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A Method of Rearing *Hexagenia* Nymphs (Ephemera).

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No one has ever succeeded in rearing mayflies of the genera *Hexagenia*, *Ephemera*, or their relatives through an entire life cycle. Wiebe, 1926, has described the first three larval stages of *Hexagenia bilineata* Say. In view of later taxonomic work that has been done on *Hexagenia*, it seems probable that this is a misidentification and that Wiebe was dealing with *Hexagenia occulta* Walker. Neave, in 1932, incubated the eggs of *Hexagenia occulta* and then from a statistical study of nymphs collected from Lake Winnipeg decided that the complete life history, with limited exceptions, took two years. Ide, 1935, reared the first 11, the 13th, and the last 8 instars of *Ephemera simulans* Walker, but missed the intervening stages. Many other workers have hazarded guesses as to the length of the life cycles of these burrowing forms, but they have not had sufficient evidence to establish their statements.

There seems to be fairly general agreement on the following points: (1) that the eggs of the burrowing species can be obtained by stripping and can be readily incubated; (2) that the later stages, up to and including the emergence of the subimago, can be successfully handled under experimental conditions; (3) that the rate of incubation of the egg, the development of the nymph, and consequently the length of the life cycle are dependent mainly upon the external temperature. Thus the main difficulty seems to be that no one has devised a suitable method of handling the nymphs during the younger and intermediate stages, at the same time keeping them in a habitat similar to their normal environment. The methods recorded in the following pages seem to offer a solution to this difficulty.

At Lake Wawasee, Indiana, *Hexagenia occulta* usually emerges in great numbers at various times during the month of July. These swarms appeared in 1927 on July 3, 6, 13, 18, and 27th. Scattered specimens were collected during the last week of June and the first week of August. The subimagoes appear in the early evening, at which time they are positively phototropic and collect in great numbers around electric lights along the shore. The next day they can be found on the under sides of leaves, in tall grass, cat tails, and other forms of vegetation. Often they are located several hundred yards from the shore of the lake.

During the afternoon, the subimaginal skin is cast. Just at dusk the imagoes appear for the nuptial flight. The males first collect in groups, usually near the shore, and engage in the nuptial dance. By the time the majority of the females have joined them, it is almost dark and they are all concentrated near the shore. After mating in the air, the females fly out over the lake and drop two cylindrical packets of eggs.

On July 3, 1927, specimens in copula were collected from a swarm and the females stripped of their eggs. The packets of eggs were dropped into quart jars which were about half full of water. Upon touching the water, the egg packets immediately dissolved and the individual eggs fell to the bottom. The eggs apparently are coated with a sticky substance that hardens in a short time, for after a few minutes they can be removed only by force. Four such cultures were collected. Two of the jars were partially immersed in a small stream, in a completely shaded location. The others were kept in the building that served as a laboratory, where the temperature was much higher. In the latter cultures, the nymphs emerged after 15 days and in the former they emerged after 20 days.

The newly hatched nymphs were observed to be negatively phototropic, and since they belonged to a species whose nymphs burrow they were assumed to be positively thigmotropic. Several cake pans 4" x 4" x 8" were procured and filled about half full of mud from the bottom of a small stream. This mud was similar to that of the lake shore, but was taken from

a locality where no females had been observed to oviposit, nor had any individuals been seen mating in this vicinity. The mud was carefully gone over to remove any large organisms, especially predaceous ones. Two days after the nymphs emerged, they were placed in the pans and kept in a cool place for 12 hours. At the end of this period the pans were immersed in a gently flowing stream. Thus the nymphs were in a habitat similar to their normal one, and yet in order to escape they must leave the mud and swim over the edge of the pan. This they did not do.

From time to time, the pans were taken from the water and a sample of the mud removed. This was put into a white enamelled pan with a small amount of water and carefully searched for nymphs. Because of the small size of the nymphs, this was difficult at first, but within a few weeks they had reached such a size as to be easily located (see chart).

At the end of August a large washtub was partially filled with mud and all of the cultures added to it. The tub was then placed in an unused pond of the State Fish Hatchery at Lake Wawasee. This pond was supplied with fresh water by a natural spring and an inlet from a lake. On returning to Lake Wawasee on October 15, 1927, it was found that there were a great many nymphs in the tub. They had grown enormously during the intervening period (see chart). It was impossible to see the culture again until June of 1928. At that time there were no nymphs in the tub. Whether they had emerged, migrated, died, or had been killed by predators, it is impossible to say.

The average length of the newly hatched individuals was approximately 0.9 mm., whereas full grown nymphs vary from 18 to 27 mm. for males, and from 20 to 30 mm. for females. An inspection of the table shows that the specimens in the cultures grew rapidly, but that within a group of the same age a great amount of disparity in size was soon observable. Furthermore, within three months some of the individuals were half grown. It seems difficult to conceive that these specimens would not have ordinarily emerged the following summer. In

point of fact, there seems to be no reason why a species in the northern part of its range might not take two years to mature, while in the southern part of its range one year would be sufficient.

The following table gives a fair picture of the rate of growth of the specimens in the cultures. Unfortunately the number of specimens collected was not large.

Age-days from hatching to preserving	Number Specimens	Average Length	Minimum Length	Maximum Length
10	3	1.5	1.5	1.5
14	2	1.75	1.5	2.0
16	11	2.61	2.25	3.0
21	2	2.8	2.25	3.75
26	1	3.75	3.75	3.75
28	3	3.2	2.5	4.0
30	2	3.5	3.0	4.0
33	7	4.8	4.5	5.5
88	16	11.3	7.5	16.0

SUMMARY.

(1) A method has been presented for rearing the nymphs of burrowing mayflies. The method seems entirely feasible provided the investigator has facilities where he can watch his cultures over a period of time.

(2) In view of the fact that the rate of development is mainly dependent upon temperature, there seems to be no reason why the length of the life cycle should not vary in different parts of the range within which the group is found.

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