lowing 2 machines were used. They had been previously tested by Brown and Watson (1953).

TIFA: The Todd Insecticidal Fog Generator was operated with the insecticide pressure at 25 p.s.i., the fuel pressure at 60 p.s.i., and the particle size selector at 11; the emission rate with diesel oil was 20 g.p.h.

Husman Pneumatic Sprayer: This machine is described in detail by Husman (1953). It was run with the insecticide at 17 p.s.i. and atomized at 25 p.s.i.: the emission rate with diesel oil was 20 g.p.h.

SOME NOTES ON THE BEHAVIOR OF FOURTH INSTAR *ANOPHELES QUADRIMACULATUS* SAY (DIPTERA, CULICIDAE)

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Information on the behavior and activities of normal insects is necessary for properly evaluating their responses to many physiological and toxicological stimuli, yet, so far as the writer is aware, no one has given a complete qualitative descriptive list of the larval activities for even a single species of the Culicidae. Hocking's paper (1953) on *Aedes communis* seems to come closest to giving such general information. Many isolated observations on different species of Culicidae are scattered in the literature, and much of this information has been reviewed by Bates (1949) and Hopkins (1952). Many activities of mosquito larvae do not seem to lend themselves to a quantitative approach. Others, e.g. reflex diving, are more readily subjected to experimental analysis. There are a number of excellent quantitative studies on larval responses to a variety of stimuli, notably those of Ivanova (1940), Thomson (1940), Folger (1946), and Thomas (1950).

The present notes are concerned with qualitative descriptions of the behavior and activities of normal fourth instar *Anopheles quadrimaculatus* Say under more or less uniform, and hence highly artificial, laboratory conditions. The data are assembled partly from detailed notes taken from controls in current experiments dealing with the effects of salts, drugs, and poisons on various organ systems in fourth instars. The information is also based on numerous observations made from time to time during the four-year period in which this species has been under study in this laboratory. However, no attempt has been made to elucidate any of the complex factors involved in the behavior observed. Quantitative data on some of the activities listed in these notes will be given in a subsequent series of papers that deal with physiological responses to abnormal stimuli.

METHODS. The larvae of *Anopheles quadrimaculatus* were reared as described by Peffly *et al.*, (1946).

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Both macroscopic and microscopic observations were made. Macroscopic studies were made on both individual specimens and on groups of larvae in beakers, Petri dishes, rearing pans, or in the wells of micro slides. Microscopic observations were made on individual larvae placed in micro slides containing distilled water with and without food. All studies were conducted at 27± 2° C.

Detailed notes were taken only on fourth instars. All ages within this stage were examined, physiological ages being roughly estimated by a method given by Jones (1953).

The terminology and classification used in this study were necessarily arbitrary and are, therefore, tentative.

RESULTS. Fourth instar *A. quadrimaculatus* have at least 14 different types of activities or general behavior patterns under routine laboratory conditions.

(1) Complete rest at surface.—Larvae lie straight and completely motionless suspended at the air-water interface, their spiracular plates, palmate hairs, notched organs, and mouth brushes lightly indenting the surface film. Mechanical forces tend to keep many of them perpendicular to the edge of ordinary glass and enamel containers as pointed out by Renn (1941). However, resting larvae may be seen both parallel to the edge or in the center of containers. They may lie at complete rest with either dorsal or ventral face of the head uppermost, most commonly the former.

(2) Quiescent feeding.—When abundant food is present, larvae lie quietly as described in (1), the ventral face of the head being uppermost after rotation through a 180° angle, with only the mouth parts being in motion. Rotation of the head is usually counter-clockwise. Very frequently the head also moves from side to side in quiescent feeding. When these movements are made in the absence of food they can be termed "imitative."

Nuttall and Shipley (1901) describe rotation of the head in *A. maculipennis* larvae. According to Smith (1914) these rotations are always counter-clockwise. Renn (1941) gives detailed descriptions of "free" ("eddy") feeding, "film," and "interfacial" types of feeding in *A. quadrimaculatus* larvae.

(3) Radius feeding.—Frequently when larvae are quietly feeding as described under (2), their bodies may sway horizontally through a 5–60° arc or momentarily bend to either side, their posterior ends remaining at a fixed point as they feed.

(4) Sub-surface feeding without locomotion.—When little or no food is present in the surface film, larvae have been observed to bend the anterior end of the body downwards, their stigmatic apparatus being still attached to the surface film, and feed either on the bottom of shallow containers or in the intervening space between bottom and surface in deeper containers (figure 1).

(5) Swimming excursion.—Often larvae may be observed to swim forward. Such excursions are of two main types: In (a) "simple excursion" larvae move parallel to the horizontal water film and either do not feed as they float; or, more commonly, they feed after rotation of the head as they move. The second type of excursion may be referred to as (b) "subsurface grazing." In this type of activity they bend downwards as described under (4) and swim about vigorously feeding on the bottom but the head does *not* rotate (figure 1). They engage in this latter type of activity generally only when there are no particles in the surface film.

Smith (1914) describes this activity in *A. punctipennis* larvae: ". . . when feeding on a filament, usually the mouth parts are underneath and the head is bent downwards."

(6) Positional movements. — Larvae, which are resting or feeding at the surface, may often exhibit a variety of complex but slight body movements which mainly seem to keep them properly positioned at the surface. They may move slightly backwards and forwards (generally while feeding vigorously), or move slightly from side to side, or rock along their horizontal axis. Occasionally they may flick the median plate of the stigmatic apparatus or

stretch along the longitudinal axis *in situ*. These movements tend to be spasmodic. In the present study they were observed to make these movements when gently blown about over the surface in a weak air current, or while feeding on a surface containing an excess of food particles.

(7) Homostrophic reflex.—One of the most clearly defined responses given by larvae after a mechanical disturbance is the homostrophic reflex (figure 2). In giving it, the body of the larva still lies parallel to the water surface but bends from its straight position (2a) to assume an S-shape (fig. 2b). When larvae are touched laterally with a pointed applicator stick they tend to bend away from the point of stimulation. However, larvae may be approached with an applicator stick or glass rod and repeatedly stroked along their dorsal surface without eliciting this response. They may even be shifted about by a weak air current or with an applicator stick over a considerable distance without giving this reflex.

If a ripple is made at a distance from a resting or quietly feeding larva that is facing the direction of the stimulus, it generally remains quiet until after the ripple and its reflections have passed and then gives the homostrophic reflex, or else wriggles violently and/or falls to the bottom of a shallow test container. But larvae may maintain the S shape for a long time if the disturbance is continued. Normally they do not feed immediately following the reflex but may do so shortly thereafter while still in the "S" position.

When the container in which larvae are resting or feeding quietly is lightly but sharply tapped, larvae have been sometimes observed to arc the anterior end of the abdomen downward while keeping their stigmatic apparatus, notched organs and head at the surface as shown in figure 3. This response is included tentatively with the homostrophic reflex.

No description of this reflex was found in a survey of the literature on mosquitoes.

(8) Sudden swimming movements.—One of the principal means of locomotion of larvae is through sudden, vigorous, backward-directed swimming movements. Removal of the hairs of the ventral brush on the anal segment did not interfere with these movements. In the present studies swimming movements were mainly observed in larvae deprived of food for a short while, and in larvae which were mechanically disturbed. There seems to be a definite connection between these movements and defecations in freshly starving larvae, for almost invariably they immediately follow defecations. However, when abundant food is present sudden swimming movements do not necessarily follow defecations. Sometimes starving larvae may go into a veritable frenzy of sudden swimming movements, which then closely resemble convulsions. Generally such larvae may be rapidly quieted by feeding them.

Wriggling movements are included under this category because the pattern of locomotion appears to be fundamentally identical. Wriggling tends to last longer than simple sudden swimming movements. Larvae ordinarily begin instant and violent wriggling when the surrounding medium is withdrawn so that they touch the bottom of the container.

(9) Crawling.—When larvae are dried off so as to leave only a thin film of water about them on a glass slide, they make *forward* crawling movements. When completely dried off they cannot move around but are still capable of flipping about vigorously.

Nikolsky (1924) has described the ability of *Anopheles maculipennis* larvae to crawl. He found that they used their "oral appendages," especially the labrum, as points of support and "crawled" by means of peristaltic (forward telescopic) movements.

(10) Reflex "diving."—If *A. quadrimaculatus* larvae are greatly disturbed, e.g. by sudden violent jarring or shaking of the test container, they tend to fall rapidly downward.

According to Nuttall and Shipley (1901), in *A. maculipennis* larvae, the median flap of the stigmatic apparatus ". . . folds suddenly back [as] the animal

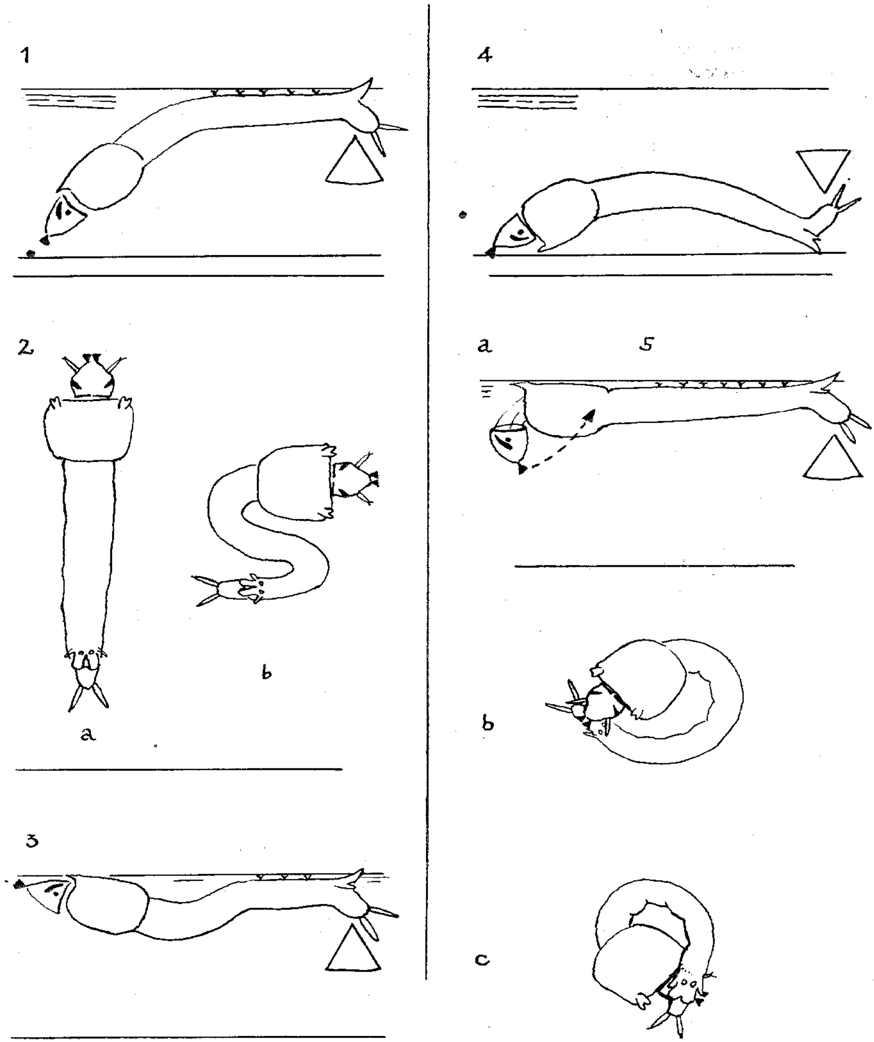

Plate 1.
(Diagrammatic)

FIG. 1.—Lateral view of larva engaging in sub-surface grazing. FIG. 2.—Dorsal view of larvae in (a) normal resting position (complete rest), (b) homostrophic reflex position. FIG. 3.—Lateral view of larva arching anterior end of abdomen downward as a result of mild disturbance (see text). FIG. 4.—Lateral view of larva in state of akinesis. FIG. 5.—(a) Lateral view of larva cleaning about thorax. (b) Dorsal view of larva cleaning about the stigmatic apparatus. (c) Dorsal view of larva showing position assumed in cleaning abdomen.

suddenly darts below. . .". That this is not the case with *A. quadrimaculatus* larvae can be shown after slipping a cover-glass over them in a well slide so as to completely exclude them from contact with the air-water interface. When this is done it can be seen that the lateral edges of the median dorsal plate of the stigmatic apparatus curl inwards to form a kind of shallow scoop. The median plate can be elevated by two muscles which are inserted medially at a point directly between the spiracular openings, but this elevation is not an essential feature and can occur without diving being involved. Further, removal of the median dorsal flaps does not alter the ability to fall ("dive") from the surface film, although it does seem to interfere slightly with re-attachment to the surface film after akinesis.

(11) Akinesis.—When larvae fall downward from the surface film in shallow water (less than ½ inch) the body is usually dorsally arched as shown in figure 4. They come to lie on the bottom, generally on their dorsal surface, but have also been observed to fall on their ventral surface in very shallow water. They have even been seen to fall onto their lateral surface (their bodies arched laterally) and briefly swim forwards for a short distance in this position on the bottom of the container. If quiescent larvae are briefly shaken in deep water (more than 2 inches) they fall downwards as described above, but many begin vigorous swimming movements shortly after leaving the surface and return to the air-water interface without ever reaching bottom.

Although akinetic larvae tend to lie quite motionless at the bottom of shallow water, they have been seen to move the mouth brushes, defecate, and give the homostrophic reflex. Rarely, they were observed to rotate the head through 180° but they apparently do not actually feed in this position.

They remain akinetic for only a few seconds or, more commonly, for prolonged periods of time in shallow water. They return to the surface through vigorous swimming movements.

If akinetic larvae are illuminated only from the bottom in a beaker of deep water in an otherwise shaded room, the time required for all in a lot of ten to return to their position at the surface is around 8 minutes even though 50% of them surface in approximately 90 seconds. If, on the other hand, akinetic larvae are lighted only from the top (beam directed vertically down into the open beaker), *all* in a lot of ten surface in from 10–40 seconds. It is noteworthy, however, that larvae at either complete rest or engaged in quiescent or radius feeding did not orient themselves in relation to variously directed beams from the same artificial light; nor do quiet, dark-adapted larvae visibly react to sudden flicking on of a strong beam of light.

(12) Cleaning activities. — *A. quadrimaculatus* larvae perform the most remarkable contortions when engaged in cleaning the body surface (figure 5). The "neck" (cervical membrane), which cannot ordinarily be seen in larvae at complete rest or when they quietly feed, can be stretched outward to such an extent that they can clean about the thorax (figure 5a). In cleaning the abdomen, larvae bend themselves so as to reach and brush the anal papillae and the ventral brush. They usually do this while still attached to the surface by the stigmatic apparatus. They do not ordinarily fall from the surface film when cleaning about the stigmatic apparatus. Mouth brushes are principally used in cleaning but cephalic and thoracic hairs probably aid in the process.

Smith (1914) briefly describes cleaning activities in *A. punctipennis* larvae as follows: "Frequently it [the larva] bends and brushes over its whole body as far as it can reach."

(13) Offensive behavior.—Larvae under crowded conditions frequently appear to "attack" each other, nipping vigorously and repeatedly at different parts of the body. Occasionally two larvae may briefly engage their mouth parts so as to appear to be struggling one with the other. From time to time, two or more larvae have

been seen apparently "attacking" a third one. Not infrequently the third larva is damaged in some way or appears otherwise abnormal.

(14) Molting behavior.—Only two aspects of the behavior prior to pupation will be described. Prior to ecdysial peristaltic movements (forward-directed contractions) themselves, the lateral walls of the last few abdominal segments—along the dorso-pleural line—rhythmically expand and collapse like a bellows, doubtless reflecting internal pressure changes. Backward-directed longitudinal stretches of the body, especially noticeable between the seventh and eighth abdominal segments, may also occur.

SUMMARY AND CONCLUSIONS. The behavior and activities of normal fourth instar *Anopheles quadrimaculatus* Say as they occur under more or less uniform laboratory conditions are briefly described and categorized as follows:

(1) Complete rest at surface; (2) quiescent feeding; (3) radius feeding; (4) subsurface feeding without locomotion; (5) swimming excursions: (a) simple excursions (b) sub-surface grazing; (6) positional movements; (7) homostrophic reflex; (8) sudden swimming movements; (9) crawling; (10) reflex diving; (11) akinesis; (12) cleaning activities; (13) offensive behavior; and (14) molting behavior.

These qualitative descriptions furnish a basis for quantitative studies on the effects of physiological and toxicological stimuli on different activities.

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