# SWIMMING AND BURROWING ACTIVITIES OF MAYFLY NYMPHS OF THE GENUS HEXAGENIA[1]

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The fact has already been established by bottom fauna studies (Adamstone, 1924; Rawson, 1930; Neave, 1932) that lake dwelling species of *Hexagenia* are inhabitors of soft, organic muds of deeper-water areas contiguous to the shallower sandy or rocky littoral region. Although specimens may be found on a sandy bottom or on a bottom of fine sand and mud intermixed, by far the greater numbers occur on bottoms of a distinctly soft, muddy character. As no investigations have been made on the ability of *Hexagenia* to burrow in various kinds of bottom, and since the character of the bottom is considered by the writer to be of primary importance as a factor influencing distribution of these nymphs as found in Douglas Lake, Cheboygan County, Michigan, a series of experiments was conducted.

## FREE-SWIMMING ACTIVITIES

*Hexagenia* nymphs are not strong, agile swimmers. Their swimming ability is easily shown by artificially circulating the water in an aquarium containing specimens. The nymphs appear passively rheotropic, orienting themselves as best they can facing against the current, at the same time performing the characteristic swimming movements and even then they are carried along almost completely at the mercy of the current. In swimming the nymphs hold the fore legs together anteriorly. The fore tarsi touch each other on the median line just anterior to the mandibular tusks. The middle legs are directed anteriorly, held close to the sides of the thorax and head, and overlap the bases of the fore legs. The hind legs are directed posteriorly, and are held arch-like, but closely pressed to the dorsum of the first three abdominal segments, and the distal tips of the femora almost touch over the middorsal line. The head and thorax are usually arched ventrally thus causing the anterior portion of the body to be directed downward. In a dish containing water this usually results in the nymph constantly striking the bottom of the container or swimming along with the fore legs touching and with the waving abdomen at a slight angle above the bottom. The nymph may turn to the right or left by tilting the body slightly toward the opposite side. Should the nymph in swimming turn entirely over on its back, it leaves the bottom and comes toward the surface. This tilting or turning over of the body at times results in a circular or corkscrew path. If the nymph, while swimming in a straight line, elevates the head and thorax slightly, the course changes to upward or becomes parallel to and just above the bottom. The lateral tails, ordinarily spread apart at about a 45° angle with the

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[1] Contribution from the Biological Station and Department of Zoology, University of Michigan.

median line when the nymph is at rest, appear to be a distinct aid in swimming. During the first swimming movements the outer tails are brought together toward the median one thus causing the setal fringes of all three to overlap and forming a more proficient propelling structure. The bringing together of the lateral tails seems to be carried out by intentional muscular movement on the part of the nymph preparatory to swimming and is not due wholly to current pressure from the sides of the nymph as it passes through the water. Swimming is accomplished through strong dorso-ventral undulations of the abdomen. The abdomen was not observed to sway laterally.

The gills are waved during swimming. These waves begin anteriorly and progress posteriorly. The gill movements made in swimming are not identical to those made while the nymph is resting. In swimming the gill motion appears to be a result of abdominal movements as a whole and not due to contractions of the gill muscles used to move them when the body is at rest. The gills do not extend laterally at right angles to the body when the nymph is at rest or swimming, but are directed upward and curved posteriorly straight back over the abdomen or slightly toward the middorsal line.

## BURROWING ABILITY

A large, deep aquarium containing water was supplied with soft bottom mud from about 13 m. depth and allowed to settle. The resulting bottom deposit was then further consolidated by gently jarring or shaking the aquarium from side to side. As a result the heavier particles settled through toward the bottom while the lighter, more or less flocculent materials remained near the top, thus more closely simulating natural conditions in the lake. After the mud had thoroughly settled and the surface-water had cleared, a number of nymphs, one-half to three-fourths grown, were introduced and their activities observed. These nymphs immediately settled gently to the surface of the mud and began swimming by means of strong, undulating, dorso-ventral movements of the abdomen. The fore and middle legs were held together anteriorly, and the head and thorax were directed downward. While swimming in this manner the body is so tilted that the anterior end is slightly lower than the posterior, consequently, entrance into the mud was made. Digging motions by the fore legs were then begun, and the strong, steady, undulating abdominal movements ceased, except for occasional waves which propelled the nymph forward at intervals after the way had been cleared by the fore legs. The gills were kept in constant motion during the digging process, producing a current of water which passed posteriorly over the body and out through the burrow opening. Mud particles thrown backward by the fossorial legs were picked up by this current and transported from the burrow. After burrowing to a depth of 10–15 cm., most of the digging movements were suspended and only gill motion continued. As long as the legs were in motion the sides of the burrow constantly caved in and the loosened particles were carried out by the water current. This caving in subsided as soon as the leg movements ceased. However, the mud in front of the nymph continued to be drawn backward over the gills by the current and carried out through the burrow opening behind the insect. The gill movements at this time became perceptably stronger, thus causing the mud particles directly in front of the nymph to be drawn toward it and swept by the current over its back. As this process continued, a caving in of the mud from the surface along an upwardly curved line was initiated. A current of water was finally produced in front of the nymph along this curved line which cleared a passageway in the mud and formed the anterior portion of the burrow. The water current also provided the nymph with a source of oxygenated water. The front and back ends of a burrow were easily distinguished since the former had a smoothly rounded edge, while the posterior one had a small mound of mud surrounding it. Branches from the original burrow were made in much the same manner. Nymphs were not observed to leave their burrows to come to the mud surface either during the day or at night. Attempts to make artificial tunnels in the mud by the insertion of various objects met with no success, for as soon as the object was withdrawn, no matter how slowly and gently, the sides immediately caved in. A short time after a nymph had deserted a burrow for a branch or if gill movements were suspended for a time, collapse of the walls occurred. The burrow was cleared of mud again when gill movements were resumed.

In order to determine the ability of nymphs to burrow into various kinds of bottom found in Douglas Lake, the following experiments were performed:

*Experiment No. 1.*—Clean sand, collected near the shore line, was placed in a large culture dish and covered with water. Several medium- to large-sized nymphs were introduced into it. They immediately began the typical swimming movements as described above, trying to gain entrance into the sand. All efforts to penetrate the sand were futile and, as the nymphs grew progressively tired, swimming gradually slowed down and finally ceased. They remained quiet for a short time, moving the gills intermittently and making no effort to hold to the sand with their claws. They were easily pushed to one side or turned completely over by another nymph swimming by and usually made no effort to right themselves at the time. After some time, swimming was resumed and further burrowing efforts were made. Some nymphs would occasionally stop swimming and begin digging with the fore legs, pushing the sand grains laterally. However, very little headway was made due to the constant tumbling of the sand grains back into the small depression as it was deepened. A solid footing for thrusting the head into the depression could not be obtained on the loose sand. Undulations of the abdomen were of no aid to the nymph in forcing the anterior portion of the body into the sand.

When a small stone (1–3 cm. diameter) was placed on the sand, a nymph coming in contact with it began to burrow. By the united efforts of digging with the legs, thrusting the head under the stone by abdominal movements, pushing downward with the dorsum of the thorax braced against the underside of the stone, and pushing with the hind legs, a cavity was made sometimes large enough to conceal only the head and thorax leaving the abdomen exposed, at other times large enough to conceal the entire body of the nymph. The nymph then remained in this cavity for a time, usually making no further attempts to burrow. Another nymph, trying to burrow in the same place, either forced the first one to leave its burrow or caused it to move further on under the sand until it was no longer under the stone. In this event the latter did not remain completely submerged but came close enough to the surface so that the gills were exposed and might be moved freely leaving the rest of the body still covered by a thin layer of sand grains. A nymph covered in this manner remained thus for some time but would ultimately break out and begin swimming around the dish again in seeming effort to find a more suitable environment.

If covered artificially with sand, a nymph usually remained quietly submerged for a short period and very little if any gill movement was observed. Finally digging started and the nymph came up near the surface of the sand as described above. When covered by a thick layer of sand, the nymph did not escape by digging and soon died.

*Experiment No. 2.*—In order to determine if the size of sand grains were significant in the ability of the nymphs to burrow, some of the sand from the first experiment was sorted by means of a 35 mesh (opening 0.417 mm.) Tyler Standard Scale screen into two grades: (1) sand retained by the 35 mesh screen, and (2) sand which passed through it.

TABLE I

PHYSICAL ANALYSIS OF BOTTOM MATERIALS, EXPERIMENT NO. 3

Sample of 500 cc. washed through a set of 6 Tyler Standard Scale screens. Measurements of wet volume. Loss in washing, 35 cc.

| Meshes per Inch | Size of Mesh in mm. | Materials Retained by Screen in cc. | Percentage of Total | Materials |
|---|---|---|---|---|
| 4 | 4.699 | 2 | 0.4 | Shells and sticks |
| 8 | 2.362 | 4 | 0.8 | Large marl flakes |
| 14 | 1.168 | 18 | 3.6 | Mostly marl flakes |
| 35 | 0.417 | 128 | 25.6 | Coarse sand and marl |
| 65 | 0.208 | 275 | 55.0 | Fine sand and marl |
| 100 | 0.147 | 33 | 6.6 | Very fine sand and marl |
| Pan | ............ | 5 | 1.0 | Finest sand and marl |

It was found that nymphs could not burrow into either of these two grades of clean, sorted sand. Their reactions on both grades were the same as described in the first experiment.

*Experiment No. 3.*—Since nymphs were sometimes found in more or less sandy areas in shallow water, their burrowing ability in this type of bottom was tested. A sample of sand was collected at a depth of 1.5 m. from a place where the nymphs were known to occur. Nymphs were placed on this sand in a large culture dish containing water. They had some difficulty burrowing into the sand but all of them finally gained entrance and remained under it. From one end to the other, the burrows were made almost entirely by digging with the fossorial legs and were relatively short and shallow. The time required to complete a burrow was longer than it was for those made in mud. A 500 cc. sample of this sand was then graded by means of a set of six Tyler Standard Scale screens in order to determine more exactly the composition of the sand. From the data given in Table I, it may be seen that the principal constituent, other than sand, is marl.

That sand and marl retained by the 35 mesh screen and that which passed through were used for further experiments. It was found that nymphs could burrow very readily into the fine sand and marl which passed through the 35 mesh screen. However, only about 50 per cent of the nymphs placed on coarse sand and marl retained by the 35 mesh screen could burrow into it by deliberate digging movements.

Clean sand was placed in a dish and covered with a layer of marl. It was found that nymphs burrowed into the marl but not into the sand layer below. If the same sand and marl were intermixed, the nymphs burrowed readily into the mixture.

*Experiment No. 4.*—Between the sandy bottom in shallow water and the muddy bottom of deep water, a zone occurs in which the sand and mud are intermixed, and the mud forms a comparatively thin layer on top. Dredgings were taken in this intermediate zone at 6–10 m. water depth. This material was placed in a dish containing water and the sand settled quickly to the bottom allowing a layer of mud to form on top. When nymphs were placed on this sandy mud, they burrowed into it readily. A 500 cc. sample of this same mud was graded using the Tyler screens. The results are given in Table II and show that finely divided organic detritus is the main constituent other than sand.

TABLE II

PHYSICAL ANALYSIS OF BOTTOM MATERIALS, EXPERIMENT NO. 4

Sample of 500 cc. washed through a set of 6 Tyler Standard Scale screens. Measurements of wet volume. Loss in washing, 56 cc.

| Meshes per Inch | Size of Mesh in mm. | Materials Retained by Screen in cc. | Percentage of Total | Materials |
|---|---|---|---|---|
| 4 | 4.699 | 3 | 0.6 | Wood |
| 8 | 2.362 | 4 | 0.8 | Wood, shells, stones |
| 14 | 1.168 | 6 | 1.2 | Wood, small stones |
| 35 | 0.417 | 140 | 28.0 | Coarse sand, organic debris |
| 65 | 0.208 | 253 | 50.6 | Fine sand, organic debris |
| 100 | 0.147 | 27 | 5.4 | Very fine sand, organic debris |
| Pan | ............ | 11 | 2.2 | Finest sand, organic debris |

Clean sand was then placed in a dish and covered with a layer of mud with no intermixing of the sand and mud. Nymphs placed on this burrowed into the mud but only one specimen was observed to enter the sand layer. When this same sand and mud were thoroughly mixed, nymphs easily burrowed into it.

## DISCUSSION

The series of experiments described above show that the character of the bottom is one of the most important environmental factors influencing the distribution of *Hexagenia* nymphs.

Morgan and Grierson (1932) seem to have definitely established experimentally that the tracheal gills of *Hexagenia recurvata* Morgan are respiratory organs and that nymphs from which the gills had been removed showed little or no efforts to burrow but remained on the surface of the mud. Wingfield (1939) in his discussion of the experimental work on the respiratory function of the tracheal gills of mayfly nymphs, stated that, "nothing is known of any relation between function and habitat."

At least two important functions of the gills of *Hexagenia*, besides respiration, have been brought out in the author's experimental work. First, they are used indirectly to build the anterior portion of the burrow made in mud and to keep the entire passageway clear of mud particles once it is built. The fact that the walls of the burrow begin to collapse after gill movements cease seems to indicate that the integrity of the burrow is somehow dependent upon the current of water. It may be true that in the natural environment the compactness or adhesive quality of the mud is sufficient to sustain the walls of the burrow at depths greater than 8–10 cm. from the mud surface. However, the uppermost stratum of mud in Douglas Lake is almost always composed of flocculent, semisuspended particles which do not possess the compactness of the deeper mud. Second, the gills insure a more or less steady supply of oxygenated water for use in respiration. The oxygen supply in the water surrounding the nymph would undoubtedly be rapidly exhausted were it unable to renew the supply of water. It was stated under Experiment No. 1 that when a nymph was covered by sand, the gills did not move freely. This was due to the weight of the sand particles which constantly fell back onto them almost as soon as they were pushed away; consequently, the nymph was forced to come to the surface or suffocate. In Morgan and Grierson's work, it may be that the reason the nymphs made no effort to burrow after their gills had been removed was because they had no means of obtaining a fresh supply of water in the burrow and would therefore suffocate. Also, without gills they would have no way of keeping the burrow clear, even if they could build it.

It is known that the nymphs of *Hexagenia* do not occur in the profundal regions of Douglas Lake (Eggleton, 1931), and they do not extend beyond 15 m. depth. This is also substantiated for other large bodies of water in the bottom fauna studies of Adamstone (1924), Rawson (1930), and Neave (1932). One of the significant reasons for this limited depth distribution seems to be indicated by the dissolved oxygen relations in the South Fishtail depression of Douglas Lake. From the physico-chemical data published for Douglas Lake by Welch (1928), and Welch and Eggleton (1932; 1935), it may be seen that in the South Fishtail depression, beginning about the first of July and continuing through the summer, the normal limits of the thermocline vary within about 12 to 16 m. In these data it is also shown that there is a distinct and rapid drop in the dissolved oxygen content of the water between the upper and lower limits of the thermocline, the oxygenless zone usually beginning at about 20 m. Thus, there appears to be a correlation of the habitat occupied by the nymphs (11–13 m.) with the limits of the thermocline and the amount of dissolved oxygen present. From the selected data given for the years 1929–1933 (Welch and Eggleton, 1932; 1935) the average drop in dissolved oxygen within the thermocline limits was 4.4–1.4 cc. per liter. Therefore, at depths greater than 15 m. the nymphs in their burrows would have difficulty obtaining sufficient oxygen from the available water. The character of the bottom materials beyond 15 m. is not sufficiently different to lead one to the conclusion that this alone would preclude the nymphs from migrating into greater depths.

The preceding experiments also show that the nymphs cannot burrow into clean sand taken from the wave-washed area just below the water line but can burrow into marly or muddy sand taken at greater depths. When the marly and muddy sand grains were carefully examined under a microscope, their otherwise smooth surfaces were found to be coated with a thin layer of either marl or fine particles of mud. Thus, the marl and mud appear to act as binders or adhesives holding the grains in place and preventing them from falling back into the burrow once it is cleared. In the instance of the clean, more or less smooth sand grains there is nothing to hold them in place after they are piled up during the digging process. Consequently, they fill the depression as fast as it is dug. Tables I and II show that by far the greater proportion of the sand-marl or sand-mud was of the finer type, and therefore more suitable to the nymphs for burrowing than the coarse sand-marl or sand-mud. The fact that when the clean sand was mixed with either marl or mud, the nymphs could readily burrow into it further supports the conclusion that marl or mud affects the character of the sand in some way which renders it suitable for burrowing.

## SUMMARY

1. Free-swimming and burrowing activities of *Hexagenia* nymphs are described in general.
2. Gills are used for burrow construction and to maintain a more or less constant current of water through the burrow once it is built.
3. Burrowing experiments performed with nymphs on various kinds of bottom show that the character of bottom is one of the most important factors influencing distribution of *Hexagenia* nymphs.
4. The greatest water depth at which *Hexagenia* nymphs occur in Douglas Lake is correlated with dissolved oxygen relationships within the thermocline.

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