THE FIRST THREE LARVAL STAGES OF HEXAGENIA BILINEATA SAY.

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INTRODUCTION.

Although the study of the Ephemeridæ was begun by J. Swammerdam in the seventeenth century, our knowledge of the developmental stages through which these insects pass from the time they leave the egg until they appear on land as sub-imagos is still very limited. Quite a number of papers have appeared dealing with the morphology, embryology, taxonomy, biology, and the distribution of Ephemeridæ, but very little has been done towards working out the different stages in their post-embryonic development. The first work of this nature was done by Lubbock (1863–66) on the development of *Chloen dimiditum*. This was followed by the work of N. and E. Joly (1878) on *Paligenia virgo* and by that of Vayssiere (1882) on *Heptagenia longicauda*. More recently Murphey ('21) and Gros ('23) have worked out the post-embryonic development of *Baetis posticatus* and *Ecdyonurus forcipula* respectively.

THE MATERIAL.

The material on which the study of the first three larval stages of *Hexagenia bilineata* has been based was obtained at the Ohio State University Lake Laboratory at Put-in-Bay, Ohio. The larvæ were hatched in the laboratory from eggs that had been fertilized in normal salt solution and then incubated in lake water. The time required for incubation was nine days; this is one day less than the shortest period of incubation that I observed during the summer of '24 in the same laboratory. Clemens ('13) states that the time required for incubation is forty days. The length of time required for hatching varies perhaps with the individual eggs as well as with the temperature. Under identical conditions eggs from the same female fertilized by spermatozoa from the same male differed in the length of time required for incubation

from ten to twenty-four days. A low temperature would, I think, prolong the period required for incubation, for Murphey ('21) has shown that if in the development of *Baetis posticatus* the average monthly temperature is reduced from 62.4° to 45° the length of the aquatic existence is increased by fifty percent.

EGGS.

The female deposits on an average about 1500 eggs. Due, perhaps, to the crowded conditions in the abdomen of the female, the eggs are quite variable in shape; some are long ovals, some are elongate with square ends, again others have concave sides, etc. The eggs are whitish in appearance and measure about .27 mm. in length and .17 mm. in width. The chorion is rather heavy and is surfaced with regular hexagonal figuring. When hatching, the shell splits lengthwise to allow the emergence of the larvæ. The young larvæ become quite active soon after crawling out of the shell.

Because of the transparence of the body-wall, the alimentary canal—filled with yolk globules—can readily be seen. In the head it is a narrow tube, but it widens out abruptly in the prothorax and from there on it gradually decreases in width until the beginning of the hind-gut in the eighth abdominal segment. The beginning of the hind-gut is marked by the origin of the Malpighian tubules and the presence of the pyloric valve. The hind-gut is a short, straight tube. The Malpighian tubules, two in number, extend anteriorly along the ventral abdominal wall until they reach the metathorax, where they are bent dorsally and then extend posteriorly for a distance of several segments.

The dorsal vessel is also seen quite readily and one can see the blood corpuscles enter it and watch their passage from one chamber to the other.

STAGE I.

The newly hatched larvæ (Fig. 1) measure about .88 mm. in length, not including the antennæ and the caudal filaments. The head is almost quadrangular in shape except that the anterior border is more or less rounded off. From the anterior border of the head between the antennæ arises a small projection, (the clypeo-cephalic prolongement of Lestage). The head is .16 mm. wide and .14 mm. long. It is almost as wide

as the prothorax and rather wider than the meso- or the metathorax. The head bears five ocelli; an anterior pair, a posterior pair, and one unpaired median ocellus at the base of the clypeocephalic prolongement. All are approximately equal in size and circular in outline.

The antennæ (Fig. 5) .28 mm. in length, are composed of five segments of which the first is the shortest and of uniform thickness throughout, the second is rather longer than the first and increases in thickness towards the apex. All the three remaining segments are approximately equal in length and each about twice as long as the second. The second and third segments each bear one bristle near the apex, the fourth and fifth each has a pair of bristles in the same relative position.

The thorax measures .26 mm. in length and its three divisions show very plainly. The prothorax is slightly wider, as well as longer than either the meso- or the metathorax.

The abdomen measures .48 mm. in length and is composed of ten segments. Each of these is very distinct except the first one, which is so intimately joined to the metathorax that it is rather difficult to make out the presence of ten segments. The segments decrease in width, but increase in length from the first to the tenth. The length of the last four segments is half that of the entire abdomen.

The last abdominal segment bears three caudal filaments, (Fig. 6). Each of these is .32 mm. in length and composed of four segments of which the first three are approximately equal in length and the three together are slightly longer than the fourth. The first and second each has a finely toothed ridge near its distal end. The latter also bears one bristle near the apex. The fourth segment tapers down to a point and bears one terminal bristle. In *Ephemera simulans* the last segment bears two terminal bristles.

The fact that the lateral and the median filaments are equally well developed in Stage I distinguishes *Hexagenia bilineata* from several other species that have been described. Lubbock (1866) found that in the first stage of *Chloen dimidiatum* the two lateral filaments were composed of nineteen segments each. The median filament appeared as a minute knob in the second stage and did not show segmentation until the larvæ reached the sixth stage. Vayassiere (1882) found that in *Heptagenia longicauda* the median filament is much shorter than the lateral ones. Gros ('23) found just the opposite

relation in *Ecdyonurus forcipula*. Murphey found that in *Baetis posticatus* the median filament does not appear until the fifth stage.

The three pairs of legs (Fig. 4) are all the same and are already well developed; serving as organs of locomotion. The coxa and the trochanter are very short and well defined. The femur is likewise well developed and its length approaches one-third the length of the entire leg. The tibia is almost as long as the femur, and it bears two bristles. The tarsus is about half as long as the trochanter and likewise bears one bristle. Each leg terminates in a long, heavy claw.

The mouth-parts are in the first stage already fairly well developed. The labrum (Fig. 14) is a simple quadrangular shield bearing on the dorsal side of each anterior angle a well developed bristle. The mandibles (Fig. 8) show two well developed canines on the outer anterior border and a well marked molar surface on the inner edge. The maxilla (Fig. 7) bear a number of strong bristles on their free ends and show a slight development of the palpi. The labium (Fig. 9) as viewed from the ventral side appears like six lobular projections arising from a rectangular base. The two lateral lobules, destined to become the labial palpi, are a little longer than the remaining lobules which form the ligula, and each bears two small bristles, one near and one at the distal end.

This first stage, which lasts about four days, showed as far as I could discover, no trace of either trachea or gills.

STAGE II.

Specimens of this stage measure about (length not constant) .96 mm. in length. The antennæ have increased by the addition of a sixth segment and now measure .30 mm. in length. The caudal filaments have added one segment and are .36 mm. in length. The legs bear two additional bristles on the tibia and one on the femur. The ocelli show no modifications. The front of the head is modified by a decided increase in the clypeo-cephalic prolongement. However, the most important change has taken place in the abdomen. Along the posterior angles of segments 2 to 7 the gills (Fig. 2) have made their first appearance as little evaginations of the body wall. Apparently the gills arise from the dorsal part of the segment. There seems to be considerable difference of opinion on the origin of

the gills in Ephemeridæ. It has generally been assumed that they are pleural or even ventral in their formation. B. Duerken ('07), however, maintains that they arise from the tergi, while Carl Borner ('09) has claimed that they are modified coxa. On segments eight to nine no gills appear, but a long hair arises in the positions occupied by the gills in the preceding six segments.

The simultaneous appearance of six pairs of rudimentary gills in the second stage is remarkable and it distinguishes *Hexagenia bilineata* from most other species in which the early stages have been studied. Vayssiere found that in the first stage of *Heptagenia longicauda* rudiments of gills appeared on segments four and five. In the second stage those of the third and sixth segments appeared well formed, whereas, those of the second segment were rudimentary. Not until the third stage were six pairs of gills visible. In *Ecdyonurus forcipula* Gros ('23) found that gills appeared on segments five and six in the second stage and on four and seven in the fourth stage. It was not until the sixth stage that six pairs of gills were present. In *Chloen dimidiatum* Lubbock found no traces of gills until the third stage, when they were present on segments two to six, those on the third and fourth showing the highest degree of development. Heymons ('96) gives a figure of a young larvæ of *Ephemera vulgata* that shows an equal development of gills on segments two to seven. He does not say what stage it is, but from its appearance and comparing it with my larvæ of *Ephemera simulans*, I conclude that it is not beyond the second stage. Murphey ('21) states that in *Baetus posticatus* "gills show as tiny out-pockets of body wall," but does not mention the number.

In my larvæ of *Hexagenia bilineata* of the second stage the gills are all in the same stage of development and there is yet no trace of the seventh pair. No traces of tracheæ are found in either the gills or the body cavity.

The mouth parts of the second stage differ but little from those of the first stage. The canines and molar surfaces of the mandibles (Fig. 10) as well as the bristles and the palpi of the maxilla (Fig. 11) have increased in size. The labial palpi (Fig. 12) are now definitely separated from the ligular portion of the labium and are composed of two or three segments. The hypopharynx (Fig. 13) was not studied in the first stage,

although it undoubtedly exists there. It is in this stage composed of one median lobe and two lateral lobes which partially overlap the median lobe.

This second stage, like the first stage, lasts about four days.

STAGE III.

The antennæ show no change except a small increase in length. The caudal filaments have added two segments. The legs bear six hairs on the tibia and three on the femur.

There are still only six pairs of gills, but they are much larger (Fig. 14) and have become irregular in outline. The division between the gills and the abdominal segments is now much more definite and partakes of the nature of a joint. The gills move freely in an undulating manner.

In this stage the tracheal system makes its first appearance. Two longitudinal trunks, more lateral than median, extend the entire length of the body. Branches extend into the antennæ, towards the eyes, and towards the mouth parts. Branches extend also into the legs and to the muscles of the thorax. In the abdomen, branches segmental in their arrangement, are given off to the abdominal organs of the first eight segments and to the gills on segments two to seven. In the tenth abdominal segment each longitudinal trunk gives off a branch to the corresponding lateral caudal filament. I have been unable to establish definitely the origin of the tracheal branch of the median filament, but, presumably, it is similar to that in the damsel-fly, i. e., the two longitudinal trunks, after giving off the branches to the lateral filament, join and extend into the median filament.

I was unable to determine the length of the third stage as all my specimens died before the end of it. It lasts, however, at least as long as the previous stages.

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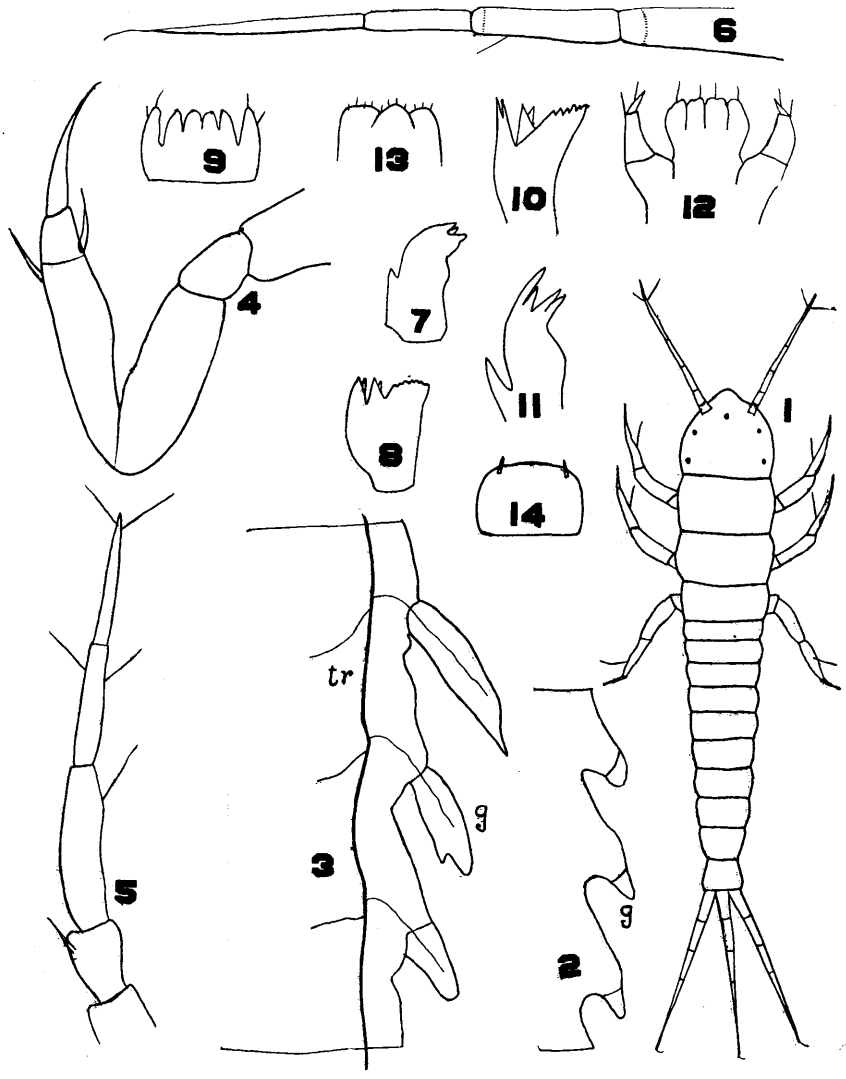
EXPLANATION OF PLATE.

Fig. 1. Larva of the first stage.
Fig. 2. Right half of abdominal segments 2–4 of the second stage showing beginning of gills.
Fig. 3. Right half of abdominal segments 2–4 of the third stage showing further development of the gills and the presence of trachea.
Fig. 4. Dorsal view of a leg of stage 1.
Fig. 5. Antenna of stage 1.
Fig. 6. Caudal filament of stage 1.
Fig. 7. Dorsal view of maxilla, stage 1.
Fig. 8. Dorsal view of mandible, stage 1.
Fig. 9. Ventral view of labium, stage 1.
Fig. 10. Dorsal view of mandible, stage 2.
Fig. 11. Dorsal view of maxilla, stage 2.
Fig. 12. Ventral view of labium, stage 2.
Fig. 13. Hypopharynx, stage 2.
Fig. 14. Dorsal view of labrum, stage 2.

Note: Fig. 1, X80; Figs. 2–14, X265. Abbreviations: g, gills; tr, trachea; lp, labial palpi.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