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# Interfacial Feeding Behavior and Particle Flow Patterns of *Anopheles quadrimaculatus* Larvae (Diptera: Culicidae)

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*The interfacial feeding behavior, mouthpart movements, and particle flow patterns of* Anopheles quadrimaculatus *larvae were investigated, using videotape recordings, high-speed microcinematography, SEM, and laboratory experiments. While positioned at the water surface, larvae demonstrated 12 behaviors associated with movements of the head. In one of these, a larva rotated its head 180° and directed its mouthparts against the air–water interface. The larva rapidly extended and retracted its lateral palatal brushes (LPBs) at a rate of 5 cycles/s (5 Hz), creating currents and allowing for the collection of particles. Particles moved toward the head at a velocity of 4.31 mm/s, in discrete stops and starts, as the LPBs beat. Our analyses determined that particle movement toward the mouth was governed by very low Reynolds numbers (0.002–0.009). This finding indicated that viscous forces predominated in* Anopheles *feeding and no inertial movement of particles occurred. According to this model, the LPBs cannot intercept particles directly, but function as paddles for particle entrainment. We did not observe the pharynx to function in particle filtration but, rather, in food bolus formation. We propose that the maxillary pilose area and midpalatal brush function as interception structures. It appeared that the LPBs do not break the surface film to feed, but collect particles from the surface microlayers. A plume of uningested particles emerged from the sides of the cibarium and descended into the water column. The plume consisted of alter-*

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*nately clear and dark, lenticular laminae formed beneath the larval head during the collecting-filtering feeding mode. A comparison of particle sizes from surface microlayers and gut contents of fourth instars showed that larvae ingested mainly small particles in the range of 1.5 to 4.5 μm in diameter. The potential significance of interfacial feeding by anopheline larvae in their aquatic environment is discussed.*

**KEY WORDS:** *Anopheles;* Culicidae; larvae; feeding; behavior; hydrodynamics.

## INTRODUCTION

Filter-feeding aquatic insects have evolved active and passive methods to remove particulate matter from suspension (Wallace and Merritt, 1980). Passive methods, used by black flies, depend on existing currents to bring food to the animal (Craig and Chance, 1982), while active filter-feeding in mosquitoes involves energy expenditure to create feeding currents (Wotton, 1990). Adaptations for filter-feeding center on specialized structures (e.g., mouth brushes, labral fans) that act as sieves or collection devices. Rubenstein and Koehl (1977) showed that sieving is only one of several mechanisms by which filter-feeding animals remove particles from water. Mosquito larvae have evolved different filtering mechanisms and morphological adaptations which provide behavioral flexibility for feeding on diverse resources (Surtees, 1959; Pucat, 1965; Harbach, 1977; Laird, 1988; Clements, 1992).

Mosquito larvae utilize "mouth brushes," or lateral palatal brushes (LPBs), on the labrum of the head to generate currents containing food particles that approach the mouth. Recently, Merritt *et al.* (1992) reclassified mosquito larval feeding modes into four categories—collecting-filtering, collecting-gathering, scraping, and shredding—based on the functional feeding-group concept applied to other freshwater invertebrates (Cummins, 1973; Merritt and Cummins, 1984). The collecting-filtering feeding mode is defined as the removal of fine particulate organic material from suspension, regardless of the filtering mechanism (Merritt *et al.*, 1992). Because most mosquito larvae occupy standing-water habitats, collecting-filtering will be governed by high drag and viscous forces at low Reynolds numbers (Dahl *et al.*, 1988; Widahl, 1992; Clements, 1992). Dahl *et al.* (1988) analyzed the collecting-filtering mechanism of suspension-feeding culicine larvae in relation to fluid conditions.

*Anopheles quadrimaculatus* Say larvae normally inhabit ponds, marshes, and impounded water with floating debris and aquatic vegetation. They feed with their body parallel to the air–water interface and their head rotated through 180°, so that the mouthparts are directed toward the water surface (Renn, 1941; Schremmer, 1949). During feeding, the larval body often orients with the pos-

terior end to a plant–water interface, mainly at downed plant stems lying on the water surface (Walker *et al.*, 1988a). Such "intersection lines" (Hess and Hall, 1943) provide potential refugia from predators (Orr and Resh, 1989) and may constitute food-rich foci at the water's surface (Merritt *et al.*, 1992). This specialized feeding mode at the air–water interface, termed "interfacial feeding" by Renn (1941), fits within the collecting-filtering mode defined by Merritt *et al.* (1992).

Although recent studies have examined the interrelationships among morphology, function, and the spatial and temporal patterns of flow and particle retention in culicine larvae (Dahl *et al.*, 1988; Widahl, 1992), no studies of this nature have been conducted on larval anophelines. Our basic understanding of the anopheline feeding process comes from observations made over 50 years ago by unaided eyes or low-power magnifying lenses (Christophers and Puri, 1929; Renn, 1941). The objectives of this study were to describe the interfacial feeding behavior, mouthpart movements, and particle flow patterns of *An. quadrimaculatus* larvae, based on an analysis of high-speed microcinematography, videotaping, and laboratory experiments. We also wanted to determine whether the LPBs function as true filters or just as collecting elements in the feeding apparatus.

## MATERIALS AND METHODS

*Mosquitoes. An. quadrimaculatus* larvae were either obtained from a laboratory colony maintained at Michigan State University or collected at the Inland Lakes Research and Study Center marsh located on the MSU campus. This study site was described by Walker *et al.* (1988a).

*Behavior of the Head During Feeding.* Fourth larval instars were collected from our field site in July–October 1990. Prior to videotaping, larvae were held in tap water in round, plastic dishes (6 × 15 cm) with friction-fitting lids and starved for 24 h.

To develop a catalog of discernable behaviors (Fagen, 1978) associated with the larval head, the head and thorax of *An. quadrimaculatus* larvae were videotaped while feeding at the air–water interface. The observation chamber was a clear glass container (6.5 × 6.5 × 2.5 cm) with a round well (capacity, 3 ml). Two milliliters of tap water was placed into the well, a single larva transferred into the well using a pipette, and a light dusting of food (beef liver powder; Difco) added to the water surface using a wooden dowl. After an acclimation time of 5 min, individuals were filmed using a Javelin Chromachip II color camera configured to a Wild M7 stereomicroscope with a monocular phototube (offering about 10× magnification). Images were recorded on an RCA VR 450 videocassette recorder and viewed with a Sony Trinitron color monitor. Behaviors of 35 larvae were recorded (ca. 7 h of recording). Later, tapes were

analyzed and behaviors of the larval head were delineated to construct the catalog.

*Mouthpart Movements and Particle Flow Patterns.* To make direct observations of larval anopheline interfacial feeding from the front and sides, we used a high-speed microcinematographic apparatus identical to that used for filming copepod feeding behavior (Alcaraz *et al.*, 1980; Vanderploeg and Paffenhöfer, 1985). This allowed the visualization of precise mouthpart movements by the larvae and particle flow patterns in the area immediately surrounding the head.

The apparatus consisted of a Locam 16-mm high-speed movie camera run at 100–250 frames-$s^{-1}$, using high-speed Eastman Ektachrome Video News Film No. 7250 (400 ASA) and Eastman Color Negative Film No. 7292 (320 ASA). The latter provided superior exposure latitude. A 25-mm Luminar lens (NA = 0.15) and 125-mm ocular were used. A 75-W xenon light source and appropriate condenser (Alcaraz *et al.*, 1980) provided Kohler illumination for bright-field observations. To enhance observation of functioning mouthparts and other internal structures, particularly food bolus formation, a deep-red filter (Wratten Filter No. 29) was employed. The entire apparatus was housed in a temperature-controlled room (20°C).

To film fourth larval instars feeding from the front and side, they were transferred from the dishes described above into smaller aquaria (2.3 × 2.3 × 2.3 cm) containing filtered pond water. Proper focusing was accomplished with a micromanipulator that moved the aquarium in the fixed horizontal optical path of the filming apparatus. To make observations from above, the camera and microscope tube with the same objective and ocular were mounted vertically above the larva. A 10-ml algal settling chamber (2.5 cm in diameter × 2.2 cm deep) served as the aquarium. The chamber was mounted on a stage removed from an inverted microscope, and illumination from below was provided by a Bausch and Lomb fiber optic light. Visualization of particle flow patterns was aided by touching the water surface with a capillary tube filled with dilute India ink. Addition of food particles (i.e., yeast) was sometimes necessary to stimulate larval feeding.

Approximately 6000 ft of developed film was examined and analyzed using a Steenbeck flatbed editing console which allowed frame-by-frame and variable-speed viewing. Positions of mouthparts and particles in the surface microlayers were traced onto clear acetate sheets. Measurements of LPB filaments and particle movements to determine velocities were made at 96× magnification to an accuracy of 0.005 mm. To measure the filament diameters and spaces between them, larvae were prepared for scanning electron microscopy as outlined by Merritt and Craig (1987) and magnified at 1000×.

To characterize hydrodynamically flow around the mouthparts, we applied Reynolds numbers (Re) calculations, as follows:

$$\mathrm{Re} = LU/\upsilon$$

where $L$ is the diameter of the filament, $U$ is the velocity of the water at the filament, and $v$ is the kinematic viscosity of water ($1.004 \times 10^{-6}$ m$^2$/s at 20°C). This is a dimensionless ratio that expresses the relationships of inertial and viscous forces in a flowing medium (Vogel, 1981). When Re is $< 1$, viscous forces predominate.

Because of its viscosity, water flowing by a stationary object will have zero velocity at the water/surface interface and increase in velocity with increasing distance away from the surface. This characteristic velocity profile is termed the "boundary layer" (Vogel, 1981). As velocity increases, the boundary layer becomes thinner. However, at Re $< 1$, a solid object produces effects over greater distances, relative to its size (Tritton, 1988), and this area has been referred to as a "zone of viscous effect." We use the boundary layer when dealing with flow in the microlayers beneath the "surface film" and the zone of viscous effect when dealing with flow involving filaments of the LPBs.

*Plume Formation and Particle Size Selection.* Observations also were made on field-collected larvae that were introduced into laboratory aquaria (34 × 20 × 26 cm) filled with tap or filtered pond water. In the presence of larvae, we placed 0.5–1.0 ml of Pelikan Drawing Ink A or 10–20 mg of carmine stain particles (Fischer Scientific, NJ) on the water surface. We observed that immediately after feeding commenced and the larva's lateral palatal brushes started beating, ink and stain particles passed in a "plume" from the mouthparts down into the water column. Based on this observation and those in the film sequences, we wanted to determine the nature of plume formation and particle sizes ingested by the larvae.

The depth that the plume descended in the water column and the time it took were measured and recorded for 34 larvae (9 third and 25 fourth instars). Each mosquito larva was placed in the aquarium and given 5 min to acclimate. At the end of this period, a small drop of India ink was pipetted onto the surface film and allowed to disperse. The depth of the plume produced by each larva was recorded every 10 s over a 2-min period.

To determine what particle sizes larvae were feeding on, we set up the above aquaria and scattered carmine stain particles over the entire water surface. These were then allowed to disperse in the surface microlayers for 15 min, while larger particles began to sink. After 15 min, the water surface was observed to have a pinkish haze, made up of very small particles which now remained trapped. The carmine particles did not dissolve during experiments.

Preliminary observations showed that only third and fourth instars produced a visibly discernible plume, therefore our experiments were limited to these instars. Ten *An. quadrimaculatus* larvae were added to the aquarium for a 10-min period. Seven of these were observed to produce plumes of carmine particles and the following procedure was implemented to examine particle size selection. A Pasteur pipette was used to siphon carefully the material from the

plume of each larva, and this was transferred to a vial. A sample of the carmine particles at the point of ingestion was then immediately collected from the surface microlayers in the same manner. The larva that was feeding was then collected with an eye dropper and immediately placed in hot water (to prevent regurgitation of gut contents) and then in formalin for preservation. Each larva was dissected after several washings in petri dishes of water. The gut was carefully removed with microforceps and the anterior portion containing carmine was excised. The gut contents were then siphoned up and down in a vial several times to break up aggregates that had been formed during ingestion.

The samples from the plume, surface microlayers, and gut were each filtered onto 0.45-$\mu$m pore-size Sartorius membrane filters. Twenty-one filters were preserved from the seven larvae (six of which were fourth instar and one third instar). The filters were covered and allowed to air-dry, after which each was mounted in immersion oil and counts of particles made. To count and measure particles, slides were examined with a microscope, and an ocular micrometer was used to measure particles lying along a random transect across the filter. One hundred particles on each prepared sample were counted in each instance and the length of their longest axis was recorded.

Particle size selection by larvae was evaluated by calculating two selectivity indices using $W$ and $E^*$ (Vanderploeg and Scavia, 1979; Lechowicz, 1982). $E^*$ is a relativized Ivlev index with a range between $-1$ and $+1$ with neutral electivity indicated by zero. $W_i$ is the conditional probability that the $i$th size category will be selected if particles in all size categories were equally abundant. Random selection would be indicated by $1/n$, where $n$ is the number of size categories. Because there were not many counts in the larger size categories, the counts were combined to create a category for particles $>4.0$ $\mu$m.

## RESULTS

### Behaviors of the Head

Observations on videotaped, fourth-instar *An. quadrimaculatus* revealed 12 distinguishable head behaviors associated with interfacial feeding. The following list provides a name and description of each behavior, and Fig. 1 represents a parasagittal section of an *Anopheles* larval head to illustrate relationships of mouthparts and associated structures. In the descriptions, "normal" refers to the position of the head relative to the rest of the body, where the dorsal surface of the head and body are aligned. Conversely, "inverted" refers to the posture where the larva has rotated its head 180° from normal, such that the ventral side of the head is aligned with the dorsal surface of the body.

*1. Inverted, Beat.* The head is inverted 180° from normal position, and

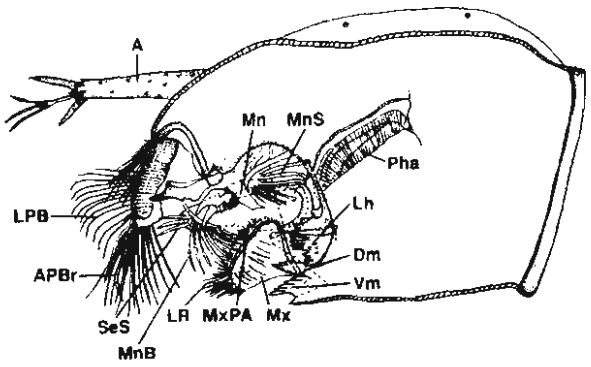


**Fig. 1.** Parasagittal section of *Anopheles* larval head, showing some mouthparts and associated structures [adapted after Harbach and Knight (1980) and modified from Merritt *et al.* (1992)]. A, antenna; APBr, anteromedian palatal brush; Dm, dorsomentum; Lh, labiohypopharynx; LPB, lateral palatal brush; LR, laciniorastrum; Mn, mandible; MnB, mandibular brush; MnS, mandibular sweeper; Mx, maxilla; MxPA, maxillary pilose area; Pha, pharynx; SeS, sellar setae; Vm, ventromentum.

the LPBs extend and retract along the air–water interface very rapidly, without full retraction of the LPB filaments into the cibarium.

*2. Inverted, LPB Shallow Adduction.* While the head is inverted, the LPBs are retracted in a shallow fashion and appear to be swept or cleaned by the mandibular brushes.

*3. Inverted, LPB Deep Adduction.* While the head is inverted, the LPBs are retracted in a deep fashion and appear to be swept or cleaned by the mandibular and possibly maxillary brushes.

*4. Inverted, Masticate.* While the head is inverted, the mandibles masticate a particle or food mass. The particles generated from mastication are swallowed, drift away from the mouth, or sink.

*5. Inverted, Rest.* The head is inverted, and the LPBs and other mouthparts are not moving.

*6. Discard.* The head is inverted; the mouthparts manipulate a particle, then the head rotates 45 to 90° toward normal and the particle is spit out. The particle usually sinks. Then the head returns to the inverted position, although the head may continue to rotate to the normal position.

*7. Rotate Down.* The larva rotates its head from the inverted position to the normal position.

*8. Normal LPB Flick.* While the head is in the normal position, the LPBs are flicked but at a slower frequency and for a shorter duration than during the "inverted, beat" behavior.

*9. Normal LPB Adduction.* The head is in the normal position and the LPBs are adducted. Shallow and deep adductions were not differentiated in this behavior, because the view of the LPBs is obscured by the head capsule.

*10. Normal Masticate.* While the head is in a normal position, the mandibles masticate a particle or food mass, and the particles generated are swallowed or drift away from the mouthparts.

*11. Normal Rest.* The head is in the normal position and the LPBs and other mouthparts are not moving.

*12. Rotate Up.* The larva rotates its head from the normal position to the inverted position.

### Mouthpart Dimensions, Movements, and Particle Flow Patterns

*Mouthpart Dimensions.* Measurement of components of the lateral palatal brushes (Fig. 1) from SEM micrographs and films gave the following dimensions. The filaments of the LPBs divide at approximately three-quarters their length into four to six very fine endings. The mean diameter of the undivided filaments was 2.03 $\mu$m (SE = 0.095 $\mu$m; $n$ = 7). The mean diameter of the terminal divisions was 0.6 $\mu$m (SE = 0.056 $\mu$m; $n$ = 8). Adoral filaments (farthest from the head) were shorter ($X$ = 0.149 mm, SE = 0.00017 mm; $n$ = 7) than aboral (nearest the head) filaments ($X$ = 0.184 mm, SE = 0.00011 mm; $n$ = 7). Filament tips splayed apart both within and between rows. When the LPBs were fully extended, the mean distance between filament tips within a row was 0.022 mm (SE = 0.0016 mm; $n$ = 8), and that between rows was 0.037 mm (SE = 0.0103 mm; $n$ = 7). The mean distance between the fine terminal divisions was 2.75 $\mu$m (SE = 0.333 $\mu$m; $n$ = 9). The fully expanded LPB spread to 0.263 mm in width and 0.358 mm in length.

*Mouthpart Movements and Bolus Formation.* We observed the following mouthpart movements in films (refer to Fig. 1 for structures). The larva had already rotated its head 180° from the normal position and directed its mouthparts to the air–water interface. It commenced with the inverted, beat behavior. When the LPBs were fully extended, just prior to retraction, the shorter adoral filaments touched the surface film, but the longer aboral rows did not. The filaments did not break through the surface film. As the LPBs moved from the fully extended to fully retracted (or flexed) position, the array of 12 or 13 filament rows retracted toward the head like the flipped pages of a book. When retraction of the LPBs was nearly complete, the longer aboral filaments did touch the surface film. After complete retraction, the compacted LPB (mean width, 0.128 mm; SE = 0.004 mm; $n$ = 9) rotated laterally, then dorsally (i.e., down) in the space between the labrum and the antenna, and then anteriorly, where it opened again in the fully extended condition.

As the LPBs retracted, the mandibles (Mn) simultaneously began to retract,

but slightly later, so that as the LPBs reached full retraction, the mandibular selar setae (SeS) swept over them. The mandibles then extended as the LPBs extended, in the same phase, and reached maximum extension just as the adoral filament rows of the LPBs began to retract again. In contrast, the maxillae (Mx) were fully extended when the LPBs were fully retracted, so the mandibles and maxillae extended and retracted in opposite phase. The maxillae had more restricted lateral movement than the mandibles. The anteromedian palatal brush (APBr) retracted as the LPBs extended. It reached maximum retraction when the LPBs reached about halfway through the lateral portion of their extension.

A single retraction of the LPBs averaged 0.10 s in duration (range, 0.09 to 0.11 s; $n = 14$), and a single extension of the LPBs also averaged 0.10 s in duration (range, 0.09 to 0.12 s; $n = 11$). There was no significant difference in duration of time spent in extension or retraction of the LPBs ($t$ test, $t = 0.007$, df = 23, $P > 0.20$). One complete cycle of LPB movement, from complete retraction to extension to complete retraction again, required 0.20 s, such that there were 5 cycles/s or a frequency of 5 Hz.

The round mass of ingested material that forms in the larval mosquito pharynx is called a bolus. Bolus formation was very rapid when the particle density in the surface microlayers was high. We observed one larva to produce a mean of one bolus every 4.43 s (range, 2.85–7.95 boluses/s; SE = 0.64; $n = 7$). These boluses were passed into the anterior esophagus, located immediately below the occipital sclerite of the head. Three or four small boluses were compacted into one large bolus before it was passed to the midgut.

*Particle Movement and Hydrodynamics.* Particles moved toward the midline of the head (Fig. 2) along curvilinear pathways. Particles moved smoothly and at even velocity during retraction of the LPBs (mean particle velocity, 4.31 mm/s; range, 3.57–5.20 mm/s; $n = 25$). Particles stopped immediately when retraction was complete, without exhibiting inertia, indicating that low Re governed particle movement during LPB retraction. At the initiation of LPB extension, particles appeared to move slightly away from the head and sometimes laterally, which may have been caused by the force exerted by the initial LPB extension. This is illustrated in Fig. 2, as the series of closely spaced dots on the particle path. At a distance of about 0.6 mm from the labrum, particles accelerated to an average velocity of 12.3 mm/s (range, 10.3 to 15.3 mm/s; $n = 8$). If not ingested, particles from the surface microlayers turned rapidly laterally and exited dorsally (i.e., downward) between the labrum and the antennae on either side of the head, forming a plume of uningested material (see below). As these particles moved laterally, they passed the maxillary brush (LR in Fig. 1) and the maxillary pilose area (MxPA), as the maxillae at this point in the mouthpart phase of movement were fully extended. Given the data on dimensions of the filaments of the LPBs (diameter at midlength and at splayed tips) and an observed velocity of 4.4 mm/s at the tips of the aboral filaments,

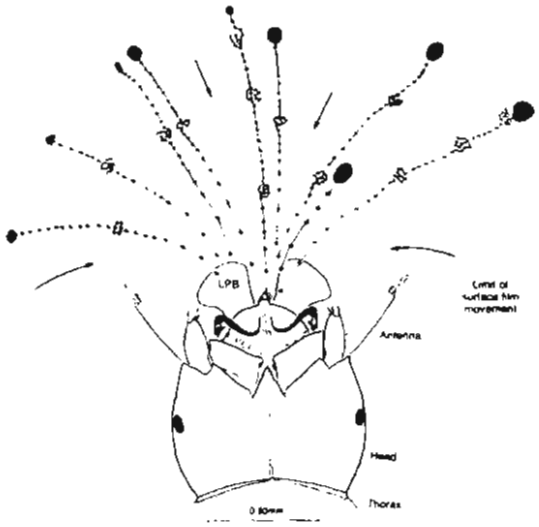


**Fig. 2.** Movement of Dayglo particles in the surface microlayers, approaching a feeding *Anopheles quadrimaculatus* larva. The ventral surface of the head is applied to the surface film. Particles move smoothly toward the head during the retraction cycle of the LPBs (connected dots) but move slightly backward and laterally during the extension cycle of the brushes. At approximately 0.6 mm from the mouthparts, the particles begin to accelerate. Water from the surface is directed medially over the midpalatal brush and then is forced laterally (curved arrows) past the maxillary brushes and down (dorsally) over the edge of the labrum and between the antennae. Time between particle positions = 0.01 s. The dotted hemispherical region is the area of the surface microlayer moved during one retraction of the LPBs. Mandibles are omitted for clarity.

we calculated an Re at the splayed tips of 0.002 and at an undivided filament of 0.009.

Approximations of velocity profiles of the boundary layer, determined by analyzing movements of particles adhering directly underneath the surface film and those in subsurface waters, are shown in Fig. 3. The thickness of the 95% boundary layer, at 1.8 mm in front of the larva, where the surface film velocity was 4.3 mm/s, was 0.86 mm. Closer to the larva, where the surface microlayer had a velocity of 12.0 mm/s, the 95% boundary layer was about 0.60 mm. The volume of water processed during movements of the LPBs was determined by estimating the area and depth of water approaching the mouthparts during one retraction of the LPBs. Because not all water in the boundary layer was moving at the same velocity, the mean velocity at the 50% boundary layer was taken as the depth of the boundary layer for this calculation. The area of surface

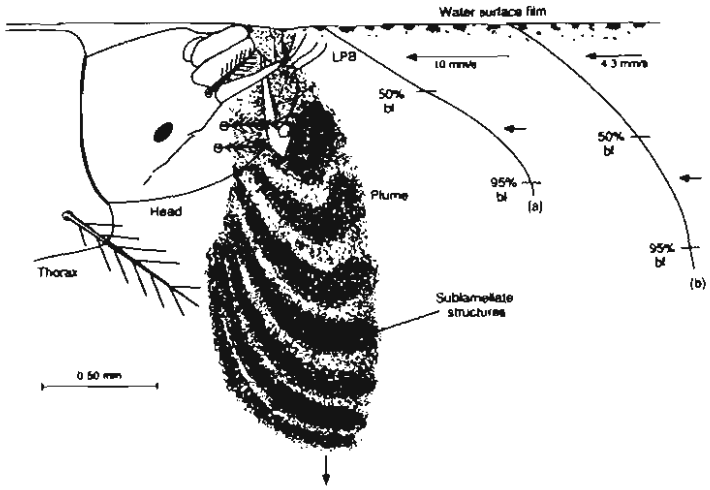


Fig. 3. Lateral view of interfacial feeding *Anopheles* larva with India ink particles in the surface microlayers. The position of filament rows of a fully extended LPB is indicated. At full extension, the longer aboral filaments do not touch the surface film. The velocity profiles of the 50 and 95% boundary layer (bl) for surface film velocities of 10.0 mm/s (a) and 4.3 mm/s (b) are shown. Water drawn in by the LPBs passes down the side of the labrum between the antenna and a large plumose seta (C-11) at the base of the mandible, to form the laminae of the descending plume below the larva.

microlayer involved was determined by tracing onto paper that area (Fig. 2) moved during one retraction of the LPBs, cutting the area out, weighing it, and determining the area from the weight of a known area of paper. The depth of the 50% boundary layer was 0.385 mm and the area of surface water moved with each retraction of the LPBs was 0.623 $mm^2$. Assuming that the water in the 50% boundary layer is processed by the mouthparts, this would yield a volume of about 0.24 $mm^3$ (0.00024 ml). At a cycle of 5.0 complete retractions per s, the amount of water processed was calculated to be about 0.0012 ml/s.

### Plume Formation and Particle Size Selection

Our filming showed that when India ink particles were present in the surface microlayers during larval feeding, a plume of alternately clear and dark laminae formed beneath the larval head (Figs. 3 and 4A). The dark water laminae represented surface microlayers and ink entrained by the LPBs. Since the Reynolds numbers involved in the brush retraction were so low, flow was laminar and there cannot be mixing of water. The clear laminae were formed from water that appeared to be from the boundary layer around the brush on the extension stroke and the water that entered the oral cavity to fill the space left by the

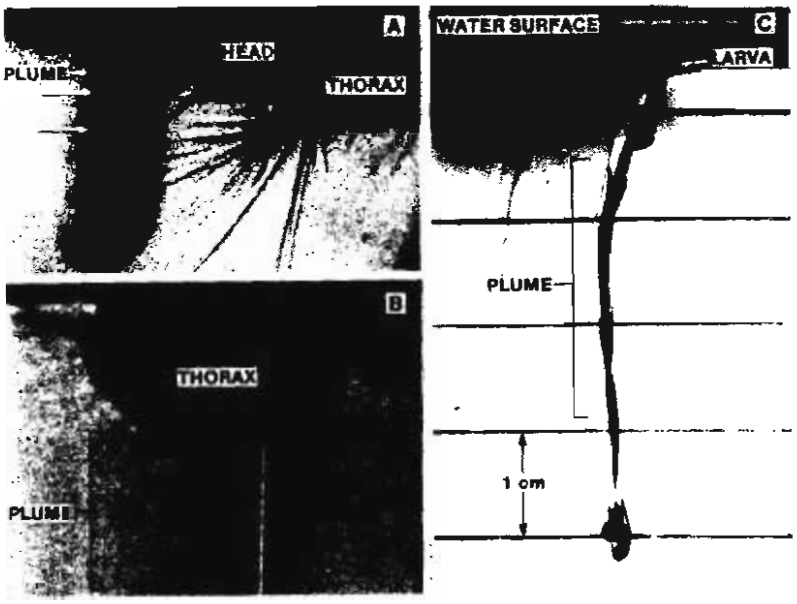


**Fig. 4.** Close-up photographs of plume formation in larvae of *Anopheles quadrimaculatus*, showing different views. The head of the larva was rotated 180° to the water surface and larvae were actively collecting India ink particles that were contained in the subsurface microlayers when these photographs were taken. The top of each photo represents the water surface. (A) Lateral view of larva feeding taken from 16-mm motion picture frame showing plume descending vertically into the water column. Arrows indicate distinct laminae formed beneath the larval head (see text for further explanation). (B) Anterior view of larva from same footage as above, showing descending plume as a series of lenticular laminae joined at the midline (head is obscured by thorax). (C) A 35-mm photograph showing descending plume in the water column, as a result of a larva feeding at the air–water interface in an aquarium. Note the formation of a toroidal vortex at the terminal end of plume (see text). Depth of plume in the water column was approximately 5 cm.

compressed LPBs as they moved laterally and dorsally during the flexion movement. A lamellated substructure (Fig. 3) within the dark laminae represented ink particles entrained by the individual rows of LPB filaments. Up to 10 of these laminae could be detected, which was in close agreement with the number of rows of filaments in the LPB.

In one film sequence, laminae could still be observed by diffraction, even though no particles were added to the surface microlayers. Such diffraction could be seen only if the refractive index of the water in the plume was different from that in the water column. This change in refractive index might be caused by incorporation of surface microlayer material into the plume water. If the plume was viewed anteriorly (Fig. 4B), it appeared as a series of lenticular laminae

joined at the midline. Our explanation for this observation was that at Re < 10, flow around an object (i.e., labrum) divides and rejoins with no vortex formation (Vogel, 1981).

Highly plumose head setae [C-II (Harbach and Knight, 1980)] basal to the antenna appeared to form a lateral barrier between the mandibles and the antennae to the exhalant water (Fig. 4A). Other plumose setae extending from the cephalic apotome (C-5, C-6, C-7) and laterally from the prothorax (P-8) also appeared to be involved in directing the plume of water down and away from the head and body. Because of the low Re involved, the water in the plume would react to these structures as if they were solid (Vogel, 1981).

A photograph of a typical plume of India ink particles produced by an anopheline fourth instar is shown in Fig. 4C. We observed plumes to form as dense columns of particles, about 1 mm in diameter, which sank and extended down several centimeters (range, 2–14 cm) to the bottom of an aquarium, ending in a toroidal vortex (Lugt, 1983). The formation of vortices of this type was homologous to vortex formation in larval suspension-feeding mosquitoes (Widahl, 1992). However, since the velocities involved for *Anopheles* were lower than those for suspension-feeding species, the vortex was much less extensive, as expected. If a larva stopped feeding or water currents were generated from disturbance, the column of particles would often meander for a time, again moving vertically downward when currents settled or feeding resumed (Fig. 4C).

The depth of plume formation over a given time period for third and fourth instars is shown in Fig. 5. We observed that after a certain time period, the

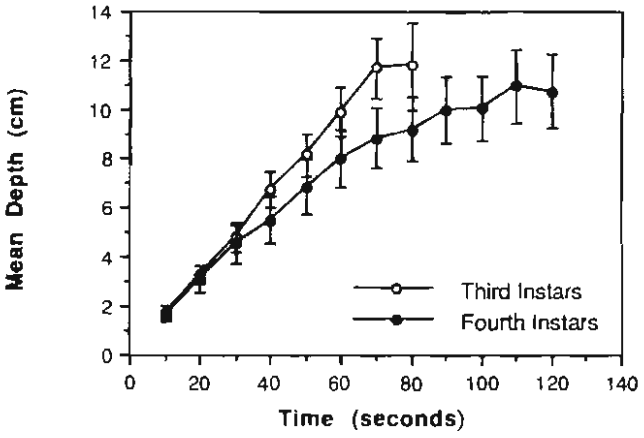


**Fig. 5.** The mean depth of the plume formation (±SE) produced by third ($N$ = 9) and fourth ($N$ = 12) larval instars of *Anopheles quadrimaculatus* over a given time period.

**Table I.** The Number of Particles Counted in Each of the Given Size Categories Sampled from *An. quadrimaculatus* Surface Microlayers (S), Gut Contents (G), and Plumes (P)[a]

| Particle size (μm) | S | G | P |
|---|---|---|---|
| 1.5 | 53.2 ± 3.80 | 61.8 ± 7.78 | 53.5 ± 6.22 |
| 3.0 | 21.5 ± 1.65 | 22.2 ± 3.16 | 22.8 ± 3.05 |
| 4.5 | 11.0 ± 1.75 | 10.0 ± 2.68 | 12.3 ± 1.56 |
| 6.0 | 5.8 ± 0.83 | 2.0 ± 0.93 | 4.2 ± 0.87 |
| 7.5 | 2.3 ± 0.71 | 0.7 ± 0.33 | 1.7 ± 0.92 |
| 9.0 | 2.2 ± 0.31 | 0.7 ± 0.49 | 0.8 ± 0.31 |
| 10.5 | 0.7 ± 0.33 | 0.3 ± 0.33 | 1.3 ± 0.61 |
| 12.0 | 0.7 ± 0.21 | 0.7 ± 0.67 | 0.8 ± 0.54 |
| 13.5 | 0.3 ± 0.21 | 0.7 ± 0.21 | 0.5 ± 0.22 |
| 15.0 | 0.3 ± 0.21 | 0.2 ± 0.17 | 0.7 ± 0.33 |
| >15.0 | 2.0 ± 0.06 | 0.8 ± 0.65 | 1.3 ± 0.76 |

[a] Mean and standard error are given for particle samples from fourth instars ($n$ = 6).

**Table II.** Particle Size Selection by Third and Fourth Instars of *Anopheles quadrimaculatus* ($n$ = 7). Expressed by Vanderploeg and Scavia's (1979) Indices, $W$ and $E^*$ ($\bar{X}$ ± SE), from Particle Size Distributions in Surface Microlayers and Gut Contents [a]

| Particle size (μm) | $W$ | $E^*$ |
|---|---|---|
| 1.5 | 0.364 ± 0.060 | 0.239 ± 0.067 |
| 3.0 | 0.303 ± 0.032 | 0.077 ± 0.059 |
| 4.5 | 0.219 ± 0.037 | −0.110 ± 0.096 |
| >4.5 | 0.113 ± 0.039 | −0.461 ± 0.132 |

[a] If selections were neutral, $W$ = 0.25 and $E^*$ = 0.

structure of the intact plume in both instars started to lose cohesion and failed to descend further in the water column. This was attributable mainly to those factors mentioned above.

The particle size analysis data are given in Tables I and II. The majority of particles in the surface microlayers, gut, and plume samples was in the smallest size categories (Table I). Although there was some variation among individuals, there also was a consistent trend toward the capture of small particles, as shown by the values of the selectivity indices for $W$ and $E^*$ within each particle size category (Table II).

## DISCUSSION

Feeding by mosquito larvae requires whole-body movements (Aly and Mulla, 1986; Walker and Merritt, 1991), various feeding modes (Merritt *et al.*, 1992), and intricate mouthpart coordination (Dahl *et al.*, 1988). The organiza-

tion of mouthpart movements during feeding involves creating flows or currents, removal and entrapment of suspended food particles from the water column or from surfaces, manipulation or mastication within the cibarium, ingestion into the true mouth, and food bolus formation in the pharynx (Rashed and Mulla, 1990; Clements, 1992; Merritt *et al.*, 1992).

During feeding at the air–water interface, fourth-instar *An. quadrimaculatus* exhibited 12 behaviors. The major focus of our study was on one of these behaviors ("inverted, beat"), in which larvae collected food particles from the surface microlayers through the action of the LPBs. The development of the 180° head rotation was a significant step in the evolution of anopheline larvae. It was paramount in allowing interfacial feeding and thus the acquisition of food from surface microlayers of natural water bodies they inhabit. However, larvae also exhibited 11 other behaviors, mostly associated with feeding at the water surface. Jones (1954) documented 14 whole-body behaviors of larvae of this species but did not specifically determine head behaviors separately. Bekker (1938), Renn (1941), and Schremmer (1949) observed a particle rejection behavior by *Anopheles* larvae, which we named "discard" and described as a brief manipulation followed by a quick turn of the head, when the particle was discarded. The means by which a larva assesses the value of a large particle, and further masticates or rejects it, are unknown.

Our catalog of head behaviors indicates that *An. quadrimaculatus* larvae do much more with their head than use the mouthparts to draw particles toward the mouth during the inverted, beat behavior. Changes from the inverted head posture to the normal posture suggested that larvae must routinely rest the muscles required to twist the neck 180° from the normal position, and maintain that position, during the various feeding activities that take place at the water surface. Other behaviors occurring in the normal head position indicate that feeding activity takes place even when the mouthparts are not directed to the air–water interface. Shallow and deep adduction of the LPBs suggested that the LPB filaments must be regularly gleaned of particulate material that accumulates on them during the various feeding movements. Alternatively, adduction may indicate that the LPBs have other functions, within the cibarium, besides generating currents to collect particles.

Analyses of collecting-filtering by mosquito larvae, other than *Anopheles*, indicate that currents drawing particles from all directions toward the head are created by a combination of LPB strokes and perhaps pharyngeal contractions (Dahl *et al.*, 1988; Widahl, 1992). In-flowing currents are caused by flexion of the LPBs, while an outflow or ejection plume, extending 10–40 mm from the larval head, was suggested to be caused by strong pharyngeal contractions (Dahl *et al.*, 1988; Widahl, 1992). These counterflows, with Reynolds numbers estimated to be below 10, form a toroid of moving water around the head of the larvae, in which particles are entrained and carried toward the mouthparts.

Current generation by *Anopheles* was first observed by Christophers and Puri (1929) and later studied in detail by Renn (1941). The latter found that, in addition to interfacial feeding, larvae also fed in a manner described as "eddy" or "free" feeding, where the movements of the LPBs created vortices laterally over each LPB and particles caught in the vortices were ingested. This type of feeding occurred at water surface tensions lower than those in natural habitats (Renn, 1941). Although we rarely observed this type of feeding behavior in our studies, it warrants further investigation.

The mechanisms by which *Anopheles* larvae capture and retain food particles are poorly understood. Schremmer (1949) assumed that the food particles were first trapped either on the filaments of the LPBs or on the spicules of the maxillary brushes and then passed along other mouthpart structures to the pharynx, where a food bolus was formed. Dahl *et al.* (1988) suggested that particles may be entrained in the boundary layers of water formed between LPB filament rows, but these particles did not adhere to the filaments themselves.

Our observations on *Anopheles* larvae using high-speed filming of LPB movements, indicated that the LPBs do not function as true filters or sieves in removing particles from suspension directly. Rather, the LPBs act as paddles (Cheer and Koehl, 1987) to create currents or flows, thereby collecting particles from the surface microlayers and bringing them to the cibarium. Furthermore, we never observed the LPBs to break the surface film to collect particles, suggesting that their feeding zone in natural waters does not normally include particles actually floating on the water surface. In contrast, the analogous structures (labral fans) in black fly larvae do function as true filtering elements that intercept particles in flowing water (Craig and Chance, 1982). The extent ($\partial$) of the zone of viscous effects caused by a cylindrical body in flow can be roughly estimated by the following relationship:

$$\partial = d/\sqrt{\mathrm{Re}}$$

where $d$ is the diameter of the fiber (Braimah, 1987). With a Re of 0.009, the zone of viscous effect around a single LPB filament would be in the order of 0.023 mm. Because the mean distance between filaments in a row is only 0.022 mm, the viscous zones around adjacent filaments overlap and little or no water will pass between filaments, even at the tips. Thus, a filament row functions as a solid body. A more sophisticated model of viscous effects of solid bodies at low Re (Tritton, 1988; Clements, 1992) also supports this conclusion. These calculations, plus our observations that food particles did not impact directly on the LPB filaments, support the findings of Dahl *et al.* (1988) for culicines that the LPBs move water during the filtering-collecting mode but do not remove particles from suspension.

If the LPBs are not serving as the major particle capture and retention mechanism in larval *Anopheles*, then what mechanism is? Although each fila-

ment row will appear to the water as a solid object because of the small filament diameter and their close spacing, water must enter the space between the rows as they are retracted row by row. This water, with entrained particles, will be squeezed out from between the rows of filaments as they reach full retraction and the LPB is compacted in the epipharyngeal region. Evidence for this entrained water from between the LPB filament rows can be seen in the lamellated substructure of each lamina in the plume (Figs. 3 and 4). This mechanism is similar to the one used by small Crustacea for filter-feeding (Cheer and Koehl, 1987). Other larval mouthpart appendages moving out of phase with each other also may play a role in generating feeding currents (Strickler, 1984).

In culicine larvae that feed suspended in the water column, the pharynx itself may provide the mechanism, through expansions that suck in particles brought to the feeding groove (i.e., cibarium) by the LPBs (Dahl *et al.*, 1988; Widahl, 1992). These particles are then sieved onto the dorsal and ventral fringes of the pharynx, and excess water is pumped out with each pharyngeal contraction. However, in *An. quadrimaculatus* larvae that feed at the air–water interface, we observed the major role of the pharynx to be food bolus formation. We did not observe filtration behavior in the pharynx, such as contractions and expansions, that might form a particle retention system.

No function was ascribed directly to the mandibles and maxillae as particle capturing structures in culicine larvae (Dahl *et al.*, 1988). Similarly, Rashed and Mulla (1990) stated that the maxillae were not involved in filtration or particle capture in *Anopheles albimanus*. However, we have shown that the currents generated by the LPBs, in which particles are entrained, change direction across the maxillae, when a lamina of the downward plume is formed. At this juncture, particles could impact upon the maxillary pilose area, and possibly the midpalatal brush, and from there may be transported by the mandibular sweepers (according to Schremmer's model) from the clypeopalatum into the pharynx. Indeed, Jorgensen (1983) commented that in marine filter feeders a change of direction in fluid flow is often associated with the filtering elements. This area requires further investigation.

The plume produced by anopheline larvae consisted of particles mixed with the surface microlayers of hydrophobic compounds that accumulate at the surface of water bodies (Hermansson, 1990). Materials that gather in the surface microlayers will thus be available to feeding larvae, and our microcinematography observations of plumes in the absence of dye may confirm this hypothesis. We observed small refractive particles passing downward from the surface in the plume, and these might have been formed from surface microlayer compounds immiscible with water. Their hydrophobic nature will result in their eventual return to the surface microlayers and this will also be true for some of the particles that become entrapped there, others descending to the sediments. On a contrasting scale, there is thus a parallel between fluxes in anopheline

ponds and oceans: the latter have a downward flux of particles from the photic zone which is compensated by an upward flux of hydrophobic particles (Smith *et al.*, 1989). We do not have an explanation for the faster downward movement of plume in third instars compared to that of fourth instars, except that third instars may reject a higher proportion of larger particles than fourth, thus making the plume descend faster. The fact that a plume was not distinguishable in early instars may suggest a somewhat different feeding mode, or that the plume was too diffuse to observe clearly.

Dahl *et al.* (1988, 1990) observed a "food string" containing a mucus adhesive that was produced by culicine larvae under surplus food conditions and expelled from the mouth. We observed a similar phenomenon in anopheline larvae, however, the origin and content of this food string were not determined. It appeared to be unrelated to plume formation in anopheline larvae. The role of mucus in feeding systems in marine invertebrates is well documented (Jørgenson, 1966) and was reported to occur in the feeding systems of larval black flies and mosquitoes (Ross and Craig, 1980; Merritt and Craig, 1987). However, recent research by Dahl *et al.*, 1990 and K. Fry (personal communication) indicates that mucus is not being produced by either of these insects.

Previous studies on mosquito feeding (Dadd, 1971; Merritt *et al.*, 1978; Merritt, 1987) and our data on particle size selectivity have shown a preference by larvae for the ingestion of smaller particles. The extraction of material from the surface microlayers will provide the larvae with a diet rich in very small particles, including bacteria (cf. Walker *et al.*, 1988b). There also will be an abundance of dissolved organic matter [defined as all material that passes through a 0.45-$\mu$m-pore size membrane filter (Wotton, 1990)] since the anopheline larval habitat is characterized by living and decomposing vegetation, which will be a rich source of material in this fraction (Hinman, 1932; Walker *et al.*, 1988a). Preliminary experiments have shown that larvae fed water from the surface microlayers of a pond had higher pupation rates than those fed subsurface water (Walker and Merritt, unpublished results), confirming that this source of food is of high quality and/or quantity.

Interfacial feeding, for which anopheline larvae are well adapted, brings surface microlayer material to the mouth from a wide distance around a feeding larva, and the downward passage of the plume will ensure that this radiation toward the mouthparts is not interrupted. Furthermore, the plume may be ecologically important in the recycling and intrasystem movement of surface particulate matter and nutrients into the water column, for use by other filter-feeding invertebrates (cf. Merritt *et al.*, 1984).

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## REFERENCES

Alcaraz, M., Paffenhofer, G. A., and Strickler, J. R. (1980). Catching the algae: A first account of visual observations on filter-feeding calanoids. *Am. Soc. Limnol. Oceanogr. Spec. Symp.* **3**: 241–248.

Aly, C., and Mulla, M. S. (1986). Orientation and ingestion rates of larval *Anopheles albimanus* in response to floating particles. *Entomol. Exp. Appl.* 42: 83–90.

Bekker, E. E. (1938). On the mechanism of feeding in larvae of *Anopheles*. *Zool. Zh.* 17: 741–762. (in Russian).

Braimah, S. A. (1987). Mechanisms of filter feeding in immature *Simulium bivittatum* Malloch (Diptera: Simuliidae) and *Isonychia campestris* McDunnough (Ephemeroptera, Oligoneuriidae) *Can. J. Zool.* **65**: 504–513.

Cheer, A. Y. L., and Koehl, M. A. R. (1987). Paddles and rakes: Fluid flow through bristled appendages of small organisms. *J. Theor. Biol.* **129**: 17–39.

Christophers, S. R., and Puri, I. M. (1929). Why do *Anopheles* larvae feed at the surface, and how? *Trans. Far-East. Assoc. Trop. Med.* **2**: 736–739.

Clements, A. N. (1992). *The Biology of Mosquitoes, Vol. 1*, Chapman and Hall, London.

Craig, D. A., and Chance, M. M. (1982). Filter feeding in larvae of Simuliidae (Diptera: Culicomorpha): Aspects of functional morphology and hydrodynamics. *Can. J. Zool.* **60**: 712–724.

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

Dadd, R. H. (1971). Effects of size and concentration of particles on rates of ingestion of latex particulates by mosquito larvae. *Ann. Entomol. Soc. Am.* **64**: 687–692.

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

Dahl, C., Craig, D. A., and Merritt, R. W. (1990). The sites of possible mucus-producing glands in the feeding system of mosquito larvae (Culicidae: Diptera). *Ann. Entomol. Soc. Am.* **83**: 827–833.

Fagen, R. M. (1978). Repertoire analysis. In Colgan, P. W. (ed.), *Quantitative Ethology*, John Wiley and Sons, New York, pp. 25–42.

Harbach, R. E. (1977). Comparative and functional morphology of the mandibles of some fourth stage mosquito larvae (Diptera: Culicidae). *Zoomorphologie* **87**: 217–236.

Harbach, R. E., and Knight, K. L. (1980). *Taxonomists' Glossary of Mosquito Anatomy*, Plexus, Medford, NJ.

Hess, A. D., and Hall, T. F. (1943). The intersection line as a factor in anopheline ecology. *J. Natl. Malaria Soc.* **2**: 93–98.

Hermansson, M. (1990). The dynamics of dissolved and particulate organic material in surface microlayers. In Wotton, R. S. (ed.), *The Biology of Particles in Aquatic Systems*, CRC Press, Boca Raton, FL, pp. 145–159.

Hinman, E. H. (1932). The role of solutes and colloids in the nutrition of anopheline larvae. A preliminary report. *Am. J. Trop. Med.* **12**: 263–271.

Jones, J. C. (1954). Some notes on the behavior of fourth instar *Anopheles quadrimaculatus* Say (Diptera: Culicidae). *Mosq. News* **14**: 186–191.

Jørgensen, C. B. (1966). *Biology of Suspension Feeding*, Pergamon, Oxford.

Jørgensen, C. B. (1983). Fluid mechanical aspects of suspension feeding. *Mar. Ecol. Prog. Ser.* **11**: 89–103.

Laird, M. (1988). *The Natural History of Larval Mosquito Habitats*, Academic Press, London.
Lechowicz, M. J. (1982). The sampling characteristics of electivity indices. *Oecologia (Berl.)* **52:** 22–30.
Lugt, H. J. (1983). *Vortex Flow in Nature and Technology*, John Wiley and Sons, New York.
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 Craig, D. A. (1987). Larval mosquito (Diptera: Culicidae) feeding mechanisms: Mucosubstance production for capture of fine particles. *J. Med. Entomol.* **24:** 275–278.
Merritt, R. W., and Cummins, K. W. (eds.) (1984). *An Introduction to the Aquatic Insects of North America*, Kendall/Hunt, Dubuque, IA.
Merritt, R. W., Morland, M. M., Gersabeck, E. R., and Ross, D. H. (1978). X-ray diffraction analysis of particles ingested by filter-feeding animals. *Entomol. Exp. Appl.* **24:** 27–34.
Merritt, R. W., Cummins, K. W., and Burton, T. M. (1984). The role of aquatic insects in the processing and cycling of nutrients. In Resh, V. H., and Rosenberg, D. M. (eds.), *The Ecology of Aquatic Insects*, Praeger Scientific, New York, pp. 134–163.
Merritt, R. W., Dadd, R. H., and Walker, E. D. (1992). Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* **37:** 349–376.
Orr, B. K., and Resh, V. H. (1989). Experimental test of the influence of aquatic macrophyte cover on the survival of anopheles larvae. *J. Am. Mosq. Control Assoc.* **5:** 579–585.
Pucat, A. M. (1965). The functional morphology of the mouthparts of some mosquito larvae. *Quaest. Entomol.* **1:** 41–86.
Rashed, S. S., and Mulla, M. S. (1990). Comparative functional morphology of the mouth brushes of mosquito larvae (Diptera: Culicidae). *J. Med. Entomol.* **27:** 429–439.
Renn, C. E. (1941). The food economy of *Anopheles quadrimaculatus* and *A. crucians* larvae. Relationships of the air-water interface and the surface-feeding mechanisms. In *A Symposium on Hydrobiology*, University of Wisconsin, Madison, pp. 329–341.
Ross, D. H., and Craig, D. A. (1980). Mechanisms for fine particle capture by larval blackflies (Diptera: Simuliidae). *Can. J. Zool.* **58:** 1186–1192.
Rubenstein, D. I., and Koehl, M. A. R. (1977). The mechanisms of filter feeding. Some theoretical considerations. *Am. Nat.* **111:** 981–994.
Schremmer, F. (1949). Morphologische and funktionelle analyse der mundteile und des pharynx der larvae von *Anopheles maculipennis* Meig. Österr. Zool. Z. **3:** 173–221.
Smith, K. L., Jr., Williams, P. M., and Druffel, E. R. M. (1989). Upward fluxes of particulate organic matter in the deep North Pacific. *Nature (London)* **337:** 724–726.
Strickler, J. R. (1984). Sticky water: A selective force in copepod evolution. In Meyers, D. G., and Strickler, J. R. (eds.), *Trophic Interactions in Aquatic Ecosystems*, Westview Press, Boulder, CO, pp. 187–239.
Surnees, G. (1959). Functional and morphological adaptations of the larval mouthparts in the subfamily Culicidae with a review of some related studies by Montschadsky. *Proc. R. Entomol. Soc. Lond.* **34:** 7–16.
Tritton, D. J. (1988). *Physical Fluid Dynamics*, Clarendon Press, Oxford.
Vanderploeg, H. A., and Paffenhöfer, G.-A. (1985). Modes of algal capture by the freshwater copepod *Diaptomus sicilis* and their relation to food-size selection. *Limnol. Oceanogr.* **30:** 871–885.
Vanderploeg, H. A., and Scavia, D. (1979). Two electivity indices for feeding with special reference to zooplankton grazing. *J. Fish. Res. Bd. Can.* **36:** 362–365.
Vogel, S. (1981). *Life in Moving Fluids*, Willard Grant Press, Boston, MA.
Walker, E. D., and Merritt, R. W. (1991). Behavior of *Aedes triseriatus* (Diptera: Culicidae) larvae. *J. Med. Entomol.* **28:** 581–589.
Walker, E. D., Merritt, R. W., and Wotton, R. S. (1988a). Analysis of the distribution and abundance of *Anopheles quadrimaculatus* (Diptera: Culicidae) larvae in a marsh. *Environ. Entomol.* **17:** 992–999.
Walker, E. D., Olds, E. J., and Merritt, R. W. (1988b). Gut content analysis of mosquito larvae (Diptera: culicidae) using DAPI stain and epifluorescence microscopy. *J. Med. Entomol.* **25:** 551–554.