SAMPLING, SEASONAL ABUNDANCE, AND MERMITHID PARASITISM OF LARVAL *COQUILLETTIDIA PERTURBANS* IN SOUTH-CENTRAL MICHIGAN

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ABSTRACT. Seasonal abundance of immature *Coquillettidia perturbans* was studied at 3 sites in south-central Michigan in 1987 and 1988. A modified boat bilge pump and funnel separators proved to be good sampling and sorting devices. Pupae were found in late May to early July, first and second instars were most abundant in July and August, while third and fourth instars were present year-round. Overall, larvae were most numerous in August and September, indicating that *Cq. perturbans* has a single generation per year in Michigan, with midsummer to fall as the main period of larval development, and with third and fourth larval instars as overwintering stages. Parasitism of *Cq. perturbans* larvae by a previously undiscovered mermithid nematode was documented at 2 sites in late summer.

INTRODUCTION

Larvae and pupae of mosquitoes belonging to the genera *Coquillettidia* and *Mansonia* obtain oxygen from roots and stems of emergent aquatic vegetation, and consequently remain submerged for most of their lives (Smith 1908, McNeel 1932, Laurence 1960, McDonald and Lu 1973). This aspect of the ecology of these mosquitoes presents barriers for sampling and evaluation of control methods. *Coquillettidia perturbans* (Walker), a widely distributed mosquito in the United States and Canada (Darsie and Ward 1981), is a common biting pest in Michigan, both in inland and coastal areas. A recent report suggested that *Cq. perturbans* has a role as epizootic vector of Eastern equine encephalomyelitis virus in the state (Francy 1982). There is currently little information available on biology of the immature stages of this mosquito in Michigan; thus, control measures aimed at larvae are poorly developed.

The objectives of this study were to: 1) evaluate the efficiency of bilge pump sampling and cylinder sorting methods for *Cq. perturbans* larvae associated with cattail roots; 2) examine the seasonal occurrence, abundance and distribution of *Cq. perturbans* larvae at sites in south-central Michigan over a 2-year period and 3) document and describe the seasonal incidence of mermithid nematode parasitism of *Cq. perturbans* larvae.

MATERIALS AND METHODS

Study Sites: Three study sites with cattails (*Typhus latifolia, T. angustifolia,* or mixed stands of both with hybrids) were used during the study period (March 1987 to December 1988). Drumheller (site 1, Clinton County) was a 0.20 ha marsh with cattails dispersed in 4 main stands. Gibbs Pond (site 2, Clinton County) was 0.44 ha in area. Approximately 75% of the 0.38 ha of cattails at this site were dead for an unknown reason, and sampling was confined to live cattail stands. In 1988, there were no living cattails in this pond. Maple River flooding (site 3, Gratiot County) was a 0.10 ha section of a large marsh forming part of the Maple River State Game Lands. This site was added in the summer of 1988 when it became apparent that the 2 sites used in 1987 no longer supported populations of *Cq. perturbans* larvae.

Collecting and Sorting Larvae: A hand-operated, plastic, boat bilge pump ("Thirsty Mate" no. 136 PR, Beckson Marine Inc., Bridgeport, CT), modified to function as a syringe by removing the distal one-way valve, was used to collect larvae from the base of cattail stems (Walker and Crans 1986). The sampled water/sediment mixture was transferred to a floating basin, and then poured into 4-liter jugs for transporting to the lab. Samples were poured in small amounts through a series of Nalgene sieves of decreasing mesh size (4, 1 and 0.25 mm mesh). The 0.25 mm sieve was sufficiently fine to insure retention of all instars (Nemjo and Slaff 1984), which were washed with water into another container and sorted. Plant debris caught on the filters was rinsed thoroughly to remove any entangled larvae.

Two experiments were performed at site 2 during the summer of 1987 to determine how many suctions were required to remove the majority of attached larvae from a stem. In the first experiment, 6 sequential suctions were collected from each of 3 randomly chosen cattail stems. Each of the 6 suctions from each stem were held separately to determine the average number of larvae per sequential suction. In the second experiment, 10 sequential suctions were taken

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from each of 3 stems, and samples from suctions 1 through 4 were consolidated into one sample, as were samples from suctions 5 and 6. Samples from both experiments were hand-sorted within 2 h of collection.

Hand-sorting larvae was very time consuming and laborious; therefore, we determined experimentally the efficiency of funnel separators [polyvinyl chloride cylinders with inverted funnels inside of them; see description and figure in Morris et al. (1985) in retrieving larvae from the water/sediment mixture]. A sample was poured into a cylinder, and tapwater was added to a level at least 2.5 cm above the tip of the inverted funnel at the top of the cylinder. Any debris blocking the tip of the funnel was removed. Filled cylinders were kept dark by covering the tops with aluminum foil. Larvae rose through the inverted funnel to the surface for air, became trapped in the clear water at the top of the cylinder, and could be collected and counted. Two replicate experiments testing the separating efficiency of the cylinders were conducted. For both experiments, 5 cattail stems were sampled by the pump method described above at site 2, with 4 suctions taken at each stem. Eleven cylinders were held for 16 h at 5°C, because we noticed that larvae often died within 24 h if kept in the cylinders at room temperature. After 16 hours, the water above the funnel was drawn off and replaced by fresh tapwater. The separators were then moved to room temperature (21°C). After 4 h, water above the funnel was again drawn off. The total number of larvae per water sample for each time period was counted. The separators were emptied and the number of larvae remaining in the bottom of the cylinder (representing the nonseparated larvae) were sorted and counted.

Seasonal Distribution: Sites were measured and mapped using a line transect and compass technique. Ten permanent stations were located at each site. At sites 1 and 3, where cattail stands were dispersed throughout the ponds, sampling stations were uniformly spaced at approximately 6-m intervals throughout each stand. If cattails ringed the entire perimeter of a marsh (e.g., site 2), sampling stations were spaced at approximately 30-m intervals around the entire edge.

Random sampling was accomplished using the modified point-quarter method of Mueller-Dombois and Ellenberg (1974). A section of marsh around each station was divided into 4 quadrants (northeast, southeast, northwest and southwest of each station marker) using a compass. Samples were taken weekly, biweekly or monthly depending on the season. On each sampling date, one quadrant was chosen beginning with the northeast quadrant and moving counterclockwise with each succeeding sampling period. A sample consisted of taking 4 suctions around the base of a single cattail stem. Five to 10 samples from the marsh were taken on each sampling date. Initially, the closest cattail to the station marker was sampled in the chosen quadrant so that, after 4 sampling dates, one cattail stem in each quadrant of each station had been sampled. Cattails were marked with flagging after being sampled to exclude them from resampling. Once a cattail in each quadrant was sampled, the sampling cycle was reinitiated beginning with the second closest stem in the northeast quadrant of each station.

Samples were taken every 2 weeks at site 1 from March 25 through June 30, 1987. By the beginning of July, this pond was almost dry and could no longer be sampled. Sampling at site 2 began June 25, 1987, and weekly samples were taken from June through September and biweekly samples from October to December. No samples were taken in January and February, 1988. Sampling was begun at site 2 again in March, 1988, but was discontinued after no larvae were found for 3 months (March to May). Periodic checks during the summer of 1988 showed that no larvae remained at this site. Sampling at site 3 began in May 27, 1988, and continued to November. Biweekly samples were taken in June, July and September, and one sample was taken in August and November.

At site 2 in 1987 the following measurements were taken at cattails sampled on each sampling date: 1) cattail stem diameter; 2) water depth; 3) location of the stem (whether in a clump of stems or isolated [greater than 0.3 m from any other stem]) and 4) proximity of the stem to open water. Cattail density was determined at site 2 in October 1987 by counting the number of cattails in ten 1-m^2 plots. Nature of larval distribution was examined by application of Taylor's Power Law (Southwood 1978), through regression of $\log_{10}$ (sample variance) on $\log_{10}$ (sample mean) for all collection dates in 1987.

Mermithid Parasitism: All larvae collected from site 2 in 1987 and site 3 in 1988 were examined for mermithid parasites using a dissecting scope. A record was kept of what appeared to be evidence of injury (black spirals under the cuticle of the larvae) caused either by the preparasites as they entered the larvae or by an encapsulation response by the mosquito, as well as obvious presence of a large worm in the body cavity.

In September 1988, 11 parasitized larvae collected from site 3 were held at 12°C in 500-ml plastic containers with water, a sand substrate and cattail roots for larval attachment. Larvae were checked every 2 days for parasite emergence. Upon emergence from the larvae, the postparasitic juvenile nematodes were held in

containers with sand and water (20°C) to see if they would molt to adults.

RESULTS

Sampling and Sorting: Results of the experiments testing the suction sampling method are shown in Table 1. The total number of larvae sampled from the 3 cattail stems in experiments 1 and 2 was 95 and 123 larvae, respectively. Both experiments gave similar results with the greatest proportion of larvae being collected in the first 4 of 6 suctions (92% in the first experiment, 82% in the second experiment). In the second experiment, some larvae were collected in the seventh, eighth, ninth, and tenth suctions (Table 1); however, these were probably drawn from surrounding cattail stems.

Results of the 2 experiments testing efficiency of the cylinder sorting apparatus showed that 94% of the larvae were recovered in the water above the inverted funnel after 20 h in both experiments.

Seasonal Distribution: Results of field sampling are shown in Fig. 1. In 1987, 21 pupae and 1,022 larvae were collected (19 sampling days); and in 1988, 3 pupae and 487 larvae were collected (9 sampling days). In spring 1987, third and fourth instars were found at site 1 (Fig. 1A), indicating that both instars overwintered in central Michigan. Third and fourth instars were present at site 1 until June 23, when the marsh dried. Site 1 had an average of 11.4 larvae per cattail stem during the sampling period (April to June). Pupae were found at site 1 from May 28 to June 23.

Larval numbers at site 2 peaked in mid-August at 42.4 larvae per stem and fell to 0.2 larvae per stem by December. Pupae were present on July 14 and 21; first instars were present from July 14 to August 25; second instars from July 14 to September 23, and third instars from July 14 to November 20. Fourth instars were present throughout the sampling period, but no larvae were found in the spring of 1988 at site 2 (sampling data not shown).

The pattern of larval development in 1988 at site 3 was similar to the pattern in 1987 at sites 1 and 2 (Fig. 1B). The drought did not affect water levels at site 3 because they were artificially maintained. Third and fourth instars were present, but in very low numbers in the spring samples; and 3 pupae were collected only on May 26, June 24 and June 28. First instars were present from June 24 to August 12, and second instars were present from June 25 through September 14. Third instars were most abundant in August and September, and fourth instars were most abundant in September to November. Larval numbers peaked in mid-September at 7.7 larvae per cattail stem.

Habitat Variables, Larval Abundance and Distribution: Habitat variables only at site 2 (1987) were related to larval numbers with correlation analysis or Mann-Whitney tests (Steel and Torrie 1980), where sampling stations were the observations for each variable. There was no correlation between larval numbers and stem diameter ($r = 0.20$, $n = 64$, $P > 0.05$) or water depth ($r = 0.17$, $n = 64$, $P > 0.05$), although the stations with the deepest water appeared to have the greatest number of larvae. There was no relationship between larval numbers and proximity of a stem to open water (Mann-Whitney test, $t = 1.36$, critical $t = 1.96$, $P > 0.05$), or whether a stem was isolated or in a clump (Mann-Whitney test, $t = 0.95$, critical $t = 1.96$, $P > 0.05$). Results of application of Taylor's Power Law (Fig. 2) to sampling data at site 2 indicated that the larvae and pupae were contagious in distribution, as the slope to the regression line (1.622) was greater than one.

Parasites: One thousand twenty-two larvae were examined for mermithid nematode parasites in 1987 (sites 1 and 2), and 487 larvae in 1988 (site 3). Sixty-two larvae were parasitized and 153 exhibited evidence of parasite entry in 1987 (site 2), and 19 larvae were parasitized, and 8 exhibited evidence of entry in 1988 (site 3). A higher percentage of larvae were parasitized in 1987 than in 1988. Mermithids were first observed in fourth larval instars from site 2 on July 14, 1987, and were found in third instars beginning on July 28. Parasites were never observed in first instars and were very rarely found in second instars.

Cumulative percentages of third and fourth larval instars with parasites or evidence of entry (i.e., injured) are shown in Fig. 3. These percentages were calculated as the cumulative number parasitized or injured up to sampling day t

Table 1. The total number and proportion of total *Coquillettidia perturbans* larvae collected in suction samples taken sequentially from the bases of 3 cattail stems in 2 experiments. In experiment 1, all 6 samples were sorted separately. In experiment 2, the first 4 samples were combined for sorting, the 5th and 6th were also pooled, but the 7th through 10th were sorted separately.

Experiment 1			Experiment 2		
Sample no.	Total	Proportion	Sample no.	Total	Proportion
1	36	0.38	1–4	79	0.64
2	29	0.30	5–6	17	0.14
3	20	0.21	7	1	0.01
4	2	0.02	8	8	0.03
5	2	0.02	9	14	0.07
6	6	0.06	10	4	0.03

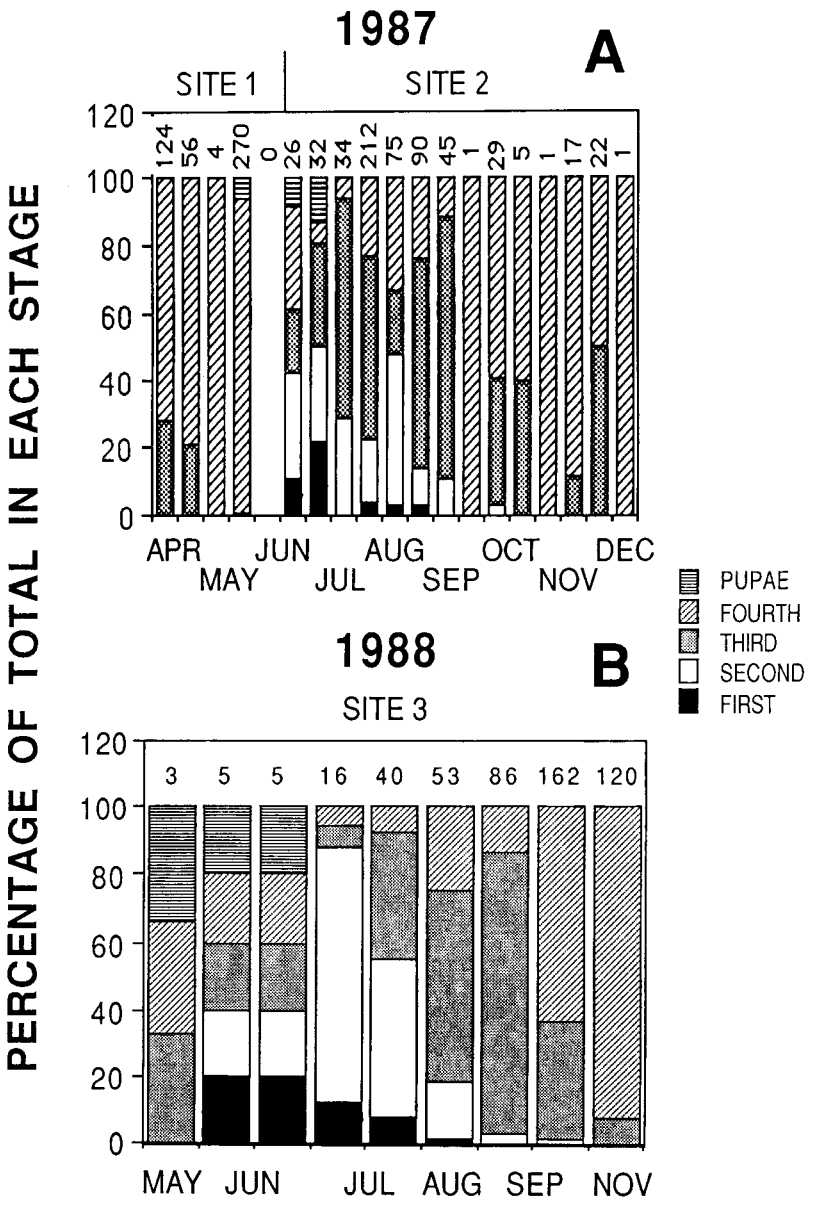

Fig. 1. Seasonal distribution of *Coquillettidia perturbans* larvae and pupae in south-central Michigan in 1987 and 1988. Data are presented as the percentage of total number of life stages at 3 sites during 1987 (A, sites 1 and 2) and 1988 (B, site 3). Numbers above each bar are the total number of individuals sampled on each date.

divided by the total number of larvae of that instar collected during the development of the summer cohort (i.e., excluding larvae which were collected in spring). Parasitism occurred mainly during the period from mid-July to mid-September. No parasitized or injured larvae were found in the overwintered cohorts. Fourth instars had higher rates of parasitism than third instars, while third instars had higher rates of injury. Peak rates of parasitism in fourth instars at site 2 were 64% (of total collected on that day) on August 18, 1987, and 67% on September 3, 1988, at site 3. In 1987 at site 2, no parasitized or injured larvae were found after October 8th, while at site 3 in 1988 one parasitized larva was found on November 3.

Parasites emerged from 5 of 11 larvae held in the laboratory; the remaining 6 larvae died. All larvae from which parasites emerged died. Observations on one larva during parasite emer-

gence revealed that the nematode emerged from the anal segment (abdominal segment 10), and not from the thorax as has been described for other mermithids in mosquitoes (Petersen 1985). Emergence occured in 1 min from when nematode movement was first noted inside the larval body. Larvae from which parasites had emerged were examined for damage to the cuticle in either the thorax or abdomen; but no emergence holes were found, suggesting that the point of emergence was, indeed, the anal segment.

Five postparasitic juveniles were observed for possible development to adulthood. In 2 cases, morphological changes were evident through the cuticle of the nematodes, however, no molts to adult nematodes occurred.

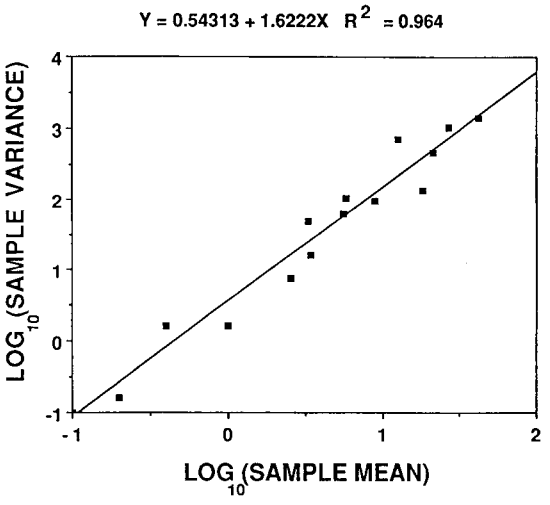

Fig. 2. Application of Taylor's Power Law to field sampling data of *Coquillettidia perturbans* from 15 dates at site 2 in 1987. Raw data were first transformed by adding one to each datum, and then means and variances were calculated.

DISCUSSION

The bilge pump was a good sampling device in the shallow cattail marshes we used as study sites. Our sampling experiments showed that four sequential suctions with the pump adequately removed most larvae from around the root zone of cattails (Fig. 1). The seasonal population data we collected (Fig. 2) indicated that the pump can be used to reveal changes in population structure of *Cq. perturbans* larvae. Both larvae and pupae were sampled with the pump, but the relative efficiency for sampling different larval instars and pupae is not known.

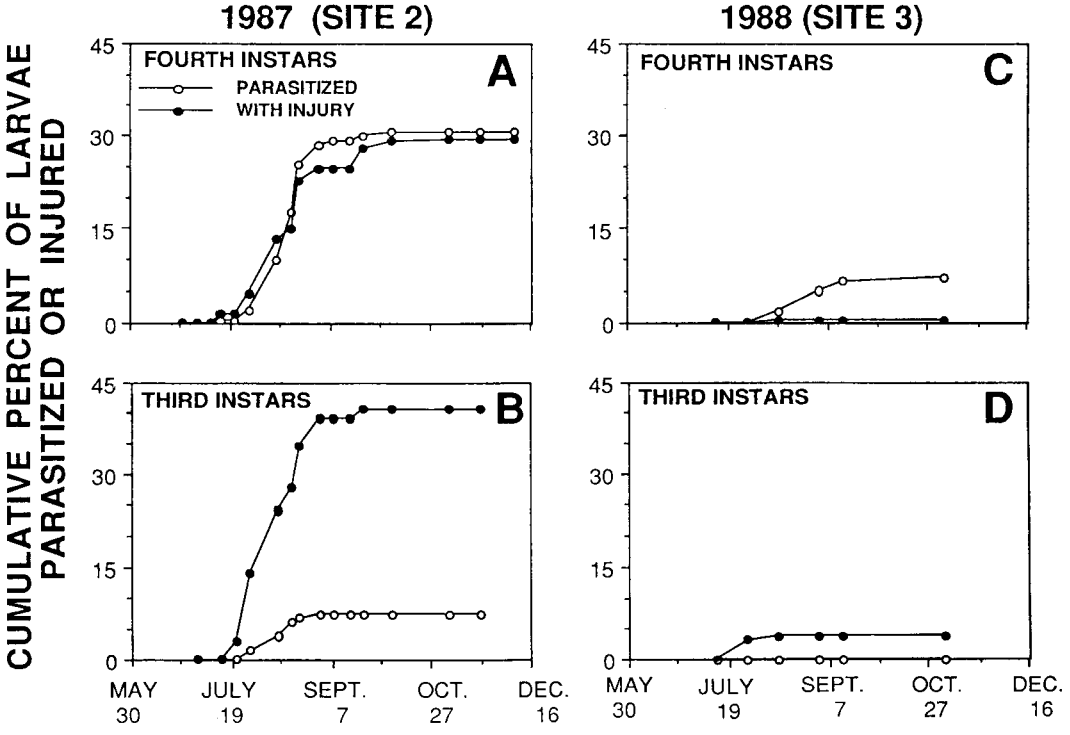

Fig. 3. The cumulative percent of third and fourth instar *Coquillettidia perturbans* either parasitized or exhibiting evidence of entry by mermithid nematodes in south-central Michigan in 1987 (A and B, site 2) and 1988 (C and D, site 3).

Cylinder sorting (Morris et al. 1985) was an efficient method of retrieving larvae from samples, and provided a good alternative to handsorting.

We documented that *Cq. perturbans* larvae were contagiously distributed at site 2 (Fig. 3); however, there was no significant relationship between several measured habitat variables and larval abundance. Site 2 changed greatly through the course of the study because of drought, so that habitat variables such as water depth, cattail stem diameter and stem condition were affected. A study in Florida (Callahan and Morris 1987) documented correlations between several physicochemical and vegetational variables and abundance of *Cq. perturbans*; however, water depth was not an indicator variable in that study.

Cq. perturbans was univoltine, with a distinct larval cohort developing from midsummer to fall. These findings agree with previous studies of *Cq. perturbans* in temperate regions (Brower 1953, Hagmann 1953, Carpenter and LaCasse 1955, Lewis and Bennett 1980, Allan et al. 1981). Collections of third and fourth instars in spring shows that both of these stages overwintered, while first and second instars did not. Pupae were present for several weeks in May to July, corresponding to the time of adult emergence recorded in studies in southern Canada (Lewis and Bennett 1980, Allan et al. 1981). First and second instars were present for a longer period of time in the summer, both earlier and later in the season, than reported by Smith (1908) and Hagmann (1953) in New Jersey. This difference may reflect extended periods of oviposition activity through the season in Michigan, or could be related to differences in sampling methods and sampling intervals among studies.

We document here, for the first time, mermithid parasitism of field populations of *Cq. perturbans*. Parasitism was only observed during the period of larval development in summer and fall, prior to overwintering. The fact that no parasitized larvae were collected after overwintering in the spring indicates that fourth larval instars are the final host stage of the parasite, so that the parasite does not persist into the adult stage. This interpretation is supported by our laboratory observations showing that parasites emerged from fourth larval instars. How the mermithids disperse among larval sites, given that adult mosquitoes are not infected, is not known and remains to be investigated.

Mermithid parasitism of *Cq. perturbans* may influence voltinism in this mosquito. In years of warm summer and fall temperatures, *Cq. perturbans* [or the European ecological homolog *Coquillettidia richiardii* (Ficalbi)] could conceivably produce a second generation of adults (Allan et al. 1981, Goshenko 1978). In this study, summer temperatures were abnormally high; yet, no late summer, second generation of pupae was observed. Selection against rapidly developing larvae owing to mermithid parasitism may limit the potential for a second generation in temperate regions. Larvae that develop slowly, or hatch from eggs laid late in the season, may have higher success through avoidance of parasitism than quickly developing larvae that hatched from eggs laid in midseason.

Our study indicated several different mortality factors acting upon *Cq. perturbans* larval populations. Values of cumulative percentage parasitism of third and fourth instars indicated that mermithids were a major source of larval mortality. Assuming that all parasitized fourth larval instars die, and that the parasite persists from earlier instars to the fourth instar, then about 40% of the larval cohort in 1987 at site 2 were killed by mermithids. At site 3 in 1988, this figure was considerably lower (<10%). The drought conditions in Michigan during the time of the study emphasized the importance of catastrophe to *Cq. perturbans*. We observed that one site dried up in spring before the overwintering larval cohort pupated and emerged as adults. At another site, the drought substantially reduced the habitable portion of the marsh, and most of the cattails at this site died during the course of the study. There was overwintering mortality at this site as well, as larvae were abundant in late summer and fall but none were collected in spring. Other studies indicated that severe winters are generally followed by summers with reduced adult *Cq. perturbans* populations (Capotosto and Boyes 1985, Walker 1985). Future quantitative population models of *Cq. perturbans* should include mortality due to drought, overwintering, and parasitism as key factors.

ACKNOWLEDGMENTS

We thank Bill Morgan, Del Richburg and Julie Greminger for help with sampling and sorting. This study was supported by a National Science Foundation predoctoral fellowship awarded to Emily J. Olds, and by National Institutes of Health grant AI-21884 awarded to Richard W. Merritt.

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