August, and it was not flooded to produce a second rice crop that year. To monitor for the presence of introduced copepods when the field no longer contained water, samples of moist soil were taken from depressions in the treatment plots in October (two months after the field was drained for harvest). The soil was placed in a bucket of water, and the water was strained through a net several hours later to collect copepods. The following February, copepods were collected from puddles in the treatment plots.

RESULTS

Laboratory Predation Tests

All tested copepod species killed substantial numbers of first-instar *An. quadrimaculatus* larvae. They usually ate all of the larva except its head capsule, but sometimes they ate only part of a larva. *Mesocyclops ruttneri* killed the most larvae, and *Acanthocyclops vernalis* killed the least (TABLE 1).

Field Survey

Almost all surveyed fields contained natural populations of *Mesocyclops ruttneri* or *Acanthocyclops vernalis*. Fifty-eight percent of the fields contained *Mesocyclops ruttneri*, and 38% contained *Acanthocyclops vernalis*. Only 9% of the fields contained both

TABLE 1. Mortality of first instar *Anopheles quadrimaculatus* larvae due to copepod predation in the laboratory.

Copepod Species	Number of Replicates	Larval Mortality[1]
Acanthocyclops vernalis	8	14.7 ± 3.0
Macrocyclops albidus	14	23.1 ± 3.9
Mesocyclops longisetus	10	23.5 ± 2.0
Mesocyclops ruttneri	14	36.4 ± 2.1
Megacyclops viridis	10	25.1 ± 2.3
Controls (no cyclopoid)	8	4.1 ± 1.3

[1]Average number (± SE) of larvae dead after 24 hours. Fifty larvae were provided to one copepod in each replicate.

species. *Macrocyclops albidus* was found in 6% of the fields.

The following cyclopoid copepods, which are not large enough to be significant predators of mosquito larvae, were also encountered: *Mesocyclops reidi* Petkovski, *Tropocyclops extensus* (Kiefer), *Microcyclops rubellus* (Lilljeborg), *Eucyclops agilis* (Koch), *Eucyclops elegans* (Herrick), *Paracyclops chiltoni* (Thomson), *Paracyclops poppei* (Rehberg), *Thermocyclops inversus* Kiefer, and *Thermocyclops tenuis* (Marsh).

Samples from 81% of the fields in the survey contained *Anopheles* larvae. Average numbers of *Anopheles* larvae declined as they progressed from the first to fourth instar (Fig. 1). The higher instars were concentrated in relatively few fields; 31% of the fields contained 77% of III/IV instar larvae. *Anopheles* pupae were found in 27% of the fields, the number varying from .01 to .04 pupae/dip. The fields with pupae were the ones that had the largest numbers of III/IV instar larvae.

There was a conspicuous negative association between *Anopheles* and *Mesocyclops ruttneri*. While *Anopheles* larvae were clearly present (>10 larvae) in all fields without *M. ruttneri*, no *Anopheles* larvae were found in 35% of fields that contained *M. ruttneri* (Fig. 2). The number of second-instar *Anopheles* larvae was substantially lower in fields that contained *M. ruttneri*, compared to fields with only *Acanthocyclops vernalis* or no larvivorous copepods (Fig. 1). The difference was highly significant with a nonparametric Mann-Whitney U-test ($P = .003$, $U = 190$, $n_1 = 20$, $n_2 = 12$, Rohlf and Sokal 1995, p. 129). First and third-instar *Anopheles* larvae were also less numerous in fields with *M. ruttneri*, but the differences were not so great ($P = .10$, $U = 152$ for first instars; $P = .08$, $U = 158$ for third instars). Third/fourth-instar *Anopheles* larvae were found in 75% of the fields without *M. ruttneri*, while third/fourth instar larvae were found in only 22% of the fields that contained *M. ruttneri*. *Anopheles* pupae were found in 42% of the fields without *M. ruttneri*, while pupae were found in only 20% of the fields that contained *M. ruttneri* (Fig. 2).

Uranotaenia larvae were in 94% of the fields, but most of the *Uranotaenia* larvae were concentrated in relatively few fields. Twenty-nine percent of the fields had many more first-instar larvae than the other fields; 9% of the fields had 66% of the third/fourth instars. The fields with large numbers of third/fourth-instar *Uranotaenia* larvae were not the same fields that contained larger numbers of third/fourth-instar *Anopheles* larvae. Like *Anopheles*, average numbers of *Uranotaenia* larvae declined as they progressed from the first to fourth instar (Fig. 1). Association of larval numbers with the presence or absence of *M. ruttneri* was not statistically significant (Mann-Whitney $U = 131$, $n_1 = 20$, $n_2 = 12$ for first instars; $U = 144$ for second instars; $U = 135$ for third instars).

Samples from 42% of the fields contained *Culex* larvae. *Culex* larvae were not numerous enough for detailed analysis, averaging 0.008 first-instar larvae/dip, 0.006 second-instar larvae/dip, 0.003 third-instar larvae/dip, and no fourth instars. Fish were seldom collected in the dipping samples.

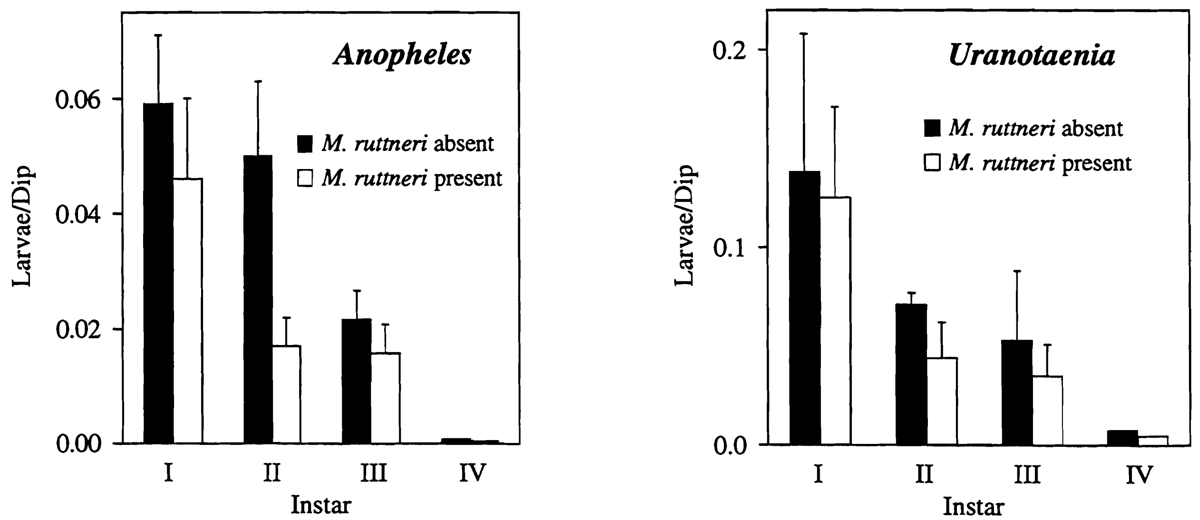

Figure 1. Average number (±SE) of mosquito larvae/dip in the September field survey. Based on 100 dips/field in 32 Louisiana rice fields. Most fields without *Mesocyclops ruttneri* contained *Acanthocyclops vernalis*.

Field Experiment

No mosquito larvae or copepods were observed in the treatment plots or control field when copepods were introduced to the treatment plots at the end of April. About half of the other newly flooded fields in the district had conspicuous populations of *Acanthocyclops vernalis* at this time.

There were a few copepods, too small to prey on mosquito larvae, in the treatment plots and control field when they were sampled in June. There were small numbers of *Anopheles* larvae (0.10 larvae/dip) in the treatment plots and control field at this time.

Every treatment plot contained adults of all five introduced copepod species when sampled in early July. *Acanthocyclops vernalis* and *Mesocyclops longisetus* were most numerous; the combined abundance of all copepod species exceeded five copepods/dip. No mosquito larvae or pupae were observed in any of the treatment plots. *Acanthocyclops vernalis* was common in the control field when inspected at the same time. No other larvivorous copepod species were observed in the control field. Second to fourth instar *Anopheles* larvae had a combined abundance of 0.07 larvae/dip in the control field.

When soil samples were taken from the drained treatment plots in October and immersed in water, adult *Acanthocyclops vernalis, Mesocyclops ruttneri, Mesocyclops longisetus*, and a small number of *Macrocyclops albidus* and *Mesocyclops edax* were swimming in the water within hours. Only *A. vernalis* and *M. albidus* were recovered from puddles in the treatment plots the following February.

DISCUSSION

Copepods

It was no surprise to find *Macrocyclops albidus* in some of the rice fields. *Macrocyclops albidus* is the most common large cyclopoid in Louisiana. It is virtually ubiquitous in drainage ditches that have at least some water throughout the year; it is less common in isolated temporary water. *Macrocyclops albidus* can survive in moist soil, but it lacks the ability of some cyclopoids such as *Acanthocyclops vernalis* to survive in drier soil for months or more. It is no surprise that *A. vernalis* is common in rice fields because *A. vernalis* is common in

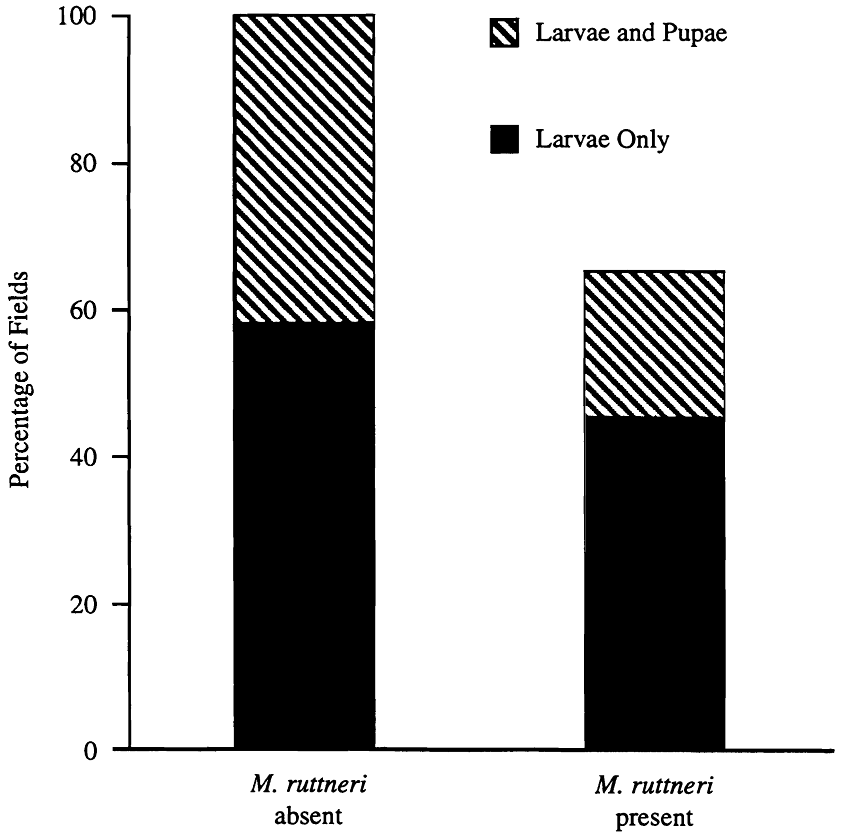

Figure 2. Percentage of fields with *Anopheles* larvae or pupae in the September field survey.

other temporary, shallow-water habitats throughout Louisiana. *Acanthocyclops vernalis* is probably frequently introduced to rice fields in irrigation water, because it is also common in canals. *Macrocyclops albidus* and *A. vernalis* should be able to survive the winter in rice fields that have pockets of moist soil because both species tolerate temperatures down to 0°C as long as the water does not freeze (Marten et al. 1994a).

One of the most striking results of the field survey was the complementary distribution of *Acanthocyclops vernalis* and *Mesocyclops ruttneri*; it was unusual to find both species in the same field. It is surprising that *M. ruttneri* is so common in rice fields because we never found this species in other shallow, temporary water in Louisiana. *Mesocyclops ruttneri* is an exotic species from Southeast Asia that lives primarily in permanent, deeper water in Louisiana. *Mesocyclops ruttneri* may be abundant in rice fields because it is abundant in irrigation canals.

It is no surprise that *Mesocyclops edax* and *Mesocyclops longisetus* do not occur naturally in Louisiana rice fields. *Mesocyclops edax* is a temperate species for which Louisiana is the southern extreme of its geographic range, and *M. longisetus* is a neotropical species not found north of Louisiana. Neither species is common in Louisiana, occurring naturally only in permanent (relatively deep) water, such as canals or large ponds, and not conspicuously abundant even there. *Mesocyclops edax* is probably associated with deeper water because of its swimming habit. Unlike most other cyclopoid copepods, which spend considerable time clinging to submerged vegetation or resting on the bottom, *M. edax* is always in motion, swimming in the water column. *Mesocyclops longisetus* is restricted to deeper water because it is killed by temperatures below 3°C (Marten et al. 1994a). Although *M. longisetus* is common in shallow water in the tropics, it survives the more severe Louisiana winter cold spells only where the water is deep enough to buffer temperature extremes.

If *M. longisetus* and *M. edax* do not occur naturally in rice fields, why did they do so well when introduced to the experimental plots? It seems the factors that prevent *M. longisetus* and *M. edax* from occupying rice fields over the long term are not operating during the summer. It also appears that neither of these two species is common enough in irrigation water to stock the fields when they are flooded.

Cyclopoid copepods have a generation time of about three weeks at late-spring temperatures, multiplying their numbers about a hundred-fold with each generation. Since it took the copepods about two months to build up their populations after introduction to the experimental plots in April, the same kind of lag should be common for natural populations as well. Because many of the fields in the study area are rotated between rice, soybeans, and fallow, there can be long periods without water, making it difficult for copepods to survive from one rice crop to the next. Moreover, we cannot expect copepods to be introduced with irrigation water pumped from underground. While *Acanthocyclops* is already present in some fields when they are first flooded in April, if they had rice the previous year, the copepod populations in most rice fields probably have to start from small numbers dispersing from nearby fields or introduced with irrigation water from canals. Several months can pass from the time copepods are introduced until they reach sufficient numbers to impact mosquito larvae.

Anopheles Larvae

If *Anopheles* oviposition was more or less continuous during the weeks preceding the field survey, the survival of each larval instar at the time of the survey can be inferred from the decline in average numbers of the instars. It appears reasonable to interpret the field survey data this way because light trap records from the survey area showed no large fluctuations in abundance of adult *Anopheles* during the weeks preceding the survey. Using the ratio of pupae to first instars, overall larval survival in the surveyed fields was <1%. It is a common observation that the survival of *Anopheles* larvae in rice fields is <5% (Roger and Bhuiyan 1990).

We can ask whether the bulk of *Anopheles* production comes from a large number of rice fields (all of which produce small numbers of mosquitoes) or from a small percentage of fields that produce many more mosquitoes than the other fields. If we consider the number of pupae to reflect the production of adult mosquitoes, a small percentage of fields in the field survey was responsible for the bulk of *Anopheles* production because pupae were concentrated in a minority of the fields.

Copepod Predation

Low survival of *Anopheles* larvae is generally attributed to predators such as fish, odonate nymphs, aquatic bugs, and aquatic beetles (Roger and Bhuiyan 1990). The field survey in this study did not include enough fields without larvivorous copepods to compare larval numbers in fields having *Mesocyclops ruttneri* or *Acanthocyclops vernalis* with numbers in fields that were entirely without copepod predation. However, the abundance of *A. vernalis* or *M. ruttneri* in the fields, combined with the large number of *Anopheles* larvae that these copepods killed in the laboratory, suggests that cyclopoid copepods are also significant predators.

The lower number of *Anopheles* larvae in fields with *Mesocyclops ruttneri* compared to fields with *Acanthocyclops vernalis* (Fig. 1) suggests that *M. ruttneri* is a more effective predator than *A. vernalis*. The substantial reduction in second-instar larvae in fields with *M. ruttneri* would be expected after heavy mortality during the first and second instars. While *M. ruttneri* is probably responsible, the presence of *M. ruttneri* could also reflect a complex of other ecological factors detrimental to early instar survival. Even if *M. ruttneri* is responsible, the first/second instar larval mortality in fields with *M. ruttneri* is not consistently strong enough for *M. ruttneri* to be of use for *Anopheles* control in the same way that copepods are now used for *Aedes* control.

The impact of copepods on *Anopheles* production in the experimental plots was quite different from what we observed with natural copepod populations in the field survey. Once the introduced copepods built up their numbers, they reduced larval populations and the production of adult mosquitoes virtually to zero. We do not know which of the introduced copepod species was responsible, or whether a combination of species was important. We can speculate that *M. longisetus* was responsible because *M. longisetus* is already known to reduce *Anopheles* larvae drastically in other situations (Marten et al. 1989). *Mesocyclops edax* may also have made a major contribution. While other cyclopoids are sedentary much of the time, the continual activity of *M. edax* in the water column should put it in frequent contact with *Anopheles* larvae hanging at the surface.

Implications for *Anopheles* Control

High larval mortality due to predation seems to be responsible, at least in part, for the very low *Anopheles* production in most rice fields. While the roles of the various naturally occurring predators are not completely clear, it seems likely that *Anopheles* production can be reduced by using cultivation practices that encourage predator populations in as many fields as possible. Pesticides that kill natural predators should be avoided. To the extent that agronomic considerations allow, the temporal and spatial arrangement of rice fields, alternate crops, fallows, and irrigation water should encourage the survival of predators from one rice crop to the next and facilitate rapid invasion by predators when fields are flooded.

One way to encourage predators is to maintain ponds in the fields while they are dry. Ponds can serve as reservoirs from which copepods and other aquatic predators spread over the fields when they are flooded. Ponds could be stocked not only with predator species that are naturally common in rice fields, but also with predators, such as *Mesocyclops longisetus* and *Mesocyclops edax*, which do not occur naturally but which thrive during the summer when introduced. The fact that these two species do not occur naturally should not be an obstacle to their use. *Mesocyclops longisetus* has proved one of the most effective copepod species for *Aedes* control, even though natural populations are seldom found in containers (Marten et al. 1994a, 1994b).

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