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thesis entitled

LIFE-HISTORY ASPECTS AND NATURAL DIET OF LARVAL

COQUILLETTIDIA PERTURBANS (WALKER) (DIPTERA:CULICIDAE)

presented by

EMILY JEAN OLDS

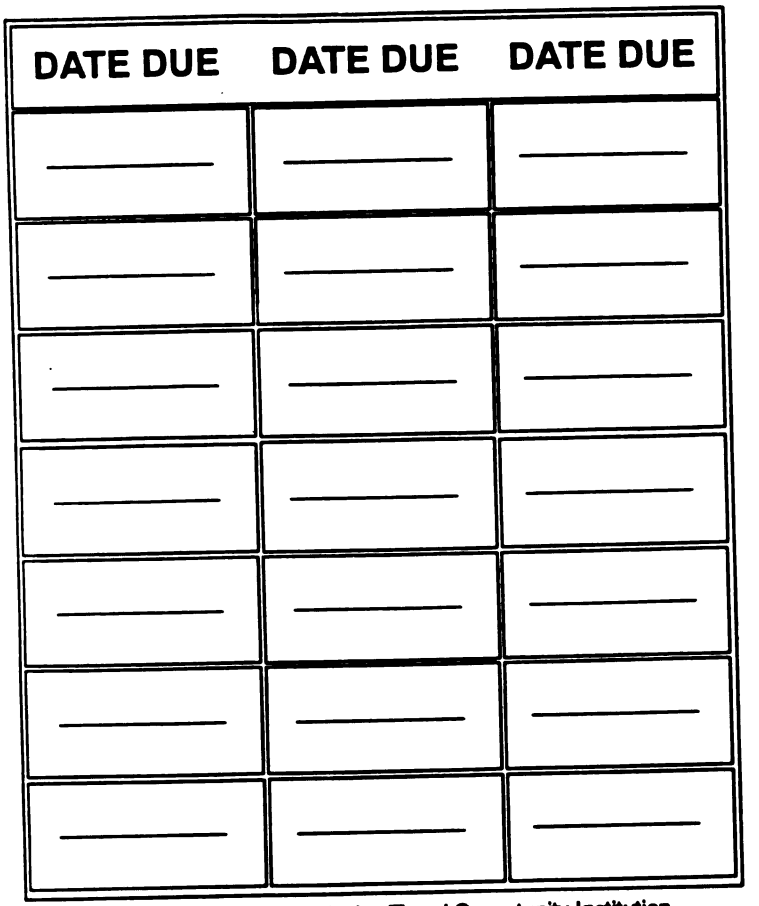
has been accepted towards fulfillment
of the requirements for

M.S. degree in ENTOMOLOGY

Richard W. Merritt

Major professor

Date 2/28/89



LIFE-HISTORY ASPECTS AND NATURAL DIET OF LARVAL COQUILLETTIDIA PERTURBANS (WALKER) (DIPTERA:CULICIDAE)

By

EMILY JEAN OLDS

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1989

ABSTRACT

LIFE-HISTORY ASPECTS AND NATURAL DIET OF LARVAL
COQUILLETTIDIA PERTURBANS (WALKER) (DIPTERA:CULICIDAE)

By

Emily Jean Olds

Coquillettidia perturbans is a medically important mosquito implicated in the transmission of Eastern Equine Encephalitis to horses and humans in the midwest and non-coastal parts of the eastern United States. Fundamental life-history and larval-feeding data necessary to design effective control programs using microbial pesticides are not currently available for this species. In this thesis, methods for locating, sampling and sorting Cq.perturbans larvae were examined. The occurrence and abundance of larval Cq. perturbans were measured at four sites over two years, 1987 and 1988. Parasitism rates were calculated at two sites for a previously undiscovered mermithid nematode parasite. The natural diet of Cq. perturbans larvae was examined and the seasonal, site and instar variations in diet were determined.

ACKNOWLEDGEMENTS

I would like to thank the following people for their help and support in this thesis. The following student workers provided much needed field assistance, and to them I extend my thanks: Dell Richberg, Chris Edens, Paul McCann and Cliff Leavitt. Thanks to Dr. Sheradan Haack for identification of bacteria. Dr. Jean Stout provided excellent editorial and scientific guidance. Special thanks for field assistance, and perspective go to Julie Greminger. I am greatly indebted to Bill Morgan for his help in many ways. I am very grateful for the help of Dr. Edward Walker during all stages of this study and would especially like to thank Dr. Richard W. Merritt for his guidance and support. Finally, for his love, tolerance, sense of humor and support, I would like to thank my husband, Tony Davis.

This research was funded by a NSF Graduate Student Fellowship and by NIH grant number AI-21884.

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PREFACE TO THESIS

Coquillettidia perturbans is a medically important mosquito. Recent evidence has begun to eludicate its role in the transmission of Eastern Equine Encephalitis (EEE). The EEE virus was first isolated from wild caught adult females in 1949 (Howitt et al. 1949). Western EE has also been isolated from wild-caught female Cq. perturbans (Sekla et al. 1980). Females transmit the EEE virus orally under laboratory conditions (Chamberlain 1957, P. R. Grimstad and R.S. Copeland, unpub. data). They have a population transmission rate of 7% to suckling mice (Boromisa et al. 1987). Clark et al. (1985) showed that infection takes place only in the adults and is not transmitted transovarially. Adult female Cq. perturbans are known to feed on a wide variety of hosts including birds, mammals, reptiles, and humans (Fallis and Wood 1957, Snow and Pickard 1957, Beckel and Atwood 1959, Hayes 1961, Downe 1962, Edman 1971). Females also are multiple feeders, biting more than one host to secure a full blood meal (Downe 1962). It has been suggested that their status as an EEE vector is enhanced by these two behaviors (Lounibos and Escher 1983), and that Cq. perturbans acts as the vector connecting the enzootic cycle of EEE in birds with the epizootic cycle in horses, as well as the epidemic cycle in humans.

Cq. perturbans is a very cosmopolitan mosquito, found throughout most of the United States and Canada (Carpenter and LaCasse 1974, Darsie and Ward 1981). The larvae of the genera Coquillettidia and the closely related Mansonia are unique among the Culicidae because they are able to obtain

their oxygen from the roots, rhizomes and stems of submerged vegetation and need not come to the surface for oxygen (Smith 1908, McNeel 1932, Laurence 1960, McDonald and Lu 1973).

Currently, control measures for Cq. perturbans are aimed primarily toward the adult stages; control measures usually involve the widespread application of a broad-spectrum insecticide (often malathion) using ultra-low volume spray apparatus (Allan et al. 1981). New microbial pesticides, specifically Bacillus thuringiensis var. israelensis (B.t.i.), as well as insect growth regulators (e.g. Methoprene) are becoming increasingly available for mosquito control progams (Cheong and Yap 1985, Sjogren et al. 1986, Walker 1987). These have the advantage of being much more specific and, therefore, less environmentally hazardous than older classes of pesticides. However, they must be used against the larval stages. Fundamental life-history and larval feeding data necessary to design effective control programs using microbial pesticides are not currently available for this species.

The objectives of this study were: 1) to locate larval habitats, and evaluate sampling and sorting methods for use in life-history and feeding behavior studies; 2) to examine the seasonal occurrence, abundance and several mortality factors of Cq. perturbans larvae; 3) to evaluate the amount of intrahabitat variation in larval abundance; 4) to determine the diet of larval Cq. perturbans in their natural environment, and elucidate possible larval feeding mechanisms.

Chapter 1

Evaluation of Methods for Sampling, Sorting and Locating the Habitat of Larval *Coquillettidia perturbans* (Diptera:Culicidae)

ABSTRACT

This paper describes a method employing the U.S. Fish and Wildlife Service's National Wetlands Inventory (NWI) maps for locating the larval habitats of Coquillettidia perturbans. In addition, the larval sampling method of Walker and Crans (1986) and sorting method of Morris et al. (1985) were assessed for their precision. NWI maps were found to be much more useful for locating larval habitat than standard U.S.G.S. topographic maps. It appeared that the water regime of wetlands, as noted on NWI maps, was a good indicator of suitable habitat, in the central Michigan area. The pump sampler of Walker and Crans (1986) sampled larvae repeatably, with 4 to 6 sequential suctions required to remove 80 to 90% of attached larvae from cattail stems. The funnel sorting method of Morris et al. (1985) repeatably separated 94% of larvae from sediments in 20 hrs.

INTRODUCTION

Coquillettidia perturbans (Walker) has been implicated as the vector for Eastern Equine Encephalitis in both the Midwest and non-coastal parts of the eastern United States (Nasci and Edman 1981, Francy 1982, Boromisa et al. 1986). However, studies of its larval stages have been largely neglected owing to the difficulties associated with locating, sampling and sorting this species (Hagmann 1953, Bidlingmayer 1954, Morris et al. 1985, Walker and Crans 1986).

The habitat requirements for Cq. perturbans larvae are rather specific; many researchers have sought to quantify them (Hagmann 1953, Bailey 1984, Batzer and Sjogren 1986). In general, Cq. perturbans larvae require a habitat that is permanently wet,.and the water must be relatively clear and stationary. The breeding area must support a high density of healthy, rooted, vascular water plants, and the bottom must be covered with a layer of soft silt (Brower 1953). Several methods have been proposed for sampling and sorting the larvae of Cq. perturbans (Bidlingmayer 1954, Morris et al. 1985, Walker and Crans 1986, Hagmann 1953). This paper describes a qualitative method used to locate Cq. perturbans larval habitats, and evaluates the effectiveness of the pump-sampling method of Walker and Crans (1986) and the inverted-funnel sorting method of Morris et al. (1985). The degree to which these two methods are quantitative also was evaluated.

METHODS

Larval Habitat Location

In 1974, the U.S. Fish and Wildlife service began the National Wetlands Inventory (NWI) program, the mission of which was to provide a database on the current status of wetlands in the U.S.A., so that future trends could be evaluated. The initial goal of the project was to completely map the wetlands using a classification system devised by Cowardin et al. (1979). Mapping was begun in 1978 and by 1987, 45 percent of the wetlands in the lower 48 states were completed. These maps are readily available to the public through the U.S. Fish and Wildlife Service's National Wetlands Inventory, the U.S. Geological Survey's National Cartographic Information Center, and some state agencies. The map legend used on NWI maps provides information regarding a wetland's hydrologic association (e.g., isolated, lake-side, river-side etc.), the life-form of the dominant vegetation in a particular area (e.g., aquatic bed, emergent, forested etc.), and its water regime; i.e., the duration of wetland flooding on a yearly basis. Examples of water regimes are: seasonally flooded, semipermanently flooded,.permanently flooded, and temporarily flooded.

During the summers of 1986 and 1987, 18 wetlands were surveyed in the central Michigan area for the presence of Cq. perturbans larvae. Based on larval habitat descriptions in the literature, only marshes or mapping units with an emergent vegetation life form (EM) were surveyed. Two water regime modifiers were most commonly associated with emergent vegetation in the central Michigan area: semipermanently flooded (F) or seasonally flooded (C). Photocopies of NWI maps were obtained from the U.S. Fish and Wildlife Service field office in East Lansing, Michigan. These maps are the same scale as U.S.G.S. topographic maps. Map quadrangles were chosen for proximity to

East Lansing and for containing the highest number of wetlands per unit area. Mapping units were color-coded and the wetlands on the NWI maps colored to match the coding which facilitated site selection for surveying. Approximately equal numbers of each of the two wetland types were selected. In addition, the selected wetlands were within 1.0 km of a road to facilitate sampling. Using these criteria, 18 wetlands were selected. All wetlands were located in the Bath quadrangle (42°45'-52'30"N. lat., 84°22'30"-30'W. long.). Ten of the 18 had semipermanently flooded water regimes and tended to be dominated by cattails (Typha spp.), while eight were seasonally flooded and contained a variety of plant species including Phalaris arundinacea, and secondarily, some Carex spp. and Typha spp.

Sites were surveyed for the presence of larval Cq. perturbans using the pumping and sieving method described in Walker and Crans (1986). A site was sampled by two people for 30 minutes or until larvae were found. When larvae were found, they tended to be collected within the first 10 minutes of sampling so that 30 minutes appeared adequate for assessing larval presence or absence. Mud from the base of a cattail stem or other submerged plant part (e.g., Carex mound) was pumped into a basin with a modified bilge pump (Walker and Crans 1986). The mud was examined for larvae on site, both by pouring small amounts of mud into white enamel pans and by pouring mud through sieves. In both cases, if larvae were present, they were readily seen moving in an agitated fashion against the dark mud background.

Sampling Method

The larval sampling method devised by Walker and Crans (1986) seemed to best suit the needs and limitations of this project. A "Thirsty Mate(R)" bilge pump that was modified to function as a syringe by removing the distal one-way valve, was used to collect larvae. The distal end of the pump was

placed at the base of a cattail stem and the pump handle was pulled out quickly. The mouth of the pump was then moved rapidly into a position over a basin where the sample was allowed to drain. The suction from the pump was sufficiently strong to remove Cq. perturbans larvae from the stem being sampled, yet it did not appear to be strong enough to remove larvae from adjacent stems.

In order to use the pump method semi-quantitatively, it was necessary to determine how many suctions should be taken from a stem to remove the majority of attached larvae. Two methods were performed at Site 3 (see Olds and Merritt 1989a) during the summer of 1987. For the first method, six suctions were collected from each of three randomly chosen cattail (Typha angustifolia) stems. The water/sediment mix from each suction was poured through a funnel into a 4-liter plastic milk jug. Each of the six suctions from each stem remained separate to determine the average number of larvae per sequential suction. The second trial involved taking a greater number of suctions per stem (10) to see if any additional larvae which may have been more tightly attached, were dislodged by further pumping. In the second trial, suction numbers one through four were consolidated into one sample as were suction numbers five and six. This facilitated the hand carrying of samples 2.0 km to a car. Samples from both trials were taken to the lab and hand-sorted within 2 hrs of collection.

Sorting Method

Hand sorting of Cq. perturbans larvae from the water/sediment mix obtained using the Walker and Crans (1986) pump method was very time consuming, with inherent error, owing to sorter fatigue and experience. Therefore separation of larvae with funnel separators described by Morris et al. (1985) was attempted. In order to use these separators for quantitative counts of larvae, the number or percent of the larvae that were not separated from the

mud in a 20-hr period (the time period suggested in Morris et al. [1985]), was ascertained in the following means: A sample was placed in the bottom of a PVC pipe cylinder, (25.5 cm in length X 7.5 cm in diameter). The PVC pipe was sealed at the bottom and three screws were screwed into the cylinder 8 cm from the top at positions equidistant around the circumference of the cylinder. A plastic funnel, (5.5 cm high with a top diameter of 7.5 cm and a tip diameter of 1.2 cm) was inverted and then rested on the screws. The cylinder was filled with tapwater to at least 2.5 cm over the tip of the inverted funnel. Any debris that appeared to be blocking the tip was removed. The cylinder contents were kept in total darkness by covering cylinders with aluminum foil. This was important since we had found Cq. perturbans larvae to be negatively phototactic. When the oxygen became depleted in the bottom of the cylinder, the larvae rose through the inverted funnel to the surface for air, trapping them in the clear water at the top of the cylinder. The water from the top of the cylinder could then be siphoned off using a turkey baster, and the larvae easily enumerated.

Two tests of the cylinder separators were made. For each test, five cattail stems were sampled by the pump method described above. Four suctions were taken at each stem for a total 20-liter volume of sampled material. This material was condensed down to a volume of approximately 10 liters by filtering off excess water with a 250-um sieve. This sieve was sufficiently fine to insure retention of all instars (Nemjo and Slaff 1984). Eleven separators were used in each trial. In Trial 1, the sample-filled cylinders were placed in a 5° C cooler for 16 hours since we have found that larvae often died within 24 hours if kept at 21°C (room temperature). Larvae were allowed to rise into the water above the inverted funnel. After 16 hours at 5° C, the water above the funnel was drawn off and replaced by fresh tapwater. The separators were then moved to room

temperature (21°C). After two hours at room temperature, water was again drawn off and replaced. After a total of four hours at room temperature the water above the inverted funnel was drawn off for a third and final time. The total number of larvae per water sample for each time period was counted. The separators were then emptied and the number of larvae remaining in the bottom was counted. In Trial 2, the separators were held at 5° C for 16 hours, as in Trial 1, then moved to room temperature (21° C) for four hours. The water above the funnel was then drawn off. The number of larvae that had risen to the surface and those that were left in the bottom of the cylinder were counted. This test was done to determine if the greater degree of disturbance in Trial 1 biased the results.

RESULTS

Larval Habitat Location

Marshes containing larval Cq. perturbans were relatively easy to locate, using NWI maps to identify potentially suitable habitats. Three of ten semipermanently flooded marshes contained Cq. perturbans larvae, but no larvae were found in any of the seasonally flooded marshes. When compared with a Fisher's Exact Test for small numbers, this result was significant at the 90% level ($X^2(1,.1)=2.71$). This indicates that semipermanently flooded marshes are more likely to be suitable habitats for Cq. perturbans larvae than are marshes with a seasonally flooded water regime.

Pump-Sampling Method

The results of the pump-sampling method for Cq. perturbans larvae are given in Figure 1. The total number of larvae sampled from three stems in Trials 1 and 2 were 95 and 123 larvae, respectively. Both trials gave similar results with the greatest proportion of larvae being collected in the first four suctions. A few larvae continued to be collected in sequential suction numbers 5 and 6. In the second trial, virtually no larvae were collected in suction number 7, and a small number were collected in suction numbers 8 through 10.

Morris Funnels

The results of the first Morris Funnel trial are given in Figure 2. In both trials, 94% of the larvae were recovered in the water above the inverted funnel after 20 hours. Seventy-five percent of the larvae rose to the surface in the 16-hour period at 5° C while 21% rose to the surface in four hours at room temperature (21° C).

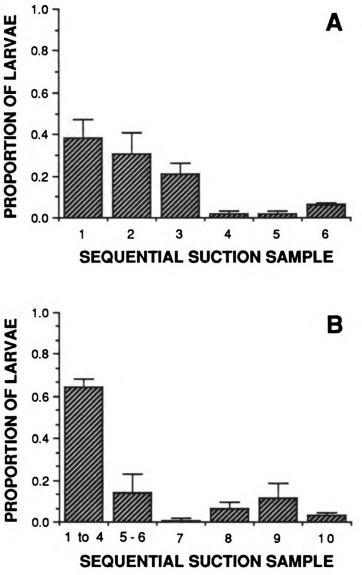

Figure 1. Proportion of larvae removed by each sequential suction sample of Walker and Crans (1986) pump sampler. A. Trial 1. Six total suctions. B. Trial 2. Ten total suctions.

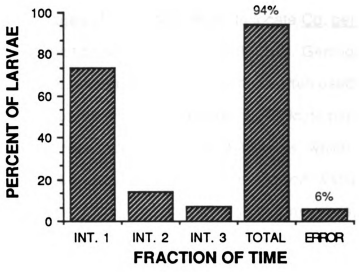

Figure 2. Percent of larvae separated from sediments by Morris et al. (1985) funnel-separator in three time intervals (INT. 1= 16 hrs, 5° C, INT. 2= 2 hrs, 21° C , INT. 3= 4 hrs, 21° C), and total separated and not separated after 20 hrs.

DISCUSSION

Survey Method using NWI Maps

Advantages of using NWI maps to locate Cq. perturbans habitat include increased resolution for wetlands over the U.S. Geologic Survey topographic maps. U.S.G.S. topographic maps are most often used by mosquito control districts to locate marshes (E.D. Walker, C. Chilcote pers. comm.). NWI maps usually include wetlands as small as 0.25 acres, which are much smaller than those on U.S.G.S. topographic maps. In addition, wetlands on NWI maps are classified by dominant vegetation type so that marshes, shrub wetlands and forested swamps can be easily differentiated, and only the relevant wetland type surveyed. In the central Michigan area, there appears to be some relationship between the water regime of a wetland, as presented on NWI maps, and its suitability as Cq. perturbans habitat. Marshes with a semipermanently flooded water regime were somewhat more likely to contain Cq. perturbans larvae than marshes with seasonally flooded water regimes. More survey work using NWI maps from different sections of the United States will be required to determine whether this result can be generalized to all potential wetland habitats containing Cq. perturbans.larvae.

Pump Sampling Method

The pump sampling method of Walker and Crans (1986) was both quantitative and simple to use. It is a very appropriate method for surveying or sampling Cq. perturbans larvae in the central Michigan area where habitats tend to be small, shallow (rarely deeper than one meter), and difficult to reach. This research demonstrated that four to six suctions per stem were adequate to provide a repeatable estimate of the number of larvae attached to an individual cattail stem. Increasing the number of suctions beyond six seems to increase

the probability that larvae will be drawn from the surrounding areas, rather than from the stem being sampled.

Lounibos and Escher (1983) have demonstrated that larvae are rather loosely attached to cattails and when disturbed, readily release. It is therefore unlikely that larvae collected in suctions eight through ten were larvae from the stem being sampled. These probably were larvae that had detached from neighboring stems and then transported into the region of the stem being sampled when increasing amounts of water were removed. It is also improbable that larvae would swim between cattails in the short sampling time, as Cq. perturbans larvae are very poor swimmers.

Sorting-Morris Funnel Method

The separator funnels described by Morris et al. (1985) were a very time-efficient method of sorting Cq. perturbans larvae. To hand sort a four-liter pump sample from one stem took a minimum of two hours when done by experienced sorters. In addition, an unknown number of larvae were lost by the hand-sorting method, owing to sorter fatigue. Using the funnel sorters, the processing time per stem was reduced to approximately 45 minutes and a repeatable 94% of the larvae in the samples were recovered.

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Chapter 2

The Life-History and Seasonal Abundance of larval *Coquillettidia perturbans* (Diptera:Culicidae) in South-Central Michigan

ABSTRACT

The occurrence and abundance of larval Coquillettidia perturbans was measured at four sites over two years, 1987 and 1988. First and second instars were present from mid-July to September (1987) and from late June to October (1988). Third and fourth instars were present for the majority of the year including the winter months. Larval populations were greatest in late August or September, with number of larvae per stem ranging from 42.4 to 7.7 in 1987 and 1988, respectively. Parasitism rates for a previously undiscovered mermithid nematode parasite were calculated at two sites. The maximum rates of parasitism for all larval instars were 21% and 9% for 1987 and 1988, respectively. The rates of parasitism for fourth instars alone (64% in 1987, 67% in 1988) was much higher and less variable between years.

INTRODUCTION

Coquillettidia perturbans is found throughout most of the United States and Canada (Carpenter and LaCasse 1974, Darsie and Ward 1981). Larvae belonging to the genera Coquillettidia and the closely related Mansonia, are unique among the Culicidae in that they are able to obtain oxygen from roots, rhizomes and stems of submerged vegetation and thus need not come to the surface for oxygen (Smith 1908, McNeel 1932, Laurence 1960, McDonald and Lu 1973).

Cq. perturbans has long been known as an annoying biter (Headlee 1945), but recent evidence has begun to elucidate its role in the transmission of Eastern Equine Encephalitis (EEE). Adult female Cq. perturbans are known to feed on a wide variety of hosts including birds, mammals, reptiles, and humans (Fallis and Wood 1957, Snow and Pickard 1957, Beckel and Atwood 1959, Hayes 1961, Downes 1962, Edman 1971). Females also are multiple feeders, biting more than one host to secure a full blood meal (Downe 1962). It has been suggested that their status as an EEE vector is enhanced by these two behaviors (Lounibos and Escher 1983), and that Cq. perturbans acts as the vector, connecting the enzootic cycle of EEE in birds with the epizootic cycle in horses, as well as the epidemic cycle in humans.

Currently, control measures for Cq. perturbans are aimed primarily toward the adult stages; control measures usually involve the widespread application of a broad-spectrum insecticides (e.g., malathion) using ultra-low volume spray apparati (Allan et al. 1981). New microbial pesticides, specifically Bacillus thuringiensis var. israelensis (B.t.i.), as well as insect growth regulators (e.g. Methoprene) are becoming increasingly available for mosquito control programs (Cheong and Yap 1985, Sjogren et al. 1986, Walker 1987). These

have the advantage of being much more specific and, therefore, less environmentally hazardous than older classes of pesticides. However, they must be used against the larval stages.

The objectives of this study were to : 1) examine the seasonal occurrence and abundance of Cq. perturbans larvae at four sites over a two year period; 2) evaluate the amount of intrahabitat variation in larval abundance; and 3) investigate the impact on larval populations of two mortality factors, drought and a previously undiscovered mermithid parasite.

METHODS

Four sites were chosen for a detailed study of the seasonal occurrence and abundance of Cq. perturbans larvae, based on a previous survey of breeding sites (Olds and Merritt 1989a). In March 1987 a severe drought occurred in central Michigan, and as a result, only two sites were sampled in the spring of 1987, a third site was sampled from June 1987 to December 1987 and a fourth site was chosen for all work conducted in the summer of 1988.

Site Descriptions

Site 1. Drumheller Pond (42° 51' 50"N, 84° 26' 30"W). This pond was located on the north side of Drumheller Rd. in Bath Township, Michigan, .8 km east of Webster Rd. The marsh was 0.5 acres with cattails (Typha latifolia) covering 0.21 acres. The cattails were interspersed throughout the marsh in four main stands with open water areas in between.

Site 2. Inland Lakes Research Center (42° 40' 55"N, 84° 28' 50"W). This natural marsh was located immediately to the west of College Rd. at the most southern edge of Michigan State University's Inland Lakes Research Center. The marsh was 1.3 acres in area with cattails forming a one to three

meter wide ring around the perimeter. The total cattail coverage was .34 acres representing 27 percent of the total pond area.

Site 3. Gibbs Pond (42° 51' 50"N, 84° 26' 30"W). This pond was located 1.0-km north of Drumheller Rd. and 0.6 km east of Webster Rd., in Bath Township, Michigan. The marsh was 1.1 acres in area with a total cattail cover of 0.94 acres. Sampling was confined to live cattail stands. Approximately 0.70 acres of the cattails were dead for an unknown reason. The living cattails were located in a U-shaped pattern around an open water area. The average cattail density was 14.7 stems m^{-2} (S.D.=4.9). In 1988, there were no living cattails in this pond. Possible explanations for the mortality of all cattails included muskrat damage, a possible herbicide influx, a cattail blight or drought stress from the previous summer.

Site 4. Maple River (43° 08' 10"N, 84° 33' 30"W). The section of marsh sampled in the study was a small, 0.25 acre parcel on the Maple River State Game Lands. It was located 10 km north of St. Johns, Michigan on Rt. 27 North. The section of the marsh used in this study was located on the northern side of the flooding, approximately 0.5 km from Rt. 27 North. This site was added in the summer of 1988 when it became apparent that the three sites used in 1987 no longer supported populations of Cq. perturbans larvae.

Population Sampling

Sites were measured and mapped using a line and compass technique. Total marsh area and vegetational cover was determined by cutting and weighing constructed maps. Ten permanent stations were located at each site, and stations were selected as uniformly as possible throughout the cattail stands. If the cattails ringed the entire perimeter of a marsh, stations were placed at regular intervals around the edge. Permanent stations were marked by placing 2 m. long iron rod station markers into the sediments.

Random sampling was accomplished using a modified point-quarter method of Mueller-Dombois and Ellenberg (1974). The section of the marsh around each station was divided up into four quadrants using a compass. The quadrants were located northeast, southeast, northwest and southwest of each station marker. Weekly, biweekly or monthly samples were taken depending on the season. For each sampling date, one quadrant was chosen beginning with the northeast quadrant and moving counterclockwise with each succeeding sampling date. Initially, the closest cattail to the station marker was sampled in the chosen quadrant. Ten replicate samples, representing one sample per station, were taken each sampling date. Over four sampling dates one cattail stem in each quadrant of each station was sampled. Cattails were marked with flagging after being sampled to exclude them from being resampled. Once one cattail in each quadrant was sampled, the sampling cycle was reinitiated beginning with the second closest stem in the northeast quadrant of each the station marker. This sampling pattern was continued throughout the season.

Cattail stems were sampled using the method of Walker and Crans (1986). Four suctions of the modified bilge pump were taken per stem. Material was collected in a floating basin, and then poured into four-liter jugs for transporting to the lab. Samples were hand sorted in the lab using a series of Nalgene$^{(R)}$ filters of decreasing mesh size (4 mm, 1 mm, and 0.25 mm mesh). All plant debris in the samples was rinsed thoroughly to remove any entangled larvae.

Samples were collected every two weeks at Site 1 and Site 2 from March 25 through June 30, 1987. By July, both ponds were almost dry, and could no longer be sampled. Sampling at Site 3 began in June 1987. Weekly samples were taken from June through September and biweekly samples were taken in October to December. No samples were taken during ice-cover in January and

February. Sampling began in March 1988 but was discontinued after no larvae were found for three sampling dates (March to May). Periodic checks during the summer of 1988 showed that no larvae remained at this site.

Sampling at Site 4 began in May 1988, and only presence/absence data were collected until July. In July, 1988 the funnel sorting technique of Morris et al. (1985) was used to separate larvae from sediments for enumeration. Larvae were counted from three buckets, each of which contained material sampled from six stems at two suctions per stem.

Habitat measurements

To determine whether within-habitat larval abundance was related to any obvious habitat features, the following measurements were taken at each cattail sampled on each sampling date: 1) water temperature at a depth of ten cm; 2) cattail stem diameter; 3) physiological state of the stem (i.e., living or dead); 4) location of the stem (e.g., in a clump of stems or isolated [greater than 0.3 m from any other stem]); and 5) proximity of the stem to open water. Water depth was measured at all sampled stems and at all station markers. Cattail-stem density was determined at Site 3 in October 1987 by averaging the number of cattails per plot in ten 1-m X1-m plots.

An experiment was conducted on 20 August 1987 to determine if larval numbers were correlated with cattail root volume. Lounibos and Escher (1985) had previously found a positive correlation with the dry weight of the cattail root mass and larval numbers. Ten cattail stems were randomly selected by the modified point quarter system described above. However, in this case, two stems each were taken from stations 1 to 5. Four suctions per stem were taken using the method of Walker and Crans (1986). Samples were hand sorted for larvae immediately after being returned to lab. Sampled stems were gently removed from the mud and care was taken to collect all of the root mass of each

stem. The rhizome which tended to anchor the stem was broken at least 0.3 m from the stem and the stems were labeled and transported to the lab for processing. Root volume was measured by measuring the amount of water that was displaced by submersing the stem in a known water volume.

Intra-habitat Variation in Abundance

A specific degree of accuracy of Cq. perturbans larval population estimates was determined by using the sampling data from 18 sampling dates from Sites 1, 2, and 3 and selecting a desired confidence interval. The number of samples taken per sampling date was either five or ten using the method of Walker and Crans (1986). The data on larvae per stem were transformed using a log (x+1) transformation to normalize the data (Steele and Torrie 1980, Southwood 1978). The mean and standard deviation were determined for each date. The chosen confidence interval was $\pm$ 25% of the mean, and the number of samples needed to provide this level of accuracy was calculated according to Steele and Torrie (1980).

Parasites

In fall 1987, a mermithid nematode parasite was observed in the thorax of a fourth instar Cq. perturbans larva. All third and fourth stage larvae collected from Site 3 in 1987 and Site 4 in 1988 were examined for this parasite using a dissecting scope at 120X. A record was kept of what appeared to be damage (i.e., black spirals in the cuticle of the larvae) caused by the pre-parasites as they entered the larvae (Petersen 1985).

In September 1988, a total of 11 parasitized larvae were collected from Site 4 over a 3-week period. They were placed in 500-ml plastic containers with water, a sand substrate and cattail roots to which the larvae could attach. Larvae were kept in growth chambers at 12^{o} C. Water was changed once per week. Larvae were checked every two days for parasite emergence. Upon

emergence from the larvae, the post-parasitic juveniles were held in containers with 500 ml water (20^{o} C) and sand to see if they would molt to adults.

RESULTS

Population Dynamics

Site 1 and Site 2.-- In spring 1987, both third and fourth instars were found at Sites 1 and 2 (see Figure 1). This indicated that both instars were able to overwinter in central Michigan. At Site 1, third and fourth instars were present until June 23 when the marsh dried. Pupae were first observed at Site 1 on May 28 and were present until the pond dried in late June. Owing to the drought conditions, no further population sampling was possible after June 23, 1987. A study was conducted to determine whether larvae burrowed into the mud to escape the drought, as some authors have suggested (Hagmann 1953). However, no larvae were found, even after sorting 40 liters of mud from an area of Site 1 known to have had very high larval densities (27 larvae per stem). At that time, the pond still held five cm of water in the areas sampled.

Site 3.-- Beginning on 25 June 1987 populations continued to be sampled at Site 3 (see Figure 1). Pupae were present from 9 July to 28 July; first instar larvae were present from 14 July to 25 August; second instars from 14 July to 1 September, and third instars from 25 June to 20 November. Fourth instars were present throughout the sampling period; however, owing to the drought and high temperatures in 1987, larval numbers became very low in winter samples and were below detectable levels in the spring 1988 samples.

Site 4.-- An additional site (Site) was selected in May 1988. The pattern of larval development at this site was similar to previous sites (See Figure 2). Third and fourth instars were present in the spring samples, again indicating

they had overwintered in these stages. Pupae were present from 26 May to 28 June. First instars were present from 24 June to 12 August, second instars from 24 June through 13 September and third and fourth instars throughout the sampling period from 26 May until sampling was terminated in November 1988.

Larval Abundance

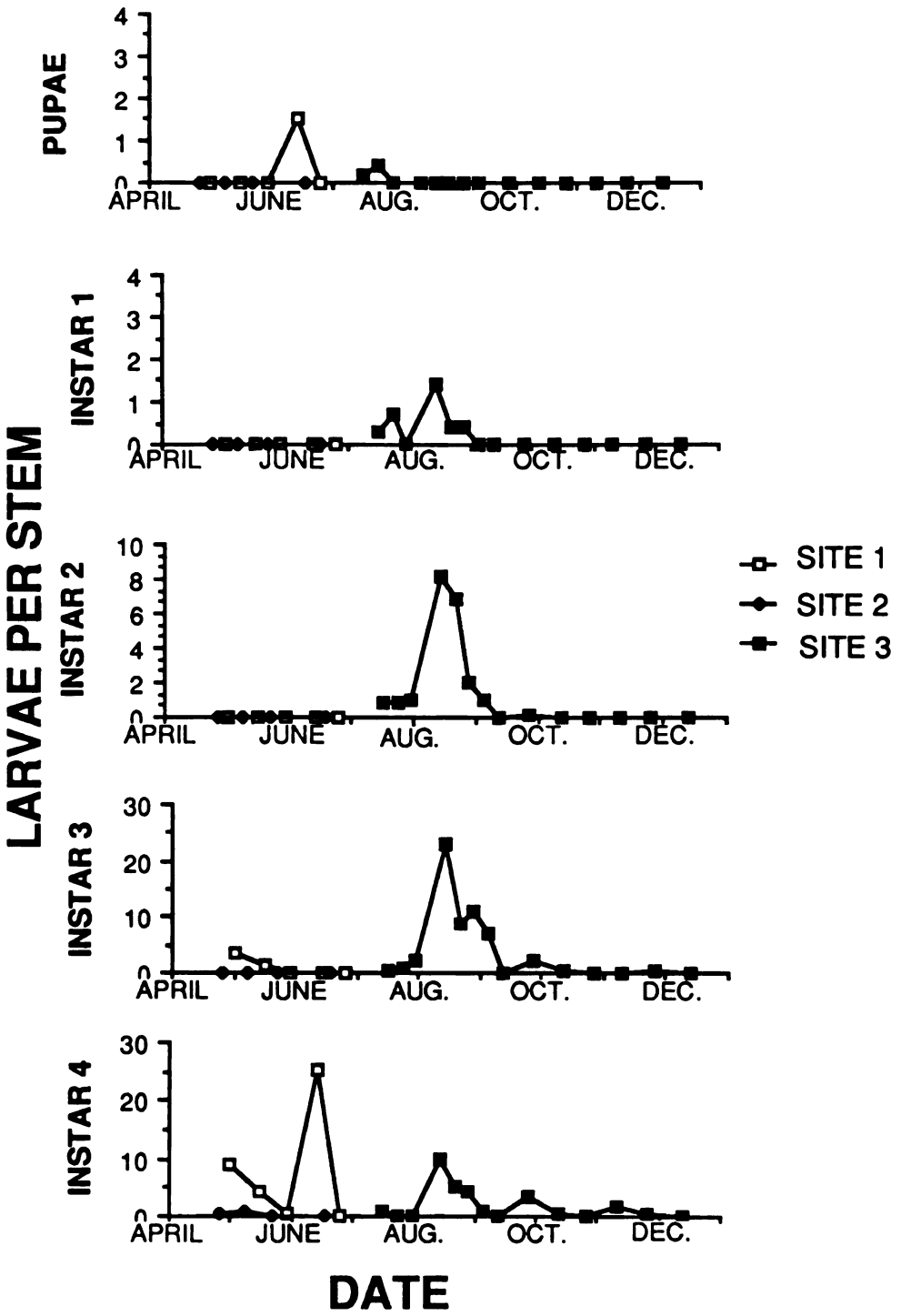
Larval abundance in Site 1 and Site 2 for the spring of 1987 is shown in Figure 3. Site 1 was much more productive than Site 2 during the three-month sampling period (April to June 1987). Site 1 contained an average of 11.4, larvae per stem while Site 2 contained an average of 0.4 larvae per stem.

The seasonal pattern of larval abundance for Site 3 is shown in Figure 3. Larval numbers peaked in mid-August at 42.4 larvae per stem but fell to 0.2 larvae per stem by December. Average total larvae for Site 3 was 114,000 larvae. This number was calculated using only the area of the living cattails. The data obtained from Site 4 in 1988 is probably more characteristic of larval habitats in non-drought years (see Figure 3). Larval numbers peaked in mid-September at 7.7 larvae per stem but only fell to 6.0 larvae per stem by November 1988. Although 1988 was the worst drought in the Midwest in more than 30 years, it did not affect water levels at Site 4, since they were artificially maintained.

Habitat Variables Compared to Larval Abundance

Habitat variables were related to larval abundance by determining correlation coefficients within each sampling date, with stations being observations for each variable. The following variables showed no or few correlations with larval numbers: stem diameter, root mass volume and stem location, both center-edge and isolated-clumped. At Site 3 the stations with the deepest water tended to have the greatest number of larvae, but no correlations existed between water depth and larval number.

Figure 1. Seasonal occurrence and abundance of pupae and larval instars at three sites (Sites 1, 2 and 3) in 1987.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LARVAE PER STEM
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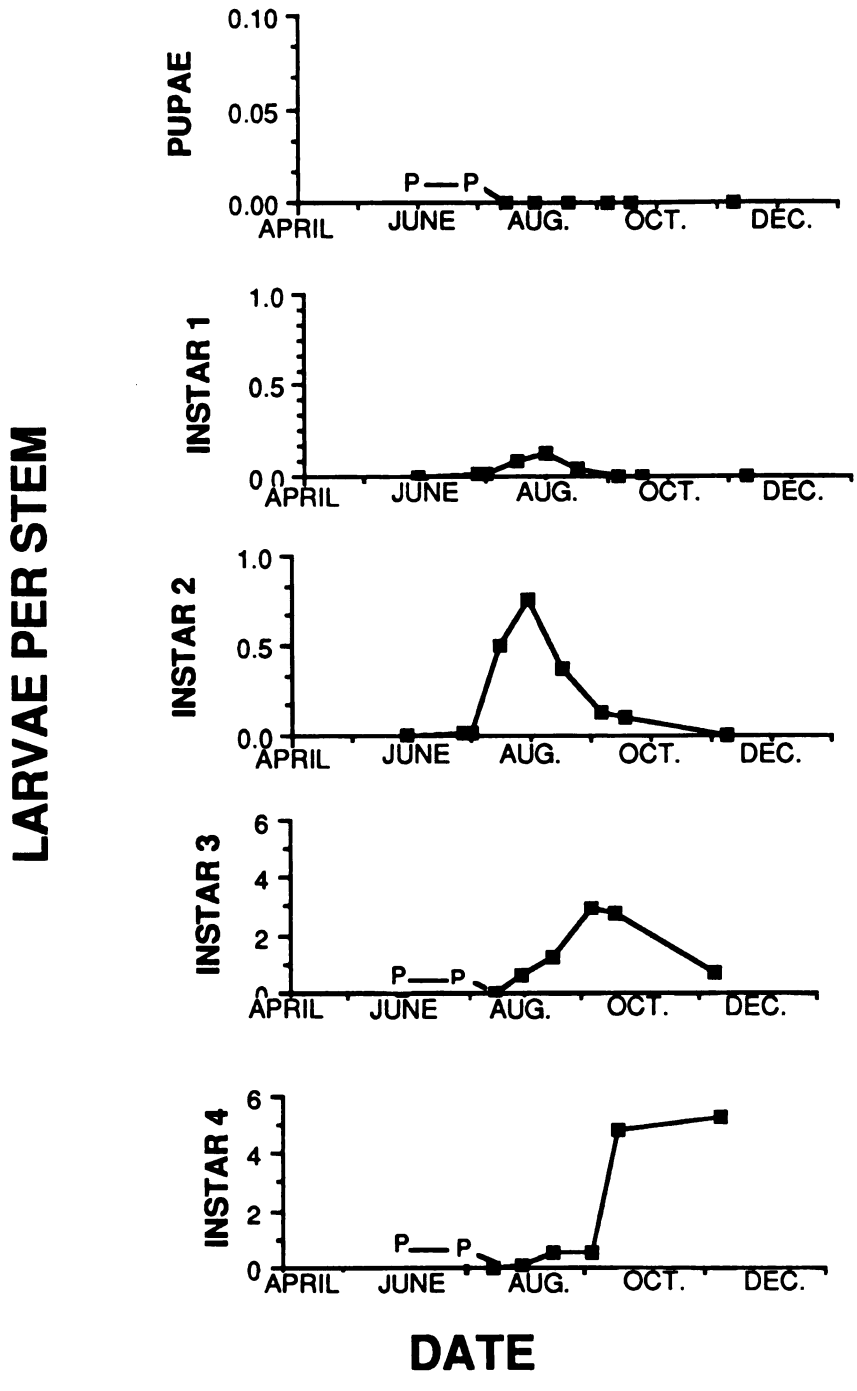
Figure 2. Seasonal occurrence and abundance of pupae and larval instars at Site 4 in 1988. P= present (Sampled only for presence/absence on May 26, June 24 and June 28).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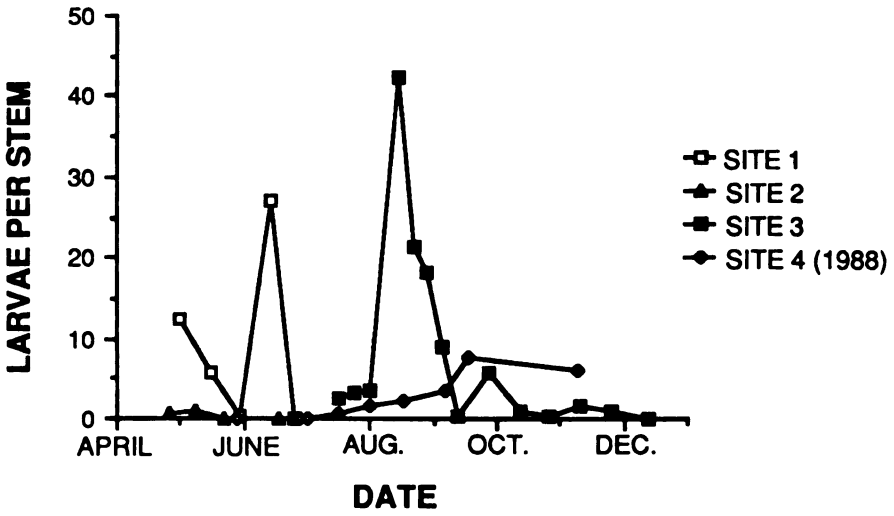

Figure 3. Total larval abundance in 1987 (Sites 1, 2 and 3) and 1988 (Site 4).

Intrahabitat Variation in Larval Abundance

The results of the estimated number of samples required to provide an degree of accuracy for larval abundance at P>F= 0.05, (with C.I. equal to ±25% of the mean) are given in Table 1. Estimates of numbers of samples required ranged from 4.7 to 184.2 with a mean of 53.3. Although this suggests a wide variation in larval numbers, it is mainly attributable to the large number of samples that lacked larvae. When the estimated number of samples is plotted against the proportion of station samples with larvae on each of the 18 sampling dates (Figure 4), it is clear that many more samples are required when most of the samples lack larvae.

Parasites

Mermithid nematode parasites were first observed on 14 July 1987 in fourth instar larvae from Site 3 (Figure 5b). Parasites were also found in third instars beginning on July 28 (Figure 5c). In addition, cuticular injury suspected to be caused by pre-parasites was first noted on 14 July and 21 July for fourth and third instars, respectively (Figure 6). This injury appeared to fit the description of encapsulation and melanization of pre-pupae described by Petersen (1985). The highest parasitism rates were recorded in mid-August . They were 64% for fourth instars (Figure 5b) and 19% (Figure 5c) for third instars; the parasitism rate for the entire population was 21% (Figure 5a). In early September, there was an abrupt decrease in the parasitism rate, especially in fourth instars. The parasitism rate fell to zero in all instars after October 8th (Figure 5a). Parasites were never observed in first instars and were very rarely found in second instars. Pre-parasite injury in second instars was rather common, with a maximum of 45% parasitized on 14 July 1987.

Table 1. Number of samples, mean number of larvae per stem(log(x+1)), standard deviation and estimated number of samples required for ±25% confidence intervals (P>t=0.05) based on results from 18 sampling dates from three sites in 1987.

No. Samples Taken	Mean	Standard Deviation	Estimated Number of Samples Required for ± 25% C.I. (P>t=0.05)
10	0.10	0.22	98
10	0.17	0.26	46
10	0.03	0.09	184
10	0.08	0.16	87
10	0.76	0.48	8.0
10	0.52	0.51	20
10	0.07	0.21	184
10	0.95	0.80	15
10	0.41	0.37	17
10	0.33	0.44	36
10	0.43	0.45	23
5	1.35	0.58	5.8
5	1.12	0.45	5.0
5	1.13	0.44	4.7
5	0.78	0.47	11
5	0.06	0.12	123
5	0.44	0.53	44
5	0.22	0.27	47

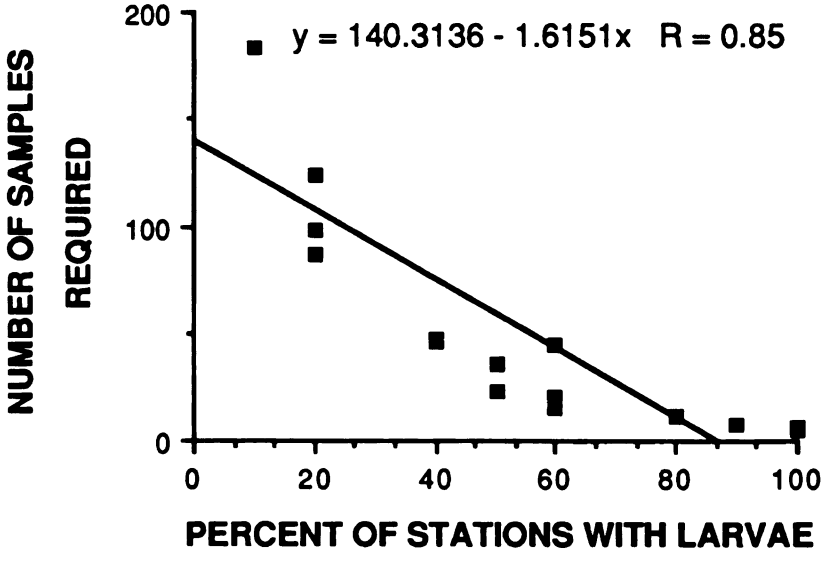

Figure 4. Regression of number of samples required to provide ±25% confidence interval for the average number of larvae per stem at P>t=0.05 against the percent of sampling stations on each sampling date whose samples contained larvae.

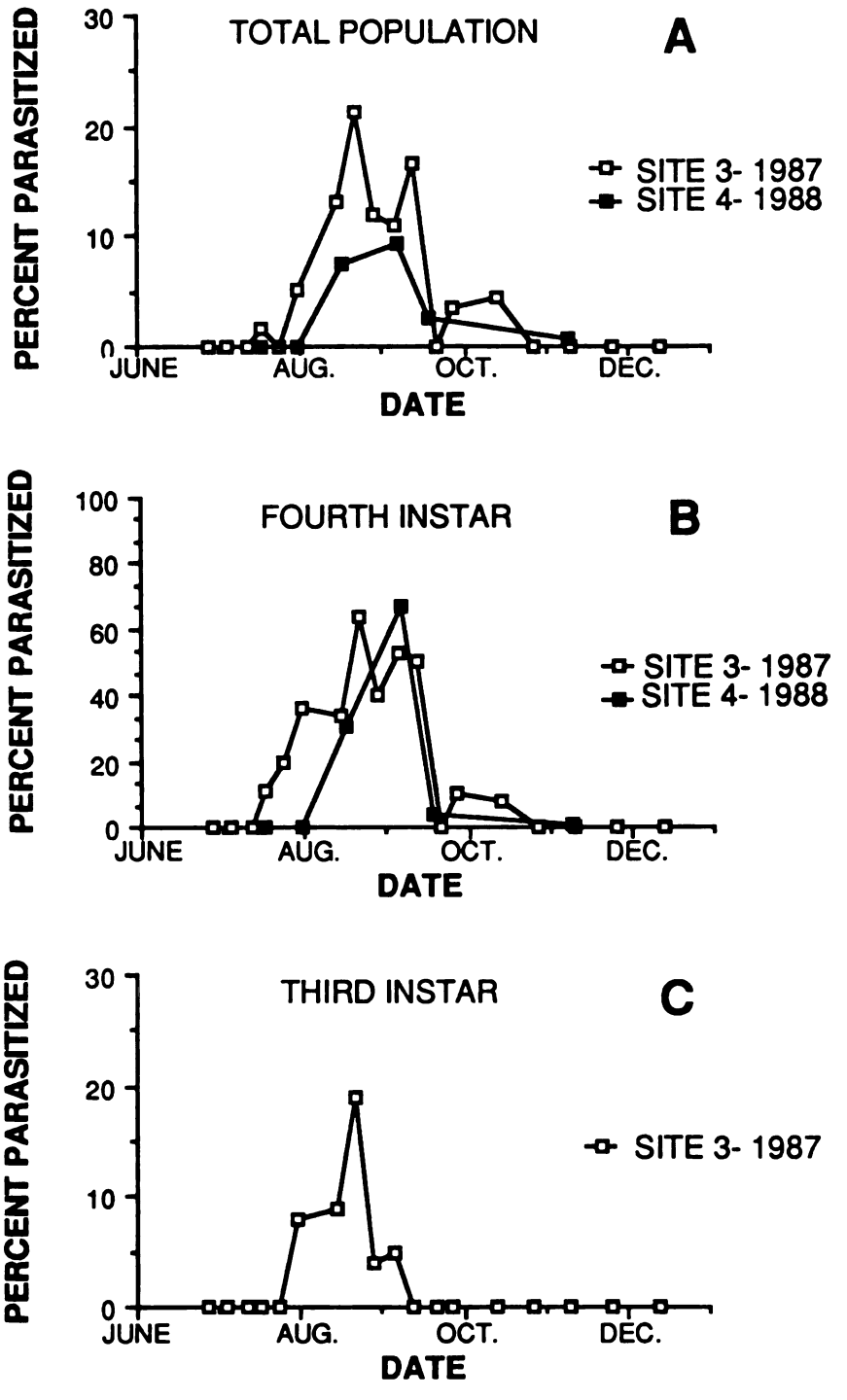

Figure 5. Parasitism rates of: A. Total larval population; B. Fourth instars; and C. Third instars (Site 3 only); from Site 3 (1987) and Site 4 (1988).

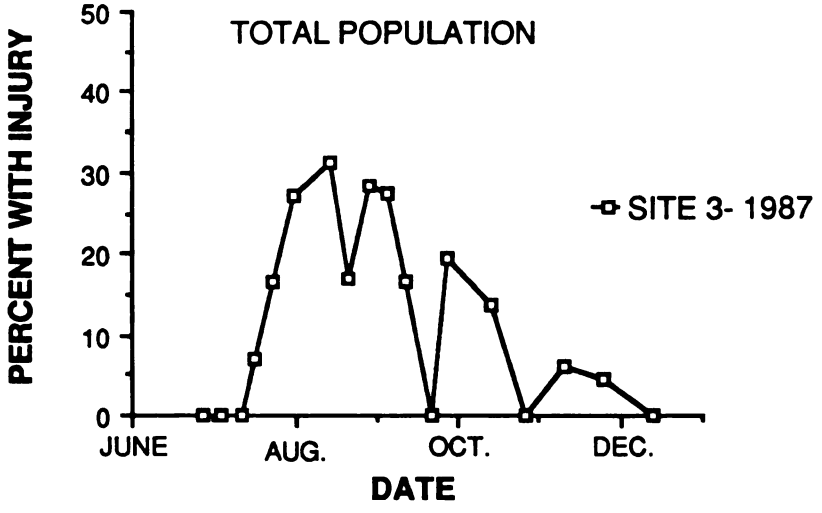

Figure 6. Percent of total larval population at Site 3 (1987) which exhibited cuticular injury as a result of penetration by mermithid pre-parasites.

In 1988, parasitized larvae were found in samples from Site 4 (Figure 5a). The dates of parasitism are similar to Site 3 in 1987, except one parasitized larva was found on 3 November 1988. The highest parasitism rate of fourth instars was also comparable (67%); however, the total population parasitism rate was lower at Site 4 (9% compared to 21.7% for Site 3) (Figure 5a). This was attributed

to two factors: the lower parasitism rate of third instars, coupled with the greater proportion of third instars over fourth instars for any given date.

Survival of parasitized larvae under laboratory conditions, until parasite emergence, was very good. A total of five parasites emerged from the larvae. In all cases, the larva from which the parasite had emerged was dead within 48 hours after the parasite's emergence. A single observation of parasite emergence revealed that the parasite did not emerge from the thorax as had been previously described by Petersen (1985), but appeared to emerge from the distal end of the abdomen, possibly from the breathing tube. Emergence occurred very quickly (less than 60 secs.). The larvae from which parasites had emerged were examined for emergence damage to the cuticle in either the thorax or abdomen. No emergence holes were found at either location. This suggested that the point of emergence was, indeed, the breathing tube.

Post-parasitic juveniles were observed for possible development to adulthood. In two cases, definite changes were seen in the grainy appearance of the nematode. The development of what might have been a vulva, as well as, elongated projections on the anterior and posterior ends of the nematodes were observed.

DISCUSSION

Population Dynamics

Cq. perturbans had a univoltine life cycle in both ponds examined throughout the year. These findings are in agreement with others who have worked on Cq. perturbans in mid-latitude regions (Brower 1953, Hagmann 1953, Carpenter and LaCasse 1974, Bidlingmayer 1968, Lewis and Bennett 1980, Allan et al. 1981). Pupae were present in Michigan ponds several weeks prior to the times described by Lewis and Bennett (1980) and Allan et al. (1981) as the onset of adult emergence in southwestern Ontario and the Nova Scotia-New Brunswick area, respectively. This period probably corresponds to the pupal to adult developmental time. The number of weeks pupae were present in ponds in Michigan was also similar to the number of weeks adult emergence occurred in southern Canada (Lewis and Bennett 1980, Allan et al. 1981). First and second instars were present for a longer period of time, both earlier and later in the season, than reported by Smith (1908) and Hagmann (1953) in New Jersey. The longer season herein reported may have more to do with improved sampling techniques and shorter sampling intervals than with any real differences in seasonal pattern.

Inherent Sampling Method Errors

Although the sampling of Cq. perturbans larvae on a per-stem basis is logical, as larvae always occur attached to macrophytes, there are certain difficulties associated with this approach. Sampling of more than 10 stems in a habitat is logistically difficult, and unless a great deal of assistance is available, this method would be best used for hypothesis testing or for comparisons in habitats with large, relatively uniform populations of Cq. perturbans larvae. An additional modification to ensure presence of larvae in all, or most samples,

would be to pool material from two to five stems in one sample, ensuring all samples would contain at least some larvae. This procedure would reduce the number of samples required for estimating population densities with reasonable confidence intervals.

Parasites

The potential for using mermithid parasites of mosquito larvae for biological control agents has been demonstrated repeatedly (Petersen and Willis 1972, Petersen and Willis 1974a, Petersen and Willis 1974b, Petersen 1975, Nickle 1979). Although the mermithid parasite of Cq. perturbans found in this study does not appear to control the mosquito populations, it may be influencing the voltinism of the population. In both 1987 and 1988, the group of mosquito larvae with the highest parasitism rates and therefore highest mortality rates was early maturing fourth instars. This group, in years of warm autumn temperatures, could conceivably produce a second generation of adults (Allan et al. 1981, Goshenko 1978). In this study, seasonal temperatures were abnormally high; yet, no second pulse of pupae were detected. Mortality caused by the mermithid parasites could have been a factor responsible for this occurrence.

The mermithid nematode which has been used most commonly in biocontrol programs is Romanomermis culicivorax. R. culicivorax appears to be limited by low dissolved oxygen (Brown and Platzer 1978b, Platzer 1981), high ions or organics (Chen 1976 but see Brown and Platzer 1978a, Petersen 1979), and polluted conditions (Mitchell et al. 1974). The habitat in which Cq. perturbans larvae live is characterized precisely by these attributes (Batzer and Sjogren 1986, Callahan and Morris 1987), suggesting that the mermithid parasite of Cq. perturbans is well adapted to these conditions. If this is correct,

biocontrol programs using this parasite might be possible under conditions unsuitable for R. culicivorax.

Applications of this research

This study showed that the best time to survey Cq. perturbans larval habitats is in the autumn. At that time, larval populations tend to be high and the two larval stages present (third and fourth instars) are more visible. If a site becomes dry or near desiccation, it can be excluded. Also, the modified bilge pump method of sampling (Walker and Crans 1986)functions best in shallower waters, which occur in autumn.

Interest exists in modeling the development and emergence of Cq. perturbans. These phenological events could be used to increase efficacy of spray regimes (Slaff 1986). Since the abundance of Cq. perturbans adults may be related to outbreaks of EEE in horses and humans (Boromisa et al. 1987), it would also be useful to model the abundance of Cq. perturbans based on some easily measured environmental parameter. Capotosto and Boyes (1984) found a good correlation between the abundance of Cq. perturbans adults and mean temperatures of the preceding winter. However, work by Rademacher (1979) suggests that larvae are not killed by low winter temperatures. Based on the larval survey and the population data collected in 1987 and 1988 presented in this thesis, it appears that drought stress is an extremely important source of mortality. Over the two and a half year period of this study, four sites containing low to very high populations of Cq. perturbans larvae had their entire population destroyed owing to pond desiccation. This undoubtedly resulted in a large decrease in adult Cq. perturbans abundance. Therefore, it is probable that population models integrating both low temperatures and drought stress (as measured by rainfall) would be much more accurate in predicting adult abundance than would low temperatures data alone.

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Chapter 3

The Natural Diet and Feeding Behavior of Larval *Coquillettidia perturbans* (Diptera:Culicidae)

ABSTRACT

The natural diet of larval Coquillettidia perturbans was examined and the season, site and instar variation determined. The numbers of bacteria, detritus and algae were very consistent in guts of fourth larval instars , within season and site. Average numbers per fourth instar gut of each food category were 1.0 X 10^6, 3.7 X 10^4, and 2.2 X 10^3, for bacteria, detritus and algae, respectively. The number of individual bacterial and algal food types per gut varied significantly over season and site. Proportions of bacteria and detritus were not significantly different among instars, however, guts of fourth instars had a greater proportion and diversity of algal food categories. Observation of larval feeding behavior showed that fourth instars fed by suspension feeding and browsing from sediments and plant surfaces. These feeding mechanisms were supported by the presence of microtrichia on lateral palatal brushes of fourth instars.

INTRODUCTION

Coquillettidia perturbans (Walker) has been implicated as the enzootic and epidemic vector for Eastern Equine Encephalitis in horses and humans in the Midwest and noncoastal parts of the eastern United States (Boromisa et al. 1987, Clark et al. 1985).

The feeding behavior of larval mosquitoes has been studied relatively little; yet, its importance to effective larval control and to the vector status of adults has been demonstrated. Grimstad and Haramis (1984) showed that Aedes triseriatus adults, developing from nutritionally deprived larvae, were more efficient vectors of the LaCrosse Encephalitis virus than those from well-fed larvae, and McComb (1980) found that an optimal laboratory larval diet increased both the size and survival of adults. Although Cq. perturbans larval control using Bacillus thuringiensis israelensis (B.t.i.) has been achieved in some locations (Sjogren et al. 1986), repeatable results have not always been demonstrated (Walker et al. 1985, Walker 1987), suggesting that some aspect of the larval feeding ecology prevented effective ingestion of the B.t.i. (Walker 1987).

Most work on larval mosquito diets occurred in the early part of this century (e.g. Coggleshall 1926, Boyd and Foot 1928, Senior-White 1928, Hinman 1930, and Howland 1930); yet, many questions about larval feeding ecology still remain. Until recently, techniques for visualizing gut contents were inadequate, resulting in certain food-types being under-represented.

Benthic-dwelling mosquito larvae can gather their food in a variety of ways by employing the following mechanisms: 1) filter-feeding; 2) browsing from a substrate surface; or, 3) actually ingesting the substrate. Many authors have described each of these feeding behaviors for different genera of

mosquito larvae (Renn 1941, Surtees 1959, Pucat 1965, Harbach 1977). Mouthpart morphology of mosquito larvae is a good indicator of their feeding strategy (Harbach 1977). For example, container-breeding Aedes, which mainly feed by browsing material rather than filtering, appear to have shorter, and thicker mandibular brushes than do pond-living, eddy-filtering mosquitoes; e.g., many Anopheles larvae (Harbach 1977, Merritt and Craig 1987).

Although mosquito larvae are not generally viewed as selective feeders (Renn 1941), studies have demonstrated a relatively high degree of selectivity for particle sizes (Clements 1963, Dadd 1971a,b, Merritt et al. 1978, Merritt 1987). This ability to select among potential food items is an important adaptation for benthic-dwelling animals. Unlike the epilimnion, where food resources are often scarce, ingestible material in the benthos is rarely lacking; the problem for benthic-dwelling organisms is that of selection for edible particles.

Larvae of Cq. perturbans and other members of the genera Coquillettidia and Mansonia are unique in that they do not need to come to the surface for air, but attach by means of a modified anal syphon to the submerged parts of rooted or floating aquatic macrophytes. They use the oxygen in the aerenchymous tissue of these plant parts for their respiration (McNeel 1932, Laurence 1960, McDonald and Lu 1973). In particular, Cq. perturbans larvae occur in the highly organic benthic zone of eutrophic ponds and marshes (Hagmann 1953, Bailey 1984, Batzer and Sjogren 1986, Smith 1908).

The habitat requirements of larval Cq. perturbans are very specific and the location, sampling and culturing of this species has been difficult to investigate (see Olds and Merritt 1989a for detailed discussion). As a result, little is known about many aspects of the larval biology of Cq. perturbans,

including its diet and feeding behavior. In addition, direct behavioral observations of this species have never been published.

Walker (1987) dissected the guts of larvae collected in his study and found nonliving particulate material and algae. The feeding behavior of the Eurasian counterpart to Coquillettidia perturbans, Cq. richiardii, has been investigated further . Guille (1976) suggested that the organic, flocculent material on pond bottoms not only serves as a food source for Cq. richiardii larvae, but also forms the medium for bacterial colonies. He suggested that bacterial colonies were the major food source of the larvae. Goshenko (1985), also working with Cq. richiardii, found that feeding varied with instar. First instars were periphytophageous on the substrate while second through fourth instars were filter feeders. However, some feeding from the substrate was observed in later instars. Goshenko (1985) implied that larvae moved their points of anchorage more frequently in response to food shortages in the water column. He also felt that substrate feeding by third and fourth instars occurred primarily under these conditions, and an analysis of the gut contents of Cq. richiardii larvae confirmed that periphyton and benthic algae were present in guts.

Laurence (1960), working with members of the genus Mansonia, showed that first instars consumed particles less than 2 um in diameter, consisting primarily of yeast, bacteria and small flagellates. Second instars ingested particles up to 20 um in diameter, including rotifers and large protozoa, and third instars consumed larger flagellates such as the chrysophycean algae, Phacus, and small rotifers up to 50 um in diameter. Fourth instars ingested plant debris up to 200 um long. Dunn (1918) and Cheong and Yap (1985) reported similar findings for members of the genus Mansonia of their regions.

This paper describes the diet of larval Cq. perturbans in their natural environment and discusses the feeding mechanisms employed by the larvae.

METHODOLOGY AND EXPERIMENTAL DESIGN

Sampling Methods and Gut Content Analysis

Larvae were collected from three sites in Ingham and Clinton Co., Michigan between March and December 1987. Two sites, Drumheller Pond (Site 1) and Inland Lakes Research Center Pond marsh (Site 2) (see Olds and Merritt 1988 for site descriptions) were sampled from March 1987 through June 1987. After that, the ponds became unsuitable habitat for Cq. perturbans larvae, except at the end of July at Site 2, owing to drought in the Midwest in 1987. Larvae from a third site, Gibbs Pond (Site 3), were collected from June 1987 to December 1987. Larvae were collected from areas of the ponds known to have high densities of larvae, using the modified bilge-pump method of Walker and Crans (1986). Five or 10 stems were sampled on each sampling date, the material was then transported to the lab in 20-liter buckets where the larvae were handsorted within 2 hours of sampling. Earlier observations showed that larvae did not feed while in the mud/water slurry in the buckets: thus, the amount of elapsed time between sampling and sorting did not affect the gut contents. Samples were also placed in a 5° C cooler upon returning to the lab to slow any digestion or other processes that might have altered the gut contents. After sorting, larvae were preserved in 10% formalin and stored in the dark at 5° C until dissection.

Dissections were performed with a modification of the Cummins et al. (1973) method. In this method, the guts were dissected out and the contents

removed from the peritrophic membrane in several washes of filter-sterilized distilled water. The contents were then placed in an acid-washed shell vial, and filter-sterilized distilled water was added to bring the total volume to 2 ml. The vial was then sonicated for 15 seconds to break-up large aggregates of material formed in the digestive tract, but not long enough to rupture any cells. The contents were then stained with DAPI (4'6 diamidino-2-phenylindole) (Porter and Feig 1980) at a final concentration of 2 ug ml^{-1} for 20 minutes in the dark. The stained material was then filtered on to a black, 0.22 um pore-size, Nalgene(R) filter using a low pressure hand-operated Millipore(R) filtration apparatus. The area of the filter covered by the sample was calculated by the standard formula for area of a circle. Filters were then mounted on slides using Cargille B immersion oil. Cover slips were applied for sample enumeration under epifluorescence microscopy.

Counts of bacteria, algae and detritus were made using the DAPI staining method of Porter and Feig (1980), which has successfully been used to identify the contents of mosquito guts (Walker et al. 1988). Algae, protozoa and bacteria were identified using keys in Ward and Whipple (1959).

Counting was performed using a Leitz Laborlux 11 microscope with epifluorescence light fittings (Walker et al. 1988). At least 15 fields containing bacteria and detritus were counted, for statistical reliability as suggested by Kirchman et al. (1982). If fewer than 200 bacteria were counted in the initial 15 fields, additional fields were counted until at least 200 bacteria were counted per slide. Counts for algae and other large, rare particles were performed by counting the particles in five transects across the widest diameter of the filter, equal to 4.5% of the total filter area.

Natural Diet

1) Seasonal Differences

Seasonal variation in the natural diet of larval Cq. perturbans was examined using fourth instars collected from Site 3 from 25 June through 2 December 1987. Forty-two larval guts were examined for bacteria and detritus, and 15 guts were examined for algae. Data were analyzed on a monthly basis to determine the effect of season on the quantity and diversity of bacteria, detritus and algae present. In June and December, larval numbers were two low to provide a reasonable sample size ($n<3$). As a result, larvae sampled in June and December were included in the July and November data, respectively.

All data from gut contents counts were transformed ($\log(x+1)$) to normalize the data (Sokal and Rohlf 1981). Data were tested for normality, skewness and kurtosis, and homogeneity of variance using Statistical Analysis System (SAS) software. MANOVA tests were performed to determine if the total food composition present in the guts varied seasonally. One MANOVA used each food category of the bacteria, as well as detritus, and a second MANOVA used only the algal food categories. The analyses were compartmentalized this way owing to the three order of magnitude difference between the number of bacteria or detritus and algae per larval gut. An ANOVA on each individual food type was performed using the SAS glm procedure for unbalanced data (SAS 1985). Tukey's Mean Separation test was used to determine which means were significantly different.

2) Site Differences

It was possible to make between-site comparisons of gut contents on two occasions: 1) fourth instars sampled from Sites 1 and 2 during the spring of 1987; and 2) third instar larvae collected on 28 and 30 July from Sites 2 and 3. Data from these ponds were analyzed in the same manner as the gut contents data from the seasonal samples. Algae, except for blue-green algae, were not enumerated in gut contents of larvae from these trials.

3) Instar Differences

All four instars were collected from Site 3 on July 28, 1987. For that period, it was possible to compare the diet among instars using gut-contents data. Counts were made of the number of each food type per gut, then transformed into the proportion of each food type, for instar comparisons. Although gut diameter and length were measured, calculations of gut volume and subsequent food type per ml of gut proved to be very difficult, especially in smaller instars. This was due to the inherent limits of resolution of the ocular micrometer used to measure gut dimensions. The proportion of each food-type per gut were normalized using an arcsin transformation (Steele and Torrie 1980), then analyzed using MANOVAs, ANOVAs, and Tukey's Mean Separation tests, where appropriate.

FEEDING BEHAVIOR

1) Natural Feeding Sites

In June 1988, an experiment was conducted at the Maple River Marsh (Site 4) (see Olds and Merritt 1988a for description), to determine whether larvae fed directly on sediments. Ten stems were sampled using the method of Walker and Crans (1986) and immediately sorted in the field. For each stem

that had larvae, a sample of the sediments surrounding that stem was taken, using a 2 cc sterile syringe. The sediment sample was carefully taken as close as possible to the surface of the sediments to prevent mixing when the sample was removed from the water. Six larval and six sediment samples were preserved immediately with formalin in the field, transported to the laboratory in a cooler, and stored at 5° C until analyzed.

The guts were dissected from the larvae, and the contents stained and counted as described above, except sediments were diluted for more reliable counts. Total detrital and bacterial particles also were counted from the diluted samples.

2) Behavioral Observations in the Laboratory

Observations of the feeding behavior of fourth instar Cq. perturbans were conducted in a chamber constructed of clear glass on the front and back, and plexiglass on the sides for support (6.7 cm high X 6.7 cm wide X 0.7 cm deep). A 2-cm layer of pond mud was placed in the bottom, and three small cattail roots (approx. 1 mm in diameter), were placed in the chamber with one end immersed in the mud and the other end extended and secured to the top of the chamber. Tap water was added to within 0.5 cm from the top of the chamber, resulting in a volume of 29.0 cm^3.

For each observation period, four larvae were added to a chamber which was placed inside a 16° C water bath. The water bath was placed inside a dark hood so that the light levels could be controlled carefully, since we had previously observed larvae to be negatively phototactic under high light conditions. Light was provided using a high intensity microscope illuminator with the tips covered with red plastic to further simulate the subdued light conditions of a pond bottom (Wetzel 1983).

After larvae were allowed to equilibrate for 30 minutes, observations were made using a Wild dissecting scope mounted horizontally in a boom. Both 60X and 120X magnifications were used. Larvae were observed for two hours per session and during that time, larval movements, location, orientation, and other feeding behaviors were recorded. The phagostimulants carmine red, charcoal and yeast extract were added on two separate occasions per phagostimulant to see if filter-feeding could be enhanced. This experimental addition was based on the work of Dadd (1970) who noted that some mosquitoes will not filter-feed in the lab unless a phagostimulant was added to their growth medium.

3) SEM

Scanning Electron Micrographs (SEM) were taken of the lateral palatal brushes and mandibular brushes of fourth instar Cq. perturbans. After larvae were collected, they were immediately sorted and stored in 3.5% gluteraldehyde. The heads were removed and dehydrated in a series of increasing ethanol concentrations, critical-point dried (with CO_2 as the transitional fluid), mounted, and gold coated. Scanning electron micrographs were taken with a JEOL JSM-356 scope at 600X, 1200X and 2400X.

RESULTS

Natural Diet

1) Particulate Food

Three categories of particulate food were found in larval guts: bacteria, detritus and algae. Protozoa were also found, but were present in few larvae at low numbers. Four types of bacteria were identified on the basis of morphology:

cocci, rods, purple sulfur bacteria, and spirochetes. Four types of algae were identified: desmids, diatoms, euglenoids (flagellated unicells) and blue-greens. When blue-green algae were particularly abundant, they were enumerated by fields (rather than in transects) with the bacteria.

2) Seasonal Differences

Results of counts of total bacteria, detritus and algae are presented in Figure 1. All three food types were present and the abundance of each was similar throughout the season. Bacteria were the most numerous food type and ranged from a monthly average of 5.37×10^5 to 1.00×10^6 cells gut^{-1}, with a maximum number of cells per gut of 1.66×10^6 and a minimum of 3.16×10^5. Detritus was the second most abundant food type, with averages ranging from 1.05×10^4 to 3.72×10^4 particles gut^{-1}. The maximum and minimum number of detrital particles in an individual larva was 1.58×10^5 and none, respectively. Algae were the least abundant food type of the three categories (including blue-greens), with monthly averages ranging from 1.12×10^2 to 2.29×10^3.

Cocci were always the most abundant bacteria food type, usually followed by spirochetes and then rods (Table 1). The most abundant algal type was bluegreens, followed by euglenoids, although the latter were more abundant in August (Table 2).

Bacteria and detritus varied significantly in larval guts among months (MANOVA, Wilk's Criterion $p<0.0031$), but algae did not (MANOVA, Wilk's Criterion $p<0.1145$). Among types of bacteria, rods varied significantly ($p<0.05$) seasonally, whereas cocci, purple sulfur bacteria and spirochetes, as well as total bacteria and total detritus did not ($p>0.05$) (Table 1). The number of rods was significantly higher in June/July than in August, September and November but was not significantly different from October (Tukey's Mean Separation test,$p=0.05$).

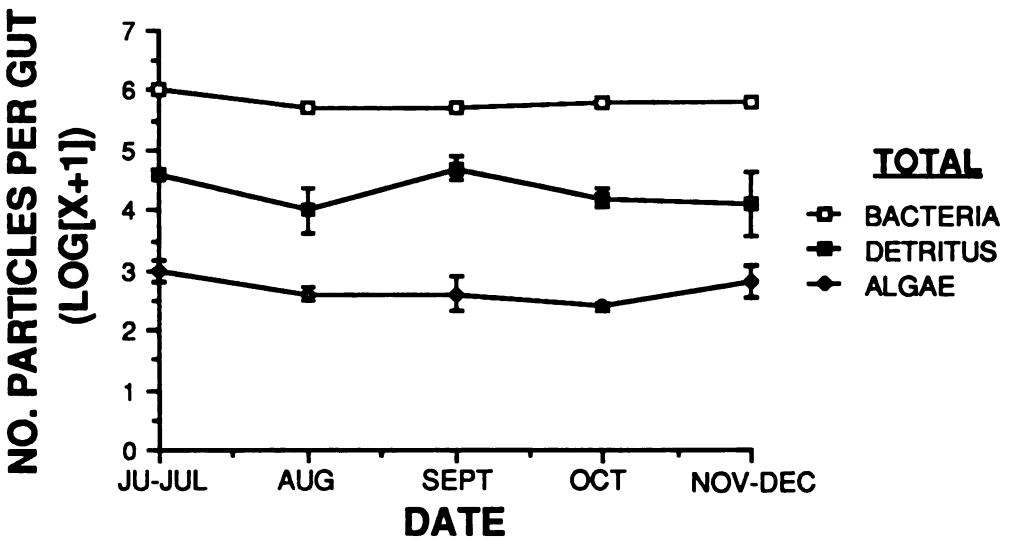


Figure 1. Total bacteria, detritus and algae in guts of fourth instar larvae on a seasonal basis.

Table 1. The number of each bacterial food type per larval gut on a seasonal basis.[a]

MONTH	N	BACTERIA			
		COCCI	RODS	PURPLE-SULFUR	SPIROCHETES
Ju./July	10	5.7 (0.08)	5.2a (0.12)	4.0 (0.67)	5.1 (0.20)
August	12	5.5 (0.06)	4.6b (0.07)	4.2 (0.41)	4.7 (0.38)
September	7	5.6 (0.05)	4.3b (0.12)	3.5 (0.66)	4.8 (0.11)
October	4	5.5 (0.03)	4.7a,b (0.06)	4.6 (0.17)	5.2 (0.15)
Nov./Dec.	9	5.7 (0.05)	4.5b (0.12)	2.0 (0.61)	4.7 (0.14)

Column means followed by the same letter are not significantly different ($P>0.05$, Tukey's Mean Separation Test). If no letter by any means in column then no significant differences were found between any means. Analysis conducted on log(x+1) transformed data.

[a] Data are means (SEM).

Table 2. The number of each algal food type per larval gut on a seasonal basis.[a]

MONTH	N	ALGAE			
		CYANOPHYTA	EUGLENOIDS	DIATOMS	DESMIDS
Ju./July	3	2.9 (0.18)	2.2 (0.14)	0.5 (0.46)	0.9 (0.46)
August	3	2.4 (0.17)	1.9 (0.04)	1.6 (0.14)	1.1 (0.60)
September	3	1.4 (0.72)	2.2 (0.09)	1.3 (0.75)	1.3 (0.75)
October	3	2.1 (0.13)	1.7 (0.15)	0.5 (0.46)	1.5 (0.15)
Nov./Dec.	3	2.7 (0.33)	1.8 (0.21)	0.0 (0.0)	1.0 (0.51)

Column means followed by the same letter are not significantly different ($P>0.05$, Tukey's Mean Separation Test). If no letter by any means in column then no significant differences were found between any means. Analysis conducted on log(x+1) transformed data.

[a] Data are means (SEM).

3) Site Differences

The number of total bacteria and total detritus in larval guts from Sites 1 and 2 are presented in Figure 2A, and from Sites 2 and 3 in Figure 2B. Total bacteria and detritus from Sites 1 and 2, which were sampled in the spring of 1987, were very similar to those in the seasonal samples from Site 3 (see Figure 1 and Figure 2A for comparison). As found in the gut contents data from the seasonal samples in Site 3 (Table 1), cocci were the most abundant type of bacteria (Table 3). and the second most abundant was again spirochetes in Site 2. However, gut contents from Site 1 had an unusually large number of sulfur bacteria, an average of 1.26×10^5 gut^{-1} (see Table 3).

The MANOVA for Site 1 and Site 2 using cocci, rods, blue-green algae, sulfur bacteria, spirochetes and total detritus, was significant (Wilk's criterion P>F=0.0059). ANOVAs using the SAS glm procedure, on each food type as well as total bacteria showed significant differences only in rods and sulfur bacteria. Rods were significantly greater in samples from Site 2 while the opposite was true for sulfur bacteria.

The MANOVA for samples from Site 2 versus Site 3, using the same variables as in previous analyses, was significant (Wilk's Criterion P>F= 0.0199). ANOVAs showed significant differences in rods and blue-green algae (see Table 4). Total bacteria and detritus were not significantly different between ponds (Figure 2). In samples from both ponds, cocci were the most abundant food type (Table 4).

4). Instar Differences

The percent of total bacteria and detritus were fairly constant among instars (Figure 3). Total bacteria was 82 to 92% of the total particles in the gut of all instars, while detritus ranged from 6 to 17% of the particles. Algae in the guts exhibited the greatest relative range among instars, with means ranging from

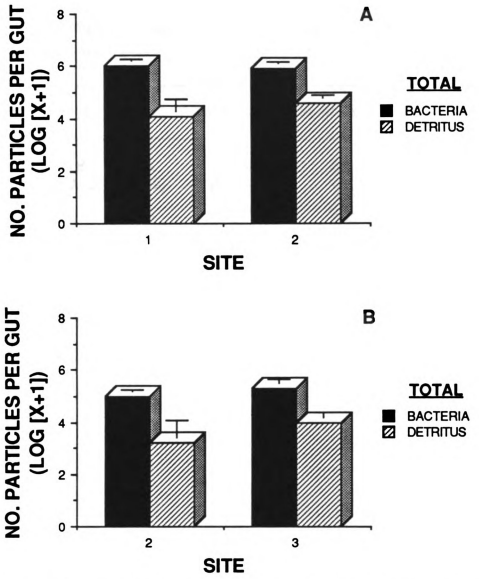


Figure 2. Total bacteria and detritus in guts of: A. fourth instar larvae from Sites 1 and 2 collected from March to June 1987; and B. third instar larvae from Sites 2 and 3 collected on 2 August and 28 July 1987, respectively.

Table 3. The number of each bacterial and algal food type per gut on a site basis-Sites 1 and 2 using guts from fourth instars.[a]

SITES	N	BACTERIA				ALGAE
		COCCI	RODS	PURPLE-SULFUR	SPIROCHETES	CYANOPHYTA
SITE 1	11	5.8 (0.05)	4.5a (0.08)	5.1a (0.11)	5.0 (0.15)	3.8 (0.39)
SITE 2	9	5.7 (0.05)	4.8b (0.07)	4.7b (0.15)	4.9 (0.15)	4.3 (0.07)

Column means followed by the same letter are not significantly different (P>0.05, T-test, two-tailed). If no letter by any means in column then no significant differences were found between any means. Analysis conducted on log(x+1) transformed data.

[a] Data are means (SEM).

Table 4. The number of each bacterial and algal food type per gut on a site basis-Sites 2 and 3 using guts from third instars.[a]

SITES	N	BACTERIA				ALGAE
		COCCI	RODS	PURPLE-SULFUR	SPIROCHETES	CYANOPHYTA
SITE 2	6	4.9 (0.06)	3.5a (0.17)	3.5 (0.72)	2.9 (0.91)	2.8a (0.58)
SITE 3	4	5.2 (0.18)	4.3b (0.20)	2.3 (0.95)	3.9 (0.21)	0.6b (0.62)

Column means followed by the same letter are not significantly different ($P>0.05$, T-test, two-tailed). If no letter by any means in column then no significant differences were found between any means. Analysis conducted on log(x+1) transformed data.

[a] Data are means (SEM).

0.1 to 1%. The MANOVA, using all bacterial types and total detritus, performed on the arcsin transformed data was not significant (Wilk's Criterion P>F= 0.5654). ANOVAs of the proportion of each bacterial type, total bacteria and total detritus showed no significant differences in any of the food types among instars (Table 5). The MANOVA performed on the algal food types was not significant (Wilk's Criterion P>F= 0.3062). However, ANOVAs of the proportion of each algal food type plus total algae showed significant differences at the 0.05 level in desmids, diatoms and euglenoids. Total algae was significantly different between instars at the 0.10 level (see Table 6). Tukey's Mean Separation Test showed that fourth instars contained proportionately more desmids and euglenoids than first, second or third instars, while fourth instars had more diatoms than first instars, but neither were different from second or third instars (Table 6).

Feeding Behavior

1) Natural Feeding Sites

Using the paired larval and sediment samples collected on 24 June 1988 from Site 4, the ratios of bacterial particles to detrital particles were calculated for larval guts and their surrounding sediments. Data were analyzed using a paired t-test . The proportion of bacteria in larval guts was significantly greater than in the sediments ($p<0.0001$) (Figure 4).

2) Behavioral Observations

The observed behaviors of Cq. perturbans larvae were divided into two classes: those when the larvae were attached to a root and those when detached. Larvae tended to spend the majority of time attached to the root or to

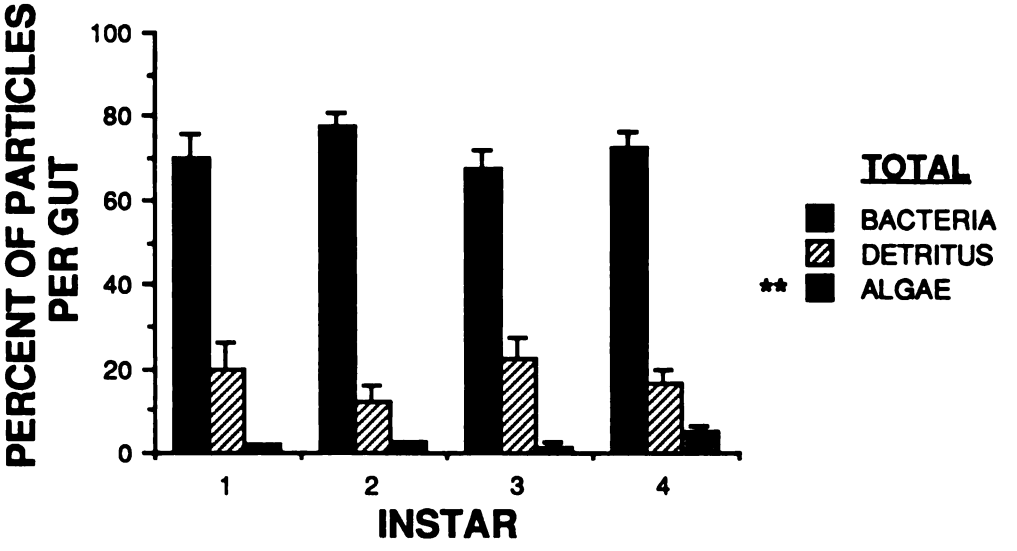


Figure 3. Percent of total bacteria, detritus and algae in the guts of first through fourth instar larvae collected on July 28, 1987. *= significant differences ($p>0.05$). Columns with same letters are not significantly different ($p>0.05$, Tukey's Mean Separation Test).

Table 5. The percent of each bacterial food type per larval gut for first through fourth instar collected on 28 July 1987.[a]

INSTAR	N	BACTERIA			
		COCCI	RODS	PURPLE-SULFUR	SPIROCHETES
1	5	59.2 (4.9)	15.4 (2.5)	5.2 (3.3)	9.3 (1.9)
2	5	63.5 (1.8)	14.7 (2.4)	3.7 (2.3)	13.5 (1.7)
3	5	53.7 (2.8)	17.4 (2.3)	6.6 (3.6)	12.9 (3.3)
4	3	50.3 (4.5)	20.5 (5.9)	15.7 (1.6)	16.7 (7.4)

Column means followed by the same letter are not significantly different ($P>0.05$, Tukey's Mean Separation Test). If no letter by any means in column then no significant differences were found between any means. Analysis conducted on proportion of each particle type per gut transformed by an arcsin(sq.rt.(Y)) transformation.

[a] Data are means (SEM).

Table 6. The number of each algal food type per larval gut for first through fourth instar collected 28 July 1987.[a]

INSTAR	N	ALGAE			
		CYANOPHYTA	EUGLENOIDS	DIATOMS	DESMIDS
1	3	1.1 (0.57)	0.3a (0.33)	0.0a (0.0)	0.4a (0.39)
2	3	2.5 (0.31)	0.3a (0.28)	0.3a,b (0.28)	0.3a (0.26)
3	3	1.3 (1.26)	0.2a (0.18)	0.2a,b (0.18)	0.0a (0.0)
4	3	4.0 (1.64)	1.3b (0.19)	1.1b (0.23)	1.7b (0.13)

Column means followed by the same letter are not significantly different ($P>0.05$, Tukey's Mean Separation Test). If no letter by any means in column then no significant differences were found between any means. Analysis conducted on proportion of each particle type per gut transformed by an arcsin(sq.rt.(Y)) transformation.

[a] Data are means (SEM).

roothairs of provided cattail stems. They usually attached with their ventral surface oriented toward the water surface, a position that placed their mouth in the path of any material settling from the water column. While attached, larvae often bent toward the cattail root and appeared to graze from around the point of attachment. Larvae never attached themselves low enough to be fully covered by mud, yet they attached with the posterior half of the body in the mud, and the mouth still in water. On several occasions, it appeared that larvae had excavated a funnel-shaped depression around the root by swimming around the area where it entered the sediments. They then attached to the root in the bottom of the depression with dorsal surfaces oriented toward the sediments. Their heads would be approximately at the level of the sediment-water interface, and their mouths were oriented to the water column.

Larvae often swam immediately to the bottom when detaching from the cattail roots. Once detached, larvae swam up or down along a root or through the water for an extended period of time, up to 10 mins. They also swam along the surface of the sediments with mouth brushes touching the actual sediment surface. Occasionally, the mouth brushes moved while larvae were swimming in this fashion.

Digging into the sediments was another common larval behavior, accomplished this by swinging the posterior halves of their bodies back and forth through the sediments. Although the sediments were 2 cm deep, they did not appear to dig more than 1 cm deep. Larvae never stayed completely covered with sediments for more than 3 to 4 minutes. They did not completely cover themselves with sediments, but rather burrowed into the sediments leaving their heads and mouth parts exposed to the sediment/water interface. While doing this, the larvae tended to orient their bodies almost parallel to the sediment surfaces with dorsal surfaces directed down.

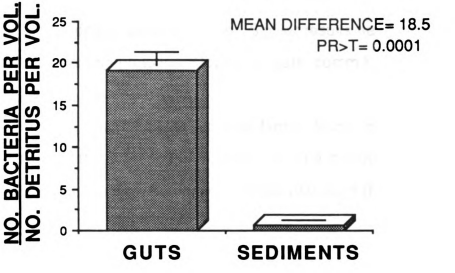


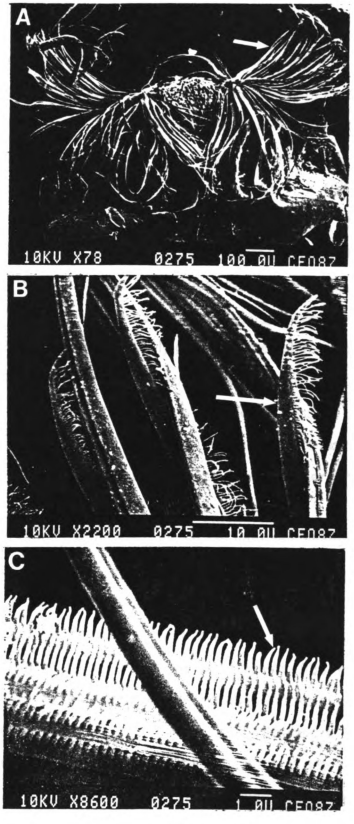
Figure 4. Ratio of number of bacteria to number of detrital particles in the guts of fourth instar larvae and the surrounding sediment.

The larvae did not filter feed when clear tapwater was provided in the observation chamber. The phagostimulants charcoal powder and carmine red did not promote or enhance filter-feeding behavior, even when added at high concentrations. However, when a slurry of yeast was added, larvae readily filtered for prolonged periods of time. Similar results have been obtained using solutions containing protozoa (Copeland, pers. comm.).

3) SEM

Micrographs of the lateral palatal fans of fourth instar Cq. perturbans larvae showed the presence of microtrichia. The microtrichia were 0.2 um at the base, 1.0 um in height with a maximum gap of 0.2 um (Figure 5).

Figure 5. Scanning electron micrograph of lateral palatal brush of fourth instar larvae showing: A. microtrichia.


A
10KV X78 0275 100.0U CE087
B
10KV X2200 0275 10.0U CE087
C
10KV X8600 0275 1.0U CE087

DISCUSSION

The diet of larval Cq. perturbans was very consistent regardless of season, site or instar. Bacteria were the most abundant food type, followed by detritus, then algae. Cocci were always the most abundant type of bacteria found in the guts. Regardless of time of year, the number of bacteria in the guts of fourth instar larvae ranged from an average of 5.37×10^5 to 1.00×10^6 cells gut^{-1}. In comparison, Nilsson (1987) found the number of bacteria per gut ranged from 6.68×10^6 to 2.18×10^7 for a variety of mosquito species. Also, Walker et al. (1988) found that the number of bacteria per gut for field-collected Aedes triseriatus and Anopheles quadrimaculatus larvae averaged 2.2×10^6 and 2.0×10^6 bacteria, respectively per gut. These latter results were obtained using the DAPI technique for visualizing bacteria (Walker et al. 1988). Larval Cq. perturbans appeared, therefore, to have fewer bacteria per gut than other mosquito larvae investigated. Possibly, they are able to compensate for consuming fewer bacteria per unit time, by having longer larval development times.

Although total bacteria in guts did not vary significantly with season or between sites, individual types of bacteria did. This is probably related to local changes of microbial populations within the small areas that the larvae browse. Several authors have shown that there is a definite seasonal component in the abundance of certain bacteria (Paterek and Paynter 1988, Holmes and Vennes 1970),and different sites can have differing abundances of microbe types within microsites (Lopez 1988, Hines and Buck 1982). Therefore, it seems improbable that larvae were selecting different types of bacteria at different sites or months. Several authors have demonstrated that mosquito larvae are able to select particles on the basis of size (Merritt et al. 1978, Merritt 1987), but only within

fairly broad categories (i.e., less than 2 um, 2 to 10 um etc.). Virtually all bacteria found in the guts of Cq. perturbans larvae were less than 5 um in the largest dimension, excluding filamentous forms (spirochetes), which were longer. Larvae did not appear to distinguish among types of bacteria in the same size range.

There was considerable variation found in certain food categories of the diet of Cq. perturbans larvae. Often, the abundance of some bacteria in larval guts, (e.g., spirochetes or purple sulfur bacteria) varied as much as 200% within site and date. This is strongly suggestive of the patchy nature of bacterial abundance and larval feeding. It is well known that many species of bacteria grow in patches in the environment (Franklin et al. 1988, Jones and Paynter 1980), and several other invertebrates and protozoans are known to feed on patches of bacteria (e.g., Sibbald and Albright 1988). Behavioral observations of larval Cq. perturbans revealed that they moved only short distances over time. Thus, differing bacterial patches may be encountered and ingested rather infrequently. This could result in the high variation in the numbers of some bacteria ingested.

Unlike Goshenko (1985), who found distinct diet differences among instars of Cq. richiardii under laboratory conditions, few major differences were found for diets of different instars of field-collected larval Cq. perturbans. Fourth instars had a higher proportion and more types of algae in their guts than did earlier instars. They may have an expanded repertoire of feeding behaviors in later compared to earlier instars, as suggested by Goshenko (1985). However, it could also be a stochastic process based on the development time of each instar and the probability of encountering and retaining algal particles in that time period. Fourth instars develop much more slowly than first and second

instars (see Olds and Merritt 1989b), and therefore would have an increased probability of finding algal particles, given a patchy distribution of algae.

The findings of this study complement similar studies conducted on the European counter-part , Cq. richiardii. Guille (1976) examined the gut contents of Cq. richiardii without using an epifluorescence, technique but concluded that bacteria represented the major food source for that species. Both Walker (1987) and Goshenko (1985) also found algae and non-living particulates in the larval guts of Cq. perturbans and Cq. richiardii, respectively. Neither used a method that allowed visualization of bacteria and, as a result, no information was available regarding bacterial abundances.

Larvae did not appear to feed directly on sediments, but rather managed to be water-column feeders and browsers of sediment and plant surfaces even while dwelling in a detritus enriched environment. Bacteria are more readily digested and assimilated than detritus (Cammen 1980, Lopez et al. 1977, Hargrave 1970, Kofoed 1975) and therefore the energetics are more favorable for feeding on bacteria rather than on detritus. This feeding strategy is not uncommon among dipteran larvae. Many chironomidae live in depositional habitats; yet, feed on resources in the water column. They do this by constructing tubes in the sediments, and weaving silken nets that cover one end of the tubes. The nets trap particles as larvae undulate their bodies, thereby pumping water and food through the tube (Merritt and Cummins 1984). Algae may have contributed greatly to larval nutrition although the total number of algal cells in larval guts was low. Algae tended to be at least an order of magnitude larger than bacteria in one dimension. On the basis on biovolume, algal volume in guts would be very similar to total bacterial volume.

Larvae appeared to have two principal mechanisms of feeding: suspension feeding from the water column, and browsing from sediment and

plant surfaces. Although Cq. perturbans larvae live in the benthos of ponds, their feeding behaviors seemed to be more similar to other water-column dwelling mosquitoes, than to other benthic-substrate dwelling organisms. The ratio of bacteria to detritus was significantly greater in larval guts than in the surrounding sediments. This indicated that larvae were not simply ingesting sediments, and supported the behavioral observations that larvae also feed by browsing on sediment and plant surfaces, and by filtering from the water column.

The results of observations of the different feeding modes used by Cq. perturbans is supported by SEM micrographs of their lateral palatal brushes (LPB) bearing microtrichia. Several authors have found that microtrichia on the rays of the lateral palatal brushes or mandibular fans coincide with a browsing and a suspension feeding mode (Merritt and Craig 1987, Aly 1983, Harbach 1977). Merritt and Craig (1987) found that the larvae of Aedes triseriatus, species that browses substrates, had lateral palatal brushes with long microtrichia (5 um height X 1 um basal width). Microtrichia on Cq. perturbans were much smaller than those found on Ae. triseriatus, indicating that while Cq. perturbans may employ browsing, its primary means of food acquisition was suspension feeding. Alternatively, the size of the microtrichia could be related to the hardness of the substrate being browsed, with harder substrates requiring larger, stiffer microtrichia. Aedes triseriatus larvae often browse from very hard substrates (i.e., bark and leaves in treeholes); whereas, Cq. perturbans larvae primarily browse the sediment surface and the surface of soft aquatic plant roots and stems.

This study illustrates the importance of examining the natural diet and feeding behavior of mosquito larvae. It is clear from this study that Bacillus thuringiensis var. israelensis and Bacillus sphaericus are not excluded from the

larval gut owing to some aspect of larval feeding behavior. If a proper formulation of B.t.i. were used that took advantage of the tendency of these larvae to both filter and to browse from plant and sediment surfaces, larval control using B.t.i. may be possible. Because of their unique mode of respiration, traditional methods of larval control are not possible for Cq. perturbans, making the development of effective microbial pesticides even more imparative. The complexity of the habitat in which Cq. perturbans larvae live (see Olds and Merritt 1988a, Batzer and Sjogren 1987) may be responsible for the failure of B.t.i. to control Cq. perturbans (Walker 1987). These factors will always pose a challenge to effective larval control regardless of control agent. With a thorough understanding of the larval biology of this species, control measures that rely on less toxic, more specific larvicides should be possible.

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