NATURAL FOOD AND FEEDING BEHAVIOR OF *COQUILLETTIDIA PERTURBANS* LARVAE

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ABSTRACT. The natural particulate food of larval *Coquillettidia perturbans* was studied through gut analysis using staining with 4′6-diamidino-2-phenylindole and epifluorescence microscopy. Bacteria (cocci, rods, spirochetes, purple bacteria and cyanobacteria), detritus, euglenoid protozoans and algae (desmids and diatoms) comprised the majority of particulate food, in order of abundance; other protozoans were rare, and hyphal forms (actinomycetes and fungi) were not observed. Abundance of food items in guts did not vary greatly among sampling months (June–December, 1987) or sampling sites. Fourth instars had a greater proportion of euglenoids and algae in their food than did younger instars. Observation of larval feeding behavior showed that fourth instars oriented upside down and fed mainly by suspension feeding, with occasional brushing from sediments and root surfaces. Microtrichia on filaments of the lateral palatal brushes of fourth instars indicated that the larvae were adapted to collect fine particles.

INTRODUCTION

Coquillettidia perturbans (Walker) is an important biting pest in many areas of the United States, and it has been implicated as a vector of Eastern equine encephalomyelitis virus (Boromisa et al. 1987). The environmentally sensitive freshwater marshes forming the habitat of *Cq. perturbans* make microbial control agents such as *Bacillus thuringiensis* var. *israelensis* serotype H-14 (*B.t.i.*) attractive; however, *B.t.i.* has failed to control *Cq. perturbans* in the field (Sjogren et al. 1986, Walker 1987), possibly owing to some aspect of its feeding ecology.

Larvae of *Coquillettidia perturbans* respire by inserting a modified siphon into the aerenchymous tissue of submerged parts of rooted or floating freshwater macrophytes (Smith 1908, McNeel 1932). The microhabitat of *Cq. perturbans* larvae is the benthic zone of eutrophic marshes (Smith 1908, Batzer and Sjogren 1986), and the "feeding zone" of these larvae appears to be a highly organic, depositional environment amongst tangled plant roots. Although little information is known of the feeding ecology of *Cq. perturbans* larvae in this zone, its Eurasian congener, *Coquillettidia richiardii* (Ficalbi), has been investigated in detail. Guille (1976) suggested that the organic, flocculent material on pond bottoms serves as a medium for bacterial colonies upon which *Cq. richiardii* larvae feed. Goshenko (1985) observed that feeding varied among instars of *Cq. richiardii*, where first instars were periphytophagous on substrates while second, third and fourth instars were mainly filter-feeders. However, some feeding from the substrate was observed in later instars as well. Goshenko (1985) suggested that larvae changed their point of attachment in response to food shortages in the water column, and he felt that substrate browsing by third and fourth instars occurred primarily under these conditions. Analysis of the gut contents of *Cq. richiardii* larvae revealed periphyton and benthic algae.

This paper describes the diet of larval *Cq. perturbans* in their natural environment and discusses the behavior and mechanisms employed by the larvae to obtain food.

MATERIALS AND METHODS

Larval sampling: Larvae were collected using the modified bilge-pump method of Walker and Crans (1986) at 3 sites (designated sites 1, 2 and 3) in Ingham and Clinton County, Michigan, from May to December, 1987, and at one site (site 4) in Gratiot County, Michigan, in June 1988. These sites were cattail (*Typha* spp.) marshes surrounded by deciduous forest; detailed descriptions are in Olds et al. (1989). Owing to drought, sites 1 and 2 had to be abandoned at the end of June 1987 except for one sample in late July at site 2. Samples were transported to the laboratory in 20-liter buckets, and larvae were sorted by hand within 2 h of sampling. The elapsed time between sampling and sorting probably did not affect gut contents because behavioral observations (see below) showed that larvae generally did not feed when not attached to roots. Samples were 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.

Gut analysis procedures: Dissections were performed using a modification of the technique described by Cummins (1973). Guts were dissected out, and the peritrophic membrane with enclosed food bolus was removed and washed several times in distilled water. The food bolus was teased from the peritrophic membrane with a minuten pin into a drop of water on a glass

slide. The material was transferred by pipette to a shell vial, and water was added to a volume of 2.0 ml. The vial was immersed in an ultrasonic cleaner for 15 sec to break up large aggregates of material. This brief exposure was to avoid any cell disruption due to sonification. The contents were stained with DAPI (4'6 diamidino-2-phenylindole), and slides were prepared following procedures in Walker et al. (1988). Acid-washed glassware and distilled, filter-sterilized (0.22 μm) water were used in all procedures to minimize contamination.

Counting of particles comprising the food bolus was performed using a Leitz Laborlux 11 epifluorescence microscope with appropriate excitation and barrier filters for DAPI applications (Walker et al. 1988). At least 15 fields containing bacteria and detritus (particles in the range of 1–50 μm) were counted, following the guidelines for statistical reliability given in Kirchman et al. (1982). If fewer than 200 bacteria or detrital particles were counted in the initial 15 fields, additional fields were counted until at least 200 particles had been counted per slide. Counts for algae and cyanobacteria were performed by transecting the widest diameter of the filter 5 times (equal to 4.5% of the total filtered area). When cyanobacteria were particularly abundant, they were enumerated by field counts instead of across transects. Field and transect counts were converted to total counts using a standard formula (APHA 1980). Algae, protozoans and bacteria were identified using Ward and Whipple (1959), Lee et al. (1985), and Staley et al. (1989). Detritus, defined as fine particulate organic matter (< 1 mm) of unknown origin (Wallace and Merritt 1980) was identified on the basis of morphology and autofluorescent properties.

Seasonal, site and instar variation: Seasonal variation in the natural diet of larval *Cq. perturbans* was examined using fourth instars collected from site 3 from June 25 through December 2, 1987. Forty-two larval guts were examined for bacteria and detritus, and 15 guts were examined for algae. In June and December, larval numbers were too low ($n < 3$) to provide an acceptable sample size. As a result, larvae sampled in June and December were included in the July and November data, respectively. To document variation of larval food among sampling sites, we compared gut contents of larvae collected at different sites on 2 occasions: 1) fourth larval instars sampled from sites 1 and 2 during the spring of 1987; and 2) third larval instars collected on July 28 and 30, 1987, from sites 2 and 3. Algae and euglenoids were not enumerated in gut contents of larvae from these trials. Data from counts were transformed [$\log_{10}(\bar{x} + 1)$] to normalize the data and reduce heterogeneity of variances (Steel and Torrie 1980). Analysis of variance (ANOVA) and Tukey's procedure were used to determine which means were significantly different among sampling dates (Steel and Torrie 1980).

All instars were collected from site 3 on July 28, 1987, allowing a comparison of gut contents among instars collected from a single site on a single date. Counts were made of the number of each food type per gut; these counts were converted into percentages of the total number of particles to compensate among instars with differing gut volumes. The proportions of each food-type per gut were normalized using an arcsin-square root transformation of the proportions (Steel and Torrie 1980) and compared among instars with ANOVA and Tukey's procedure.

Larval behavior: Qualitative observations of the behavior of fourth instar *Cq. perturbans* were conducted in a plexiglass chamber (6.7 × 6.7 × 0.7 cm). A 2-cm layer of pond mud was placed in the bottom, and 3 small cattail roots (ca. 1 mm diam) were placed in the chamber with one end immersed in the mud and the other end secured to the top of the chamber. Tap water was added to within 0.5 cm from the top of the chamber.

For each observation period, 4 larvae were added to a chamber which was placed inside a 16°C water bath. The water bath was placed inside a dark hood, and red light was provided (fiber optics illuminator with the tips covered with red plastic) to simulate the subdued light conditions at the bottom of a marsh (Wetzel 1983). Larvae were allowed to acclimate for 30 min, and observations were made using a dissecting scope (60× and 120×) mounted horizontally on a boom. Larvae were observed for 2 h per session; and larval movements, location, orientation and feeding behaviors were recorded. Carmine red, powdered charcoal and yeast extract were added on separate occasions to determine if filter-feeding could be enhanced (Dadd 1970).

Morphology of mouthparts: Heads ($n = 7$) and lateral palatal brushes were examined with scanning electron microscopy (JEOL JSM-35 operated at 15 kV). Larvae were collected, placed in Carnoy's solution, and transferred to 3.5% gluteraldehyde. The heads were then removed, washed in phosphate buffer, dehydrated in a graded series of ethanol and critical-point dried, mounted on stubs and sputter-coated with gold.

RESULTS

Particulate food: Four categories of particles were found to comprise the food bolus of *Cq. perturbans* larvae: bacteria, detritus, euglenoid protozoans (colored, flagellated unicells) and algae. Color micrographs of these particles, taken from guts of *Cq. perturbans, Aedes triseriatus*

(Say) and *Anopheles quadrimaculatus* Say larvae and treated with DAPI gut analysis procedures, are shown in Walker et al. (1988). No hyphal forms, either actinomycetes or fungi, were observed. Protozoans other than euglenoids were very rare (being found in only a few larvae at low numbers) and were not considered further. Five types of bacteria were identified on the basis of morphology and appearance: cocci, rods, purple bacteria, spirochetes and cyanobacteria. The 2 types of algae encountered were desmids and diatoms.

Seasonal variation in fourth larval instars: In fourth larval instars, all 4 food types were present and the abundance of each was similar throughout the season. Bacteria were the most abundant food type; the mean number of bacterial cells per gut was 7.07×10^5 ($n = 42$; range, 3.38×10^5 to 1.75×10^6). Detritus was the second most abundant food type; the mean number of detrital particles was 3.7×10^4 ($n = 42$; range, 0–1.58×10^5). Euglenoid protozoans were the third most abundant food type; the mean number of euglenoids was 111 ($n = 15$; range, 22–270). Algae were slightly less abundant than euglenoids; the mean number of algae was 10^4($n = 15$; range, 0–800).

Cocci were the most abundant and least variable type of bacteria counted in fourth instar guts, spirochetes and rods were about equal in abundance, purple bacteria were less abundant and cyanobacteria were the least abundant (Table 1). Numbers of rods varied significantly (ANOVA, $P < 0.05$) among the collection months, whereas numbers of cocci, purple bacteria, spirochetes, cyanobacteria, detrital particles, euglenoid protozoans, diatoms and desmids did not (ANOVA, $P > 0.05$; Table 1)

Site variation: Cocci were the most abundant type of bacteria in fourth larval instar guts at sites 1 and 2; rods, spirochetes and purple bacteria were approximately equal in abundance; while cyanobacteria were least abundant (Table 2). Larvae from site 1 had significantly (ANOVA, $P < 0.05$) greater numbers of purple bacteria and significantly fewer numbers of rods in their guts than did larvae from site 2; however, there were no significant differences (ANOVA, $P > 0.05$) in numbers of cocci, spirochetes or cyanobacteria (Table 2). Comparison of third larval instars collected from sites 2 and 3 in July 1987 showed that larvae from site 3 had significantly greater numbers of rods and significantly fewer numbers of cyanobacteria than did those at site 2 (ANOVA, $P < 0.05$); however, there were no significant differences (ANOVA, $P > 0.05$) in numbers of total bacteria, detrital particles, cocci, purple bacteria or spirochetes (Table 2).

Instar variation: There were no significant differences among instars in percentage composition of cocci, rods, purple bacteria or spirochetes in guts (Table 3; ANOVA, $P > 0.05$). Fourth larval instars tended to have a greater percentage of cyanobacteria than younger instars (Table 3), but this observed difference was not statistically significant (ANOVA, $P > 0.05$). However, fourth instars had a significantly (ANOVA and Tukey's procedure, $P < 0.05$) greater percentage of euglenoid protozoans, diatoms, and desmids in their guts than did the younger instars (Table 3).

Larval behavior: Behavior of *Cq. perturbans* larvae in the observation arena was divided into time spent unattached or attached to a root. Larvae tended to spend the majority of time attached to a root or roothair. They generally attached with dorsal surface oriented down, so that the mouth was pointed toward the water surface (Fig. 1). Attached larvae occasionally bent toward the cattail root and appeared to browse around the point of attachment. Attached larvae were not covered by sediment, but sometimes had the posterior half of the body in the sediment and the head and thorax in free water. On several occasions, larvae excavated a

Table 1. The transformed mean number (SEM) of each food type per *Coquillettidia perturbans* fourth larval instar gut on a monthly basis (1987).*

Month	Cocci	Rods	Purple bacteria	Spirochetes	Detritus	*n*	Cyanobacteria	Euglenoids	Diatoms	Desmids	*n*
June/July	5.7	5.2a	4.0	5.1	4.6	10	2.9	2.2	0.5	0.9	3
	(0.08)	(0.12)	(0.67)	(0.20)	(0.09)		(0.18)	(0.14)	(0.46)	(0.46)	
Aug.	5.5	4.6b	4.2	4.7	4.0	12	2.4	1.9	1.6	1.1	3
	(0.06)	(0.07)	(0.41)	(0.38)	(0.38)		(0.17)	(0.04)	(0.14)	(0.60)	
Sept.	5.6	4.3b	3.5	4.8	4.7	7	1.4	2.2	1.3	1.3	3
	(0.05)	(0.12)	(0.66)	(0.11)	(0.21)		(0.72)	(0.09)	(0.75)	(0.75)	
Oct.	5.5	4.7a,b	4.6	5.2	4.2	4	2.1	1.7	0.5	1.5	3
	(0.03)	(0.06)	(0.17)	(0.15)	(0.15)		(0.13)	(0.15)	(0.46)	(0.15)	
Nov./Dec.	5.7	4.5b	2.0	4.7	4.1	9	2.7	1.8	0.0	1.0	3
	(0.05)	(0.12)	(0.61)	(0.14)	(0.52)		(0.33)	(0.21)	(0.0)	(0.51)	

* Data are presented as means of $\log_{10}$ (x+1) transformed data. Column means followed by a different letter are significantly different (ANOVA and Tukey's procedure, $P < 0.05$), while no letter indicates no significant differences between means within a column. *n* indicates the number of dissected individuals used for the counts of food items in the different groups.

Table 2. Comparison of numbers of bacteria and algae in guts of *Coquillettidia perturbans* fourth larval instars and third larval instars, collected at different sites in south-central Michigan, 1987. Data are means of $\log_{10}$ (x+1) transformed data with SEM in parentheses.*

Fourth larval instars						
Sites	*n*	Cocci	Rods	Purple bacteria	Spirochetes	Cyanobacteria
1	11	5.8	4.5a	5.1a	5.0	3.8
		(0.05)	(0.08)	(0.11)	(0.15)	(0.39)
2	9	5.7	4.8b	4.7b	4.9	4.3
		(0.05)	(0.07)	(0.15)	(0.15)	(0.07)
Third larval instars						
Sites	*n*	Cocci	Rods	Purple bacteria	Spirochetes	Cyanobacteria
2	6	4.9	3.5a	3.5	2.9	2.8a
		(0.06)	(0.17)	(0.72)	(0.91)	(0.58)
3	4	5.2	4.3b	2.3	3.9	0.6b
		(0.18)	(0.20)	(0.95)	(0.21)	(0.62)

* Column means followed by a different letter are significantly different (ANOVA and Tukey's procedure, $P < 0.05$), while no letter after a mean indicates no significant differences between means in a column.

Table 3. The back-transformed mean percentage (SEM) of each food type per *Coquillettidia perturbans* larval gut by instar, collected on July 28, 1987, in Michigan.*

Instar	*n*	Cocci	Rods	Purple bacteria	Spirochetes	Detritus	*n*	Cyanobacteria	Euglenoids	Diatoms	Desmids
1	5	59.2	15.4	5.2	9.3	19.9	3	1.1	0.3a	0.0a	0.4a
		(4.9)	(2.5)	(3.3)	(1.9)	(6.0)		(0.57)	(0.33)	(0.0)	(0.39)
2	5	63.5	14.7	3.7	13.5	12.4	3	2.5	0.3a	0.3a,b	0.3a
		(1.8)	(2.4)	(2.3)	(1.7)	(3.7)		(0.31)	(0.28)	(0.28)	(0.26)
3	5	53.7	17.4	6.6	12.9	22.6	3	1.3	0.2a	0.2a,b	0.0a
		(2.8)	(2.3)	(3.6)	(3.3)	(4.8)		(1.26)	(0.18)	(0.18)	(0.0)
4	3	50.3	20.5	15.7	16.7	16.3	3	4.0	1.3b	1.1b	1.7b
		(4.5)	(5.9)	(1.6)	(7.4)	(3.6)		(1.64)	(0.19)	(0.23)	(0.13)

* Data are presented as back-transformed means from arcsin[square root(proportion)] transformed data. Column means followed by a different letter are significantly different (ANOVA and Tukey's procedure, $P < 0.05$), while no letter or the same letter following a mean indicates no significant differences between means within a column; *n* indicates the number of dissected individuals used for the counts of food items in the different groups.

funnel-shaped depression in the sediment by swimming around the area where the root entered the sediments. They then attached to the root in the bottom of the depression with the ventral surface oriented toward the water surface as previously described (Fig. 1).

Larvae that detached from a cattail root usually swam immediately to the bottom of the arena. Once detached, larvae swam vertically and horizontally through the water for an extended period of time (maximum, 10 min), with the dorsal surface of the body oriented toward the water surface. They also swam along the surface of the sediments with mouth brushes touching the actual sediment surface. Occasionally, the lateral palatal brushes beat while larvae were swimming in this fashion; but in general feeding was not observed while larvae were detached.

Larvae commonly dug into the sediments while not attached to a cattail root to a depth of no more than 1 cm; they accomplished this by swinging the posterior half of the body back and forth through the sediments. Larvae never stayed completely covered with sediments for more than 3 to 4 minutes. They seldom completely cover themselves with sediments, but left the head free in water. In this position, the larvae oriented the body parallel to the sediment surface with the dorsal surface directed down.

Attached larvae did not beat their lateral palatal brushes when clear tap water was provided in the observation chamber, nor when charcoal powder or carmine red were added. However, when a slurry of yeast was added, larvae beat the lateral palatal brushes for prolonged periods of time.

Scanning electron microscopy: Micrographs of

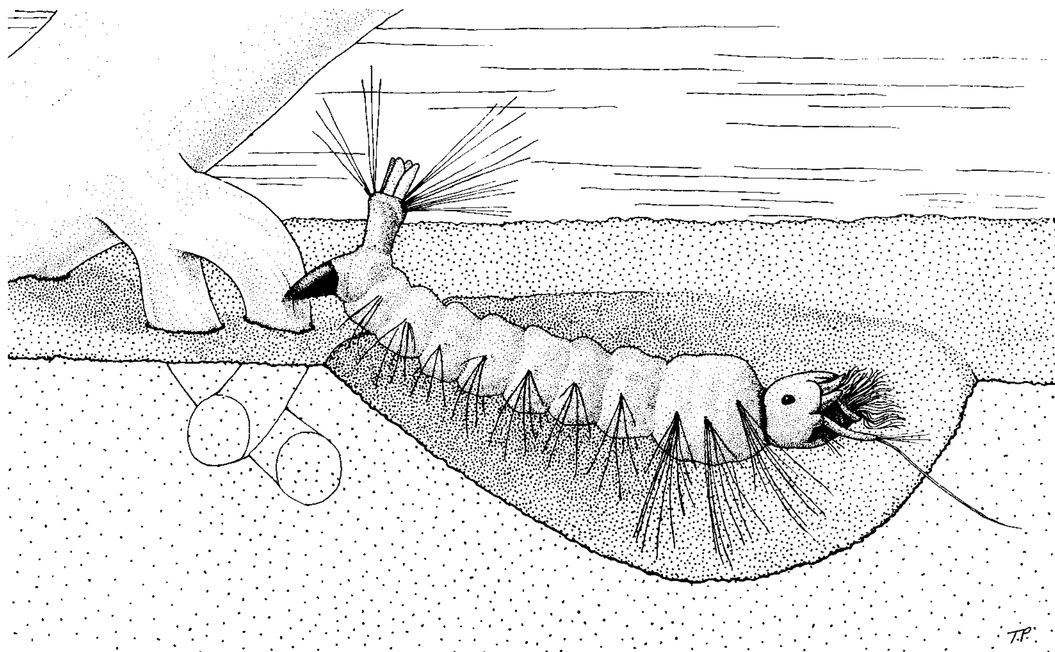

Fig. 1. Typical posture of fourth larval instar *Coquillettidia perturbans* in an observation chamber. Note the pit the larva has dug in the sediments, and the orientation of the larva with ventral side up.

the head and lateral palatal brushes of third and fourth instar *Cq. perturbans* showed many long filaments comprising the brush proper, and the presence of microtrichia on the filaments (Fig. 2). In fourth instars, the microtrichia were 0.2 μm wide at the base, 1.0 μm in length, with a maximum gap of 0.2 μm between microtrichia.

DISCUSSION

We view "gut analysis" as the quantitative examination of the particulate contents comprising the food bolus of mosquito larvae, such that these particles can be categorized and counted (Walker et al. 1988). Our techniques necessarily excluded identification of any dissolved or colloidal organic matter that may have been ingested. Gut analysis is not new to the study of mosquitoes, but earlier studies (Boyd and Foot 1928, Hinman 1930, Howland 1930, Ameen and Iversen 1978) have generally discounted the bacterial fraction of the food bolus because of the inability to visualize it. Consequently, algae have been considered as the major component of food items among the "indeterminable brown amorphous matter" making up the dissected material (Senior-White 1928). Our methodology allowed the enumeration of bacteria as well as detritus, algae and protozoans, although fragile protozoans may have already been digested, or destroyed by the brief sonification procedure we used to break up the food bolus.

Bacteria were the most abundant food type in fourth larval instar *Cq. perturbans* followed by detrital particles, euglenoid protozoans, and algae. Compared with *Ae. triseriatus* and *An. quadrimaculatus* (Walker et al. 1988), *Cq. perturbans* larvae had remarkably little detritus in their food, despite their location in the benthic zone. 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 instars ranged from less than 0.5 million to over 1.75 million. *Coquillettidia perturbans* larvae appeared to have fewer bacteria per gut than other mosquito larvae. In comparison, Nilsson[1] (using acridine orange and epifluorescence microscopy) found that the number of bacteria per gut in some *Aedes, Culex, Culiseta* and *Anopheles* species ranged from 6.68×10^6 to 2.18×10^7. Marten (1984) also visualized bacteria with acridine orange but gave no data on bacterial numbers in the food of *Ae. albopictus* (Skuse) larvae. Walker et al. (1988), using DAPI, found that the number of bacteria per gut for field-collected *Ae. triseriatus* and *An. quadrimaculatus* fourth larval instars averaged 2.2 ×

[1] Nilsson, C. 1987. Feeding and food utilization by mosquito larvae. Ph.D. Dissertation, Dept. of Zoology, Univ. Uppsala, Sweden. 269 p.

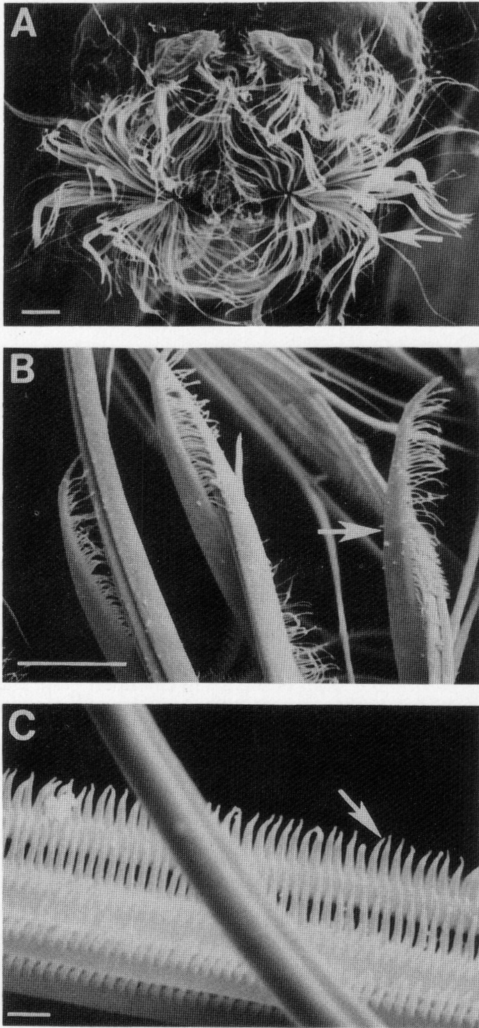

Fig. 2. Scanning electron micrographs of the lateral palatal brushes of *Coquillettidia perturbans.* A. Long filaments (arrow) comprising the brushes in a third larval instar (bar, 100 μm). B. Detail of the filaments in a fourth larval instar, showing microtrichia (arrow) (bar, 10 μm). C. Higher magnification of the microtrichia on the filaments (bar, 1 μm).

10^6 and 2.0×10^6 bacteria per gut, respectively. Guille (1976) examined the gut contents of *Cq. richiardii* without using an epifluorescence technique, but still concluded that bacteria represented the major food source for that species. Walker (1987) and Goshenko (1985) found algae and nonliving particulates in the larval guts of *Cq. perturbans* and *Cq. richiardii,* respectively, but neither used a method that allowed visualization and enumeration of bacteria. Virtually all bacteria found in the guts of *Cq. perturbans* larvae were very small (less than 5 μm in the largest dimension, excluding long, thin spirochetes). These results agree with those of Merritt et al. (1978) and Merritt (1987), who demonstrated that mosquito larvae tended to ingest smaller particles when offered a range of particle sizes.

There was considerable variation of certain food categories of the diet of *Cq. perturbans* larvae. Often, the abundance of some bacteria in larval guts (e.g., spirochetes or purple bacteria) varied as much as 200% within sampling site and date. The variation of certain types of bacteria in the food of fourth instars among sampling months or between sites may have been related to spatial variation of microbial populations within the larval microhabitat, such as documented by Lopez (1988) for microbial communities in microsites of aquatic sediments. Many kinds of bacteria occur in patches in aquatic environments (Franklin et al. 1988, Paterek and Paynter 1988), and invertebrates and protozoans are known to feed on such patches (e.g., Sibbald and Albright 1988). Because *Cq. perturbans* larvae feed while attached to a plant root, different patches of bacteria may be encountered and ingested rather infrequently. This could result in variation in the numbers and kinds of bacteria ingested. We attempted a field experiment to compare the gut contents of *Cq. perturbans* larvae with the food available in the immediate environment; however, disturbance of the larval microhabitat while sampling hampered the experiment so that results were difficult to interpret.

In contrast with Goshenko (1985), who found distinct diet differences among instars of *Cq. richiardii* under laboratory conditions, few differences were found in gut contents of different instars of field-collected *Cq. perturbans*, when numbers of particles were expressed proportionately. However, fourth instars had a higher proportion of euglenoids, diatoms and desmids in their guts than did earlier instars. This may have been due to an expanded repertoire of feeding behaviors in later compared to earlier instars, as suggested by Goshenko (1985), or possibly to differences in the food requirements among instars. The increased number of algae in fourth instars could be a stochastic process related to increased development time of successive instars and the probability of encountering and retaining algal particles in that time period. Fourth instars develop over a longer period of time than do first and second instars (Olds et al. 1989) and therefore would have an increased probability of encountering algae.

The various components comprising the food bolus indicate little about the dietary importance of these components. Bacteria are more readily digested and assimilated than detritus (Kofoed 1975, Cammen 1980) and energetics

favor feeding on bacteria rather than detritus. The contribution of algae to larval nutrition is currently controversial because algae vary in digestibility (Marten 1986, Laird 1988). Algae may have contributed greatly to larval nutrition, although the total number of algal cells in larval guts was low. We did not estimate biovolumes of the particles in this study, yet we suspect that algal and bacterial biovolumes would be similar given the differences in size since algae were at least an order of magnitude larger than bacteria in the one dimension but bacteria were far more abundant.

Coquillettidia perturbans larvae appeared to have 2 feeding modes: suspension feeding in the water column, and brushing from sediment and plant surfaces (Dahl et al. 1988). Although *Cq. perturbans* larvae live in the depositional zone, their feeding behaviors appeared to be similar to water-column dwelling mosquitoes. Larvae did not appear to feed directly on sediments, but rather were mainly suspension feeders even while dwelling in a detritus-rich environment. A suspension feeding strategy is not uncommon among benthic dipteran larvae. For example, certain chironomids inhabiting soft substrates in lotic and lentic habitats construct U-shaped burrows which are suited to suspension feeding. It involves the use of a silken tube, which houses the larva, with a conical catch-net spun across the lumen of the tube. The larva creates currents through the burrow with rhythmic undulations of the body. Periodically, the larva devours its catch-net with adhering debris that has been swept into the burrow by the water current (Walshe 1951, Wallace and Merritt 1980). That *Cq. perturbans* has 2 different feeding modes is supported by SEM micrographs (Fig. 2) of their lateral palatal brushes. Microtrichia on the rays of the lateral palatal brushes or mandibular fans coincide with both browsing and suspension feeding modes (Pucat 1965, Dahl et al. 1988). Merritt and Craig (1987) found that the larvae of *Ae. triseriatus*, a species that demonstrates both suspension feeding, interfacial feeding and brushing (Walker and Merritt, unpublished observations), had lateral palatal brushes with long microtrichia (5×1 μm). Microtrichia on *Cq. perturbans* (Fig. 2) were much shorter than those of *Ae. triseriatus*, indicating that while *Cq. perturbans* may employ brushing, 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 (Dahl et al. 1988). *Aedes triseriatus* larvae often brush from very hard substrates such as bark, whereas *Cq. perturbans* larvae primarily brushed the sediment surface and the surface of soft aquatic plant roots and stems.

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REFERENCES CITED

Ameen, M. and T. M. Iversen. 1978. Food of *Aedes* larvae (Diptera: Culicidae) in a temporary forest pool. Archiv. Hydrobiol. 83:552–564.

APHA-AWWA-WPCF. 1980. Standard methods for the examination of water and wastewater. (15th ed.). APHA, Washington, DC.

Batzer, D. P. and R. D. Sjogren. 1986. Larval habitat characteristics of *Coquillettidia perturbans* (Diptera: Culicidae) in Minnesota. Can. Entomol. 118:1193–1198.

Boromisa, R. D., R. S. Copeland and P. R. Grimstad. 1987. Oral transmission of Eastern equine encephalomyelitis virus by a northern Indiana strain of *Coquillettidia perturbans*. J. Am. Mosq. Control Assoc. 3:102–104.

Boyd, M. and H. Foot. 1928. Studies in the bionomics of American *Anopheles*. J. Prev. Med. 2:219–242.

Cammen, L. M. 1980. The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Marine Biology 61:9–20.

Cummins, K. W. 1973. Trophic relations of aquatic insects. Annu. Rev. Entomol. 18:183–206.

Dadd, R. H. 1970. Relationship between filtering activity and ingestion of solids by larvae of the mosquito *Culex pipiens*: a method for assessing phagostimulant factors. J. Med. Entomol. 7:708–712.

Dahl, C., L.-E. Widahl and C. Nilsson. 1988. Functional analysis of the suspension feeding system in mosquitoes (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 81: 105–127.

Franklin, M. J., W. J. Wiebe and W. B. Whitman. 1988. Populations of methanogenic bacteria in a Georgia salt marsh. Appl. Environ. Micro. 54:1151–1157.

Goshenko, U. A. 1985. Variability in morphological and ecological features in larvae of *Mansonia richiardii* Fic. (Diptera: Culicidae), pp. 15–17. *In:* D. A. Skarlato (ed.), Systematics of the Diptera. Oxonian Press, New Delhi, India.

Guille, G. 1976. Recherches eco-ethologiques sur *Coquillettidia (Coquillettidia) richiardii* (Ficalbi), 1889 (Diptera-Culicidae) du littoral mediterranean Francais. II. Milieu et comportement. Ann. Sci. Nat. Zoo., Paris 22:5–112.

Hinman, E. H. 1930. A study of the food of mosquito larvae (Culicidae). Am. J. Hyg. 12:238:270.

Howland, L. J. 1930. The nutrition of mosquito larvae, with special reference to their algal food. Bull. Entomol. Res. 21:431–440.

Kirchman, D., J. Sigda, R. Kapuscinski and R. Mitchell. 1982. Statistical analysis of the direct count method for enumerating bacteria. Appl. Environ. Microbiol. 44:376–382.

Kofoed, L. H. 1975. The feeding of *Hydrobia ventrosa*

(Montague). 1. The assimilation of different components of food. J. Exp. Mar. Biol. Ecol. 19:233–241.

Laird, M. 1988. The natural history of larval mosquito habitats. Academic Press, New York.

Lee, J. J., S. H. Hutner and E. C. Bovee (eds.). 1985. An illustrated guide to the protozoa. Society of Protozoologists, Lawrence, Kansas.

Lopez, G. R. 1988. Comparative ecology of the macrofauna of freshwater and marine muds. Limnol. Oceanogr. 33:946–962.

Marten, G. G. 1984. Impact of the copepod *Mesocyclops leuckarti* and the green alga *Kirchneriella irregularis* upon larval *Aedes albopictus* (Diptera: Culicidae). Bull. Soc. Vector Ecol. 9:1–5.

Marten, G. G. 1986. Mosquito control by plankton management: the potential of indigestible algae. J. Trop. Med. Hyg. 89:213–222.

McNeel, T. E. 1932. Observations on the biology of *Mansonia perturbans* (Walker) Diptera, Culicidae. Proc. N.J. Mosq. Exterm. Assoc., 19th Annu. Meet., 125–128.

Merritt, R. W. 1987. Do different instars of *Aedes triseriatus* feed on particles of the same size? J. Am. Mosq. Control Assoc. 3:94–96.

Merritt, R. W. and D. A. Craig. 1987. Larval mosquito (Diptera: Culicidae) feeding mechanisms: mucosubstance production for capture of fine particles. J. Med. Entomol. 24:275–278.

Merritt, R. W., M. M. Mortland, E. F. Gersabeck and D. H. Ross 1978. X-ray diffraction analysis of particles ingested by filter-feeding animals. Entomol. Exp. Appl. 24:27–34.

Olds, E. J., R. W. Merritt and E. D. Walker. 1989. Sampling, seasonal abundance, and mermithid parasitism of larval *Coquillettidia perturbans* in south-central Michigan. J. Am. Mosq. Control Assoc. 5:596–592.

Paterek, J. R. and M. J. B. Paynter. 1988. Populations of anaerobic phototrophic bacteria in a *Spartina alterniflora* salt marsh. Appl. Environ. Microbiol. 54:1360–1364.

Pucat, A. M. 1965. The functional morphology of the mouthparts of some mosquito larvae. Quaest. Entomol. 1:41–86.

Senior-White, R. 1928. Algae and food of anopheline larvae. Indian J. Med. Res. 16:11–30.

Sibbald, M. J. and L. J. Allbright. 1988. Aggregated and free bacteria as food sources for heterotrophic microflagellates. Appl. Environ. Microbiol. 54:613–616.

Sjogren, R. D., D. P. Batzer and M. A. Juenemann. 1986. Evaluation of methoprene, temephos and *Bacillus thuringiensis* var. *israelensis* against *Coquillettidia perturbans* larvae in Minnesota. J. Am. Mosq. Control Assoc. 2:276–279.

Smith, J. B. 1908. Notes on the larval habits of *Culex perturbans*. Entomol. News 19:22–25.

Staley, J. T., M. P. Bryant, N. Pfennig and J. G. Holt (eds.). 1989. Bergey's manual of systematic bacteriology, vol. 3. Williams and Wilkins, Baltimore.

Steel, R. G. and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach, 2nd ed. McGraw-Hill, New York.

Walker, E. D. 1987. Efficacy of sustained-release formulations of *Bacillus thuringiensis israelensis* and methoprene for control of *Coquillettidia perturbans* in Indiana. J. Am. Mosq. Control Assoc. 3:97–99.

Walker, E. D. and W. J. Crans. 1986. A simple method for sampling *Coquillettidia perturbans* larvae. J. Am. Mosq. Control Assoc. 2:239–240.

Walker, E. D., E. J. Olds and R. W. Merritt. 1988. Gut content analysis of mosquito larvae (Diptera: Culicidae) using DAPI stain and epifluorescence microscopy. J. Med Entomol. 25:551–554.

Wallace, J. B. and R. W. Merritt. 1980. Filter feeding ecology of aquatic insects. Annu. Rev. Entomol. 25:103–132.

Walshe, B. M. 1951. The feeding habits of certain chironomid larvae. Proc. Zool. Soc. London 121:63–79.

Ward, H. B. and G. C. Whipple. 1959. Fresh-water biology. W. T. Edmondson (Ed.). John Wiley and Sons, New York.

Wetzel, R. G. 1983. Limnology, 2nd ed. Saunders, New York.