DO DIFFERENT INSTARS OF *AEDES TRISERIATUS* FEED ON PARTICLES OF THE SAME SIZE?

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Microorganisms and particulate organic detritus generally constitute a major part of larval mosquito diets (Clements 1963, Ameen and Iversen 1978, Fish and Carpenter 1982). Studies suggest that there are limits to the particle sizes filter-feeding mosquitoes ingest (Clements 1963, Pucat 1965, Dadd 1971a, 1971b); most larvae ingest particulate matter from colloidal to 50 μm in size (Pucat 1965; Dadd 1971a, 1975; Wallace and Merritt 1980). The ability of mosquitoes to capture fine particles appears to be enhanced by a mucosubstance secreted onto the palatal brushes (Merritt and Craig 1987), similar to larval black flies (Ross and Craig 1980). Although particulates are, in nature, the major food of many mosquito larvae, several species can develop through all larval stages in sterile synthetic media with most of the essential nutrients in solution (Dadd 1977). However, without some solids feeding may be impaired and growth reduced, unless the dietary medium is textured by supplementation with low concentrations of colloids which increase the liquid ingestion rate (Dadd 1975, Dadd and Kleinjan 1976).

Larvae of *Aedes triseriatus* (Say), a vector of La Crosse encephalitis virus, develop in tree holes and other small containers (e.g., discarded automobile tires). The larvae not only filter particles from the water column, but abrade solids such as detrital material, bacteria and fungi from surfaces. Using X-ray diffraction analysis in laboratory studies, Merritt et al. (1978) showed that when presented a range of particles from <2 μm to 180 μm, first instar *Ae. triseriatus* ingested and retained only particles less than 2 μm in size. Second through fourth instars also took significant proportions of the smallest particles, but ingested and retained particles ranging in size from 2–50 μm as well. The objective of the present study was to determine if different instars of *Ae. triseriatus* partition particles in the <2–50 μm range, since this was the range in which the majority of particles had been ingested in the previous study (Merritt et al. 1978).

Laboratory experiments involved exposing different larval instars of *Ae. triseriatus* (WALTON strain) to minerals of different size classes within the range <2–50 μm (Table 1). The desired particle size was obtained by grinding commercially available minerals, except for kaolinite (<2 μm), in a ball mill and then sieving. Kaolinite was obtained by standard dispersion and sedimentation techniques.

Mosquito larvae were reared in the laboratory at 21°C, separated by instar, and placed into distilled water without food for 24 hr. Larvae were then distributed to petri dishes which received 0.25 g of each of the following minerals: kaolinite, labradorite, muscovite and dolomite (Table 1). The minerals were thoroughly mixed with distilled water prior to testing and the water level in each dish was adjusted to allow larvae to continually feed on the bottom while maintaining siphonal contact with the surface. Larvae in each age group (2,500 1st instars, 2,000 2nd instars, 700 3rd instars, 500 4th instars) were allowed to feed for 24 hr and then were killed in boiling ethanol. This time period was based on previous studies (Dadd 1970) which showed that mosquito larvae ingest different particulate materials at widely varying rates. Unfed control larvae were also killed in boiling ethanol. A sample from the bottom of the petri dishes served as particle size standards. The experiment was performed twice.

Samples containing different larval instars of mosquitoes were placed in crucibles, ashed at 250°C overnight, and then treated with 5 ml of 30% H_2O_2 and dried over low heat. Water was added to the residue to yield a slightly viscous paste which was then deposited on a glass slide. After air-drying, the diffraction pattern of the sample was obtained on a Phillips diffraction unit utilizing copper radiation and a goniometer which presents the diffraction peaks as a function of the Bragg angle *theta* (Whittig 1965). Mineral species have characteristic X-ray diffraction properties which enables their presence in a sample to be easily detected. Minerals were selected which are relatively stable and which possess a distinct, non-overlapping diffraction peak. If the residue from the larvae was found to contain a certain mineral by X-ray diffraction, it was evident that they had ingested particles of that particular size range. Since the diffraction intensity is proportional to the amount of a given mineral present (i.e., number of particles), an estimate can also be made of the relative proportions of minerals representing different particle sizes. Other possible applications of this technique are discussed by Merritt et al. (1978).

No X-ray diffraction peak was detected for 1st instar *Ae. triseriatus* larvae due to a low sample size or an insufficient amount of material ingested. Second instars ingested and retained only particles less than 2 μm in size (Fig. 1, Table 2), similar to results obtained by Merritt et al. (1978) for 1st and 2nd instars of the same species (i.e., all 1st instars and 83% of 2nd instars ingested particles smaller than 2 μm). Third instars ingested and retained particles of 4 different size ranges; however, a larger proportion of particles were ingested in the 2–10 μm range. Fourth instars ingested mainly particles in the 10–25 μm range and fewer particles in the <2 μm range. As shown in Fig. 1 and Table 2, there was generally an increase in coarser material ingested with increasing instar of larvae. However, a decrease in the proportion of particles ingested above the 25 μm size range occurred for 3rd and 4th instars. Results were similar for both experimental runs.

These findings raise the question of whether some type of selective feeding occurred among the larvae in that, when given a choice, the majority of particles ingested by all instars of *Ae. triseriatus* were less than 25 μm. Dadd (1971a) found that all larval instars of *Culex pipiens* Linn. generally ingested particles with mean diameters between 0.5 and 2 μm. It is well documented that, for other filter-feeding aquatic insects, the proportion of fine particles in the diet is an important factor influencing growth and population density (Carlsson et al. 1977, Wallace and Merritt 1980, Wotton 1984). Smaller particles, with high surface area:volume ratios, are more densely colonized by microorganisms than larger particles (Kondratieff and Simmons 1985). It is, however, unknown if microorganisms form the principal assimilated food item, or if perhaps other components of these fine particles are assimilated instead.

With increasing insecticide resistance and the continual development of new particulate formulations and alternative biological control agents requiring ingestion, a knowledge of the particle sizes most commonly ingested, both organic and inorganic, is of paramount importance. Identification of sizes and composition of

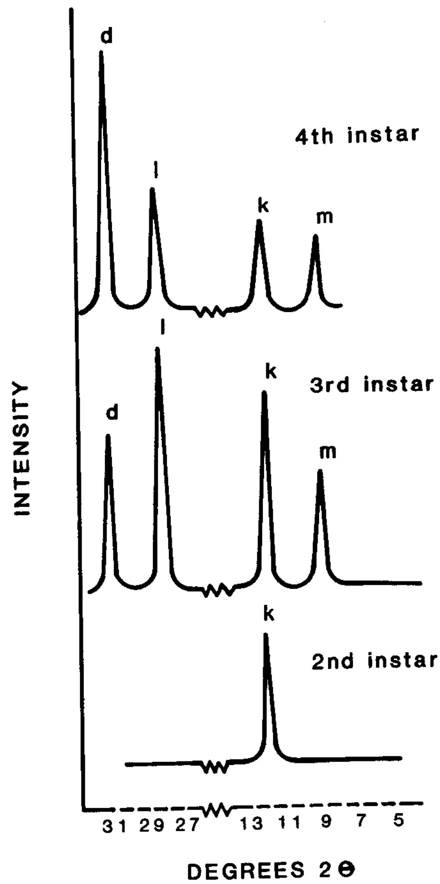

Fig. 1. The X-ray diffraction peaks for particles ingested and retained by larvae of *Aedes triseriatus* (Diptera: Culicidae). k, kaolinite (<2 μm); l, labradorite (2–10 μm); d, dolomite (10–25 μm); m, muscovite (25–50 μm). Diagnostic peak for each mineral is given in Table 1.

Table 1. Physical properties of minerals.

Mineral	Particle size	Diagnostic diffraction peak	Crystallographic indices
Kaolinite	<2 μm	7.15 Å	001
Labradorite	2–10 μm	3.20 Å	040,202
Dolomite	10–25 μm	2.88 Å	104
Muscovite	25–50 μm	10.0 Å	002

Table 2. Differences in particle size selection by mosquito larvae expressed as percentage of the peak heights in Fig. 1.

	Mineral			
Instar	% kaolinite (<2 μm)	% labradorite (2–10 μm)	% dolomite (10–25 μm)	% muscovite (25–50 μm)
2	100	nil	nil	nil
3	28	34	22	16
4	15	23	52	9

particles ingested in the field to corroborate these laboratory data is currently underway.

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