CYCLOPS VERNALIS AS A PREDATOR OF THE PREPARASITIC STAGES OF *ROMANOMERMIS CULICIVORAX*

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ABSTRACT. *Cyclops vernalis* was an avid predator of the preparasitic stage of the mermithid nematode, *Romanomermis culicivorax*. Predation rate was dependent on the nutritional state of the copepods and the volume of water. In small volumes of water, 0.2 ml, satiated copepods killed preparasites within 100 min with a median predation time (PT_{50}) of 41 min. Starved copepods killed preparasites within 70 min with a PT_{50} of 12 min. The rate of predation was inversely proportional to the water volume. In one liter of water, the infective ability of the preparasites for mosquito larvae was inversely proportional to predator density. A predator density of 53 copepods/liter reduced the number of infected mosquito larvae by 50%. These findings indicate that high densities of copepods in mosquito infested waters may interfere with mosquito control by *R. culicivorax*.

INTRODUCTION

The mermithid nematode *Romanomermis culicivorax* Ross and Smith shows promise as an efficacious biological control agent for a wide variety of mosquito larvae (Petersen 1973). However, a great deal of ecological research must be done to determine the potential of this and other mermithid nematodes. Several investigators have established some limiting abiotic conditions for the infective state of *R. culicivorax*: temperature (Brown and Platzer 1977), salinity (Petersen and Willis 1970, Brown and Platzer 1978a), oxygen (Brown and Platzer 1978b) and pH (Petersen 1979). However, little is known of the biotic factors affecting the mermithid nematodes. Mitchell et al. (1974) reported that an ostracod, *Cyprinotus dentatis* Sharpe, preyed on the infective stage of *R. culicivorax* under laboratory conditions. Stirling and Platzer (1978) reported on an epizoosis of a chytridiomycetous fungal parasite, *Catenaria anguillulae* Sorokin on laboratory cultures of *R. culicivorax*. Recently, we reported that numerous aquatic arthropods attacked and devoured the pre- and postparasitic stages of *R. culicivorax* (Platzer and MacKenzie-Graham 1978). In the present study, we demonstrate that the cosmopolitan copepod, *Cyclops vernalis* Fischer, is an effective predator of the infective state of *R. culicivorax* under laboratory conditions.

MATERIALS AND METHODS

MAINTENANCE OF ORGANISMS. *R. culicivorax* was propagated in autogenous *Culex pipiens* Linnaeus according to the procedures of Platzer and Stirling (1978). Infective larvae of *R. culicivorax* were obtained by adding 100 ml of dechlorinated tapwater (hereafter, unless specified otherwise, all water samples refer to dechlorinated tapwater) to 20 g of moist sand with eggs in a beaker. After 1–3 hrs, infective larvae were removed with a Pasteur pipette. *C. vernalis* was obtained from Dr. B. A. Federici (University of California, Riverside) and maintained with mixed bacteria and protozoa from hay infusions. Adult copepods, the only stage used in these studies, were collected with a Pasteur pipette. Adults were identified by the brevity of the last urosomal segment (Yeatman 1959). All maintenance procedures and experiments were conducted at room temperature (23–25°C).

PREDATION TIME AS A FUNCTION OF PREDATOR NUTRITION. Predatory abilities of copepods in 2 nutritional states, satiation and starvation, were studied. Satiated copepods were taken directly from culture vessels. Starved copepods were pre-

pared by placing satiated copepods in sterile water for 24 hr before initiating predatory studies. Predator-prey interactions were observed in ca 0.2 ml water placed in the depression of a Boerner slide. Individual copepods and preparasites were transferred to the depression slide with a Pasteur pipette. Each depression well contained 1 copepod and 1 preparasite and was observed for 1 min at 5 min intervals with a dissecting microscope until predation was completed. Twenty-three copepod-preparasite interactions were observed in the satiated copepod experiment and 28 in the starved copepod experiment. The time at which the preparasite was killed was recorded, the accumulated percentage of killed preparasites was calculated, and the median time for predation (PT_{50}) was calculated by probit analysis (see Statistics).

VOLUME EFFECTS ON PREDATION TIME. The effects of increasing water volume on the predation time were studied by placing the copepods and preparasites in 0.5, 1.0, 1.5, and 2.0 ml water. Small polystrene beakers (23 mm high by 14 mm in diameter) served as containers for these experiments. The ratio of preparasites to copepods was maintained at 1:1 and observations on the condition of the preparasites were made every 10 min for 2 hours. Eight-four copepod-preparasite interactions were observed. The PT_{50} was determined as described in the proceeding section.

EFFECTS OF PREDATOR DENSITY ON MOSQUITO PARASITISM BY PREPARASITES. The effects of predator density was tested in one liter of water placed in polystrene shoeboxes (17 x 30 x 8 cm; Fig. 1). Containers were set up with the

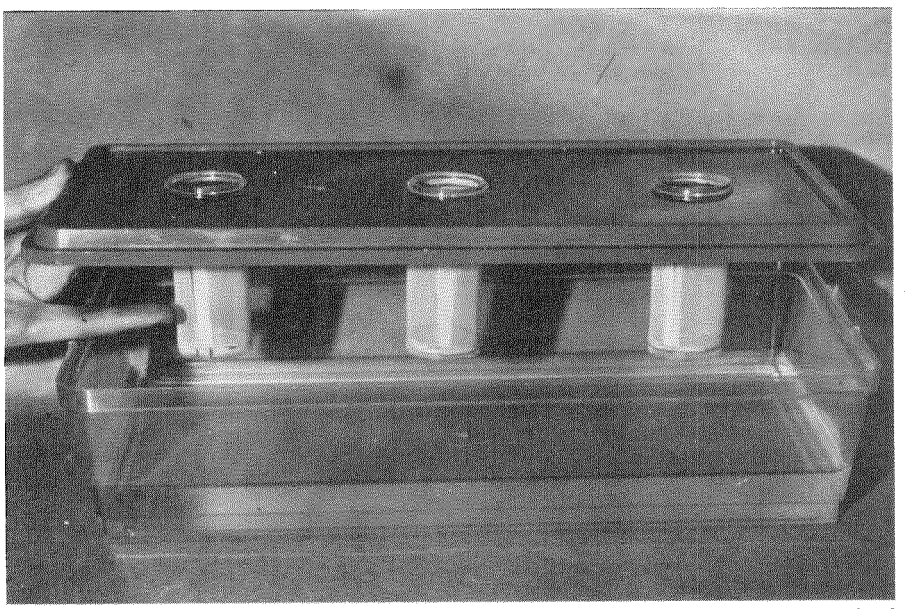

Fig. 1. The effects of predator density on mosquito parasitism by preparasites was determined in 1 liter of water in polystrene shoeboxes. The sentinel mosquito larvae were placed in the three sentinel cages supported by the plastic lid.

number of copepods increasing from 10 to 100 (10, 20, 30, 40, 60, and 100 copepods per container). The number of preparasites per container was 420. Experiments were initiated by placing copepods and preparasites in the containers. The predation in this volume of water could not be determined by direct count of dead and ingested preparasites. Instead, the effects of predation were determined by measuring a decline in infectivity of the preparasites for their host mosquitoes. Seven mosquito larvae were placed in each of 3 sentinel cages (cylindrical polystrene vials, 3 x 9 cm, with 3 openings, 3 x 5 cm, covered with 80 mesh nylon screen) supported by the plastic top of the shoebox (Fig 1.). After 48 hr, the infections of the mosquitoes were determined by dissection of the larvae (Petersen 1979). This experiment was performed 3 times and the results were pooled.

STATISTICS. Median predation time (PT_{50}) and median predation density were determined by computerized probit analysis (Brown and Platzer 1978a). The significance of the data was analyzed by analysis of variance and Duncan's multiple range test (Little and Hills 1972).

RESULTS

The following observations were made on copepods and preparasites confined to 0.2 to 2.0 ml water. Copepods found the prey in 1 of 2 ways: (1) the copepod swam about randomly without apparent interest in the preparasites and attacked the preparasite upon accidental contact; or (2) the copepod remained motionless and when the preparasite swam by the copepod attacked swiftly. After grasping the preparasite with its maxillules, the copepod would then feed, ingesting a complete preparasite within 2 min. In some cases, the copepod attacked and damaged the preparasite but lost its grasp on the nematode. The injured nematode remained motionless and died while the copepod swam about expressing no further interest in the preparasite even upon accidental contact. The presence of abundant alternative food, bacteria and protozoa from the hay infusion did not deter attacks by copepods on the preparasites. Copepods were capable of killing and eating at least 4 preparasites within 13 min.

MEDIAN PREDATION TIME AND PREDATOR NUTRITION. Median predation times were computed from the cumulative percentage of preparasites killed and/or eaten. Satiated copepods attacked all preparasites within 100 min (23 observations, Fig. 2), and a PT_{50} of 41 min was computed. In contrast, starved copepods attacked the preparasites more rapidly and in 28 observations predation was completed within 70 min and a PT_{50} of 12 min was computed (Fig. 2). The statistical difference between the $PT_{50's}$ was highly significant.

MEDIAN PREDATION TIME AND VOLUME OF WATER. The predation time for satiated and starved copepods was determined in volumes of water from 0.5 ml to 2 ml. The surface area of the water remained constant in the test containers. As

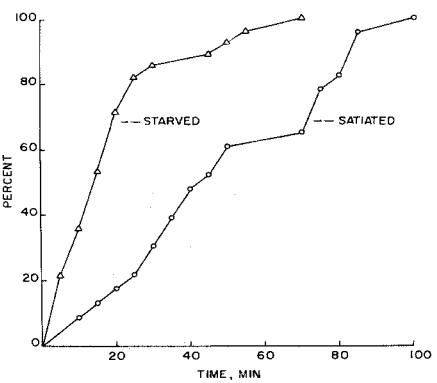

Fig. 2. Predatory success (accumulated percent of prey killed) of satiated and starved *C. vernalis* on the free-swimming preparasites of *R. culcivorax* in 0.2 ml water. The $PT_{50's}$ for starved and satiated copepods were 12 and 41 min., respectively.

in the previous study, starved copepods (33 observations) attacked the preparasites more rapidly than the satiated copepods (51 observations: Fig. 3). In addition, the $PT_{50's}$ were directly proportional to the increase in water volume and the slopes of the 2 regression lines did not differ signficantly (Fig. 3).

PREDATOR DENSITY. The effects of predator density were evaluated in one liter of water. In preliminary tests, copepods, preparasites and mosquito larvae were placed together simultaneously. Under such conditions, 50 to 100% of the mosquito larvae were killed within 24 hrs at all copepod densities tested. The presence of algae, *Chara*, did not provide a significant refuge for the mosquito larvae. This interference by the copepods was circumvented by placing the mosquitoes in miniature sentinel cages (Fig. 1). At a preparasite: mosquito larvae ratio of 20:1, all mosquito larvae were infected in the control containers. The sentinel cages did not alter the percent infection and numbers of nematodes per infected mosquito in the absence of copepods. The infection level for each copepod density tested was less than the control (Fig. 4). A significant decline in infection level occurred by 20 copepods per liter. A median predation density of 53 copepods per liter was calculated by probit analysis. The average number of nematodes per infected mosquito larva declined from 2.6 in the controls to 1.0 at 40 copepods per liter.

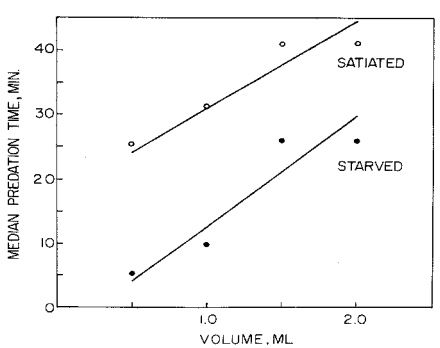

Fig. 3. Effect of water volume on the median predation times of satiated and starved *C. vernalis* on the preparasites of *R. culicivorax*.

DISCUSSION

The results of this study demonstrate that *C. vernalis* is a raptorial feeder which preys readily on the free-swimming infective or preparasitic stages of *R. culicivorax* under laboratory conditions. The physiological condition of the copepods influenced the predation rate: starved copepods attacked and ate preparasites 3.5 times (range = 1.6 to 5.2) faster than satiated copepods. Alternative food sources did not deter the predacious behavior of the copepods. Increased water volume increased the median predation time since the likelihood of ran-

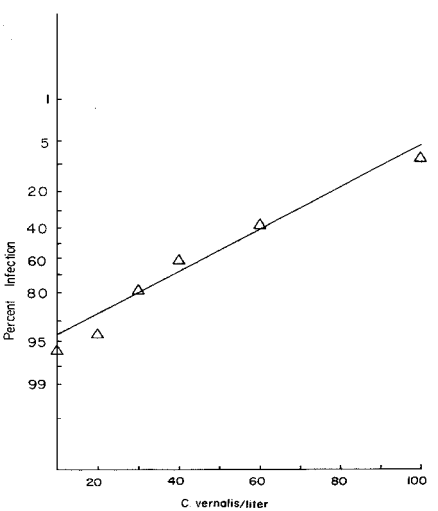

Fig. 4. Effect of increasing copepod density on percent mosquitoes (probit scale) infected by *R. culicivorax* in one liter of water. Infection in the absence of copepods = 100%. The equation for the regression line was: $y = 0.036x + 3.03$.

dom contact between the prey and predator was reduced by the increased search space. Fryer (1957a) has described the food searching behavior of cyclopoid copepods as a random hunt, i.e. there does not appear to be a directed attraction to the prey by sight or smell but reliance on accidental contact with the prey. Such typical behavior was observed during the present study although an additional food acquiring behavior was observed. Copepods usually move about with a jerky swimming motion, however, in many instances, copepods were observed lying motionless in the study containers and attacked when the prey encountered the copepod accidentally during its random swimming behavior.

Experiments with large water volumes demonstrated that the searching and predatory abilities of copepods were sufficient to interfere with biological control efforts. A significant reduction in the numbers of infected mosquitoes was observed at a copepod density of 20 per liter and infectivity was reduced to 50% by a calculated median copepod density of 53 per liter. Although these studies have not been extended to small field trials, it is reasonable to expect that high copepod populations interfere with biological control of mosquitoes by *R. culicivorax*. *C. vernalis* and other cyclopoid copepods are common inhabitants of freshwater throughout North America (Yeatman, 1959), and adult populations of 150 to 1500 *C. vernalis* per liter of shallow lake water during population peaks (Cummins et al. 1969) are not unusual.

The predation of mosquito and other insect larvae by cyclopoid copepods has been observed by others (Hintz 1951, Fryer 1957b). The intensity of the attacks observed during this study suggests that copepods should receive further evaluation in their role in the natural control of mosquito populations.

In preliminary observations, other predators consumed the preparasites of *R. culicivorax* when the prey and predator were confined to 0.2 ml water (Platzer and MacKenzie-Graham 1978). However, in tests with the cladoceran *Simocephalus vetulus*, and unidentified ostracods in one liter of water the effects on mosquito infectivity were negligible. Further studies on other potential predators of *R. culicivorax* are required to develop a better understanding of the potential of this mermithid nematode for biological control of mosquito larvae.

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FIELD EVALUATION OF PIRIMIPHOS-METHYL AS A MOSQUITO LARVICIDE IN AN URBAN AREA OF INDIA AS PART OF THE NATIONAL MALARIA ERADICATION PROGRAMME

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ABSTRACT. Following the resurgence of malaria in India, which was partially the result of an increased incidence of urban cases, alternative control methods against the vectors in urban areas were examined and adopted. During the continued examination of new insecticides, pirimiphos-methyl was tested and shown to be effective as a larvicide when applied each week at a rate of 12.5g active ingredient/hectare. Anti-larval operations with this treatment carried out in the urban areas of one large town resulted in a reduction of the transmission of malaria, lowering of the incidence among children, and decreasing the *Plasmodium falciparum* infection.

INTRODUCTION

As a result of intensive indoor spray treatments by the National Malaria Eradication Programme (NMEP) the incidence of malaria in India had been reduced by 1965 to less than 100,000 cases per annum. There was, however, a resurgence of malaria in the following years such that in 1975 over 5 million cases were recorded. The increased incidence of urban cases, and the development of strains of the vectoring species resistant to those insecticides in use, contributed to this resurgence. For example, after the use of BHC and DDT for many years large areas of India contain strains resistant to these insecticides, and even double resistance to both BHC and DDT occurs in *Anopheles culicifacies* (Brown & Pal 1971). In some areas where malathion has been used as a replacement, there is increased tolerance to this insecticide. (Rajagopal 1977.)

Malaria control methods in rural and urban areas are different; those in the former have predominantly used indoor spraying with residual insecticides to interrupt the transmission of malaria. In urban areas emphasis is placed on mosquito control using intensive anti-larval operations. For the successful control of malaria in areas where vector strains have acquired resistance to those insecticides used as indoor sprays, emphasis is now placed on finding alternative methods of