REPRODUCTION OF THE BURROWING MAYFLY, *HEXAGENIA LIMBATA* (SERVILLE), IN MICHIGAN [1]

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Burrowing mayflies of the genus *Hexagenia* are abundant in many lakes and streams throughout Michigan and constitute an important part of the bottom fauna in these waters. Because of the importance of the nymphs and winged stages as fish food and fishing bait,[2] a study of the biology and economic importance of various species which occur in the state was undertaken by the writer under a fellowship sponsored by the Institute for Fisheries Research, Michigan Department of Conservation. Pertinent information concerning the reproduction of *Hexagenia limbata* (Serville) is presented in this report. Data were obtained during the years 1947-1949 at Pine and Gun Lakes, Barry County; Big Silver Lake, Washtenaw County; and portions of the Au Sable River in Crawford and Oscoda Counties.

The present taxonomic status of some forms of *Hexagenia* is neither entirely settled nor completely satisfactory. Hexagenids encountered in this investigation which have been described as species by earlier workers are *H. limbata* (Serville), *H. viridescens* (Walker), *H. occulta* (Walker), and *H. venusta* Eaton. Since these forms were originally described they have been considered by various authors as valid species, subspecies or varieties (Eaton, 1871, 1883; Ulmer, 1921; McDunnough, 1924, 1927; Ide, 1930; Neave, 1932; Needham, Traver and Hsu, 1935; Spieth, 1941; Lyman (MS); and others). In a survey of the genus, Spieth (1941) concluded that all forms in which the male imago has strongly hooked penes, marginated cross veins and uneven coloration of the membrane in the costal margin of the mesothoracic wing, should be placed in a single species, *Hexagenia limbata* (Serville). He recognized as subspecies *californica, limbata, occulta, venusta* and *viridescens,* and stated that all of them except *californica* occur in Michigan. Although male imagoes collected from the waters investigated show great diversity in size, intensity of color, coloration and color pattern, all have the strongly hooked penis and uneven coloration of

[1] Contribution from the Michigan Institute for Fisheries Research.
[2] Large *Hexagenia* nymphs are used extensively as fish bait in winter angling for yellow perch, bluegills, and other pan fish in Michigan.

the costal margin of the forewing. Imagoes collected from the forenamed lakes show that the coloration and color patterns described for the forms *occulta*, *viridescens* and *venusta* and intergrades between the three, are present at the same time and in a single mating flight. Imagoes from the Au Sable River show color phases which agree with descriptions for *occulta* and *viridescens*, and intergrades between the two. Since the populations appear to be completely heterogeneous in their color characteristics and without discernible differences in ecology, it has been concluded that although the various described forms can be recognized they do not merit subspecific rank and should be considered as color phases or varieties of a single variable species. Therefore the author chooses to regard the species considered in this study as *Hexagenia limbata* (Serville).

The color pattern of *limbata* in the lakes studied generally tends toward that of *H. occulta* as described by Needham, Traver and Hsu (1935), and of *H. limbata occulta* listed by Spieth (1941). In the Au Sable River the color of most imagoes is somewhat darker and dorsal and ventral abdominal color patterns more pronounced and extensive than in most lake specimens. It is possible that the stream form may eventually prove to be separable from the ordinary lake form on a physiological if not on an anatomical basis. It is worthy of note that many bait dealers who handle thousands of *Hexagenia* nymphs each winter fishing season profess to be able to recognize readily nymphs which come from streams and those which come from lakes. They maintain that the stream nymphs are darker in color, are much hardier and are better fish bait than the lake nymphs.

The variability of *H. limbata* is very marked when compared to other hexagenids often encountered in Michigan. *H. atrocaudata* McD., *H. rigida* McD., and *H. recurvata* Morgan are distinct species which appear to be quite stable and show little variation in coloration or in color pattern.

Egg Production

Eggs of *H. limbata* are ellipsoid and measure 0.16-0.19 by 0.28-0.32 millimeters. All have a reticular chorionic pattern, the strands of which run nearly straight. The number of eggs produced by individual females is of considerable importance in evaluating the probable success of reproduction of the species.

Only two literature references pertaining to the number of eggs produced by *Hexagenia* are known to the writer. Needham (1920) estimated the number of eggs to be upwards of 8,000. Neave (1932) counted the eggs of two *H. l. occulta* imagoes; one contained 3,631 eggs, the other 3,388. Size of these females was not recorded. In order to ascertain more exactly the number produced, actual counts were made of eggs carried by 24 female imagoes. Preserved specimens were used and examined under a binocular dissecting microscope. Total body length was carefully measured, the abdomen opened, and eggs counted when removed from the ovaries. In most cases it was possible to count the eggs from each ovary separately. Data secured are presented in Table 1. Total number of eggs varied between 2,260 and 7,684. The greatest difference between the number in each ovary of any individual was 213. It was at once obvious that a significant relationship existed between number of eggs and body length. Plotting length and number of eggs of each individual showed a positive correlation between body length and egg production (Fig. 1). A curve drawn by inspection

TABLE 1.—NUMBER OF EGGS PRODUCED BY *H. limbata* FEMALE IMAGOES.

Locality	Body length in mm.	Wing length in mm.	Egg count		
			Right ovary	Left ovary	Total
Pine Lake	25.5	22.0	——	——	4,356
Pine Lake	24.3	21.6	——	——	3,452
Pine Lake	21.9	18.0	1,580	1,684	3,264
Pine Lake	22.4	19.7	——	——	2,731
Pine Lake	21.9	20.8	——	——	2,575
Pine Lake	22.8	20.3	1,808	1,720	3,528
Pine Lake	19.9	18.2	1,111	1,243	2,354
Pine Lake	21.2	18.3	——	——	2,841
Pine Lake	20.0	19.4	——	——	2,695
Pine Lake	20.3	17.8	1,142	1,118	2,260
Gun Lake	30.3	28.2	——	——	7,684
Gun Lake	30.2	27.6	3,653	3,440	7,093
Gun Lake	24.7	23.6	2,207	2,333	4,540
Gun Lake	27.1	25.6	3,393	3,198	6,591
Gun Lake	24.8	23.1	2,039	2,005	4,044
Gun Lake	28.4	27.0	3,299	3,230	6,529
Gun Lake	23.1	23.0	2,123	1,942	4,065
Gun Lake	24.1	22.3	1,773	1,734	3,507
Gun Lake	24.8	22.7	2,669	2,758	5,427
Gun Lake	24.7	23.3	2,406	2,417	4,823
Big Silver Lake	23.6	21.5	1,844	1,987	3,831
Big Silver Lake	27.3	24.7	2,851	2,763	5,614
Big Silver Lake	26.0	24.0	2,204	2,210	4,414
Big Silver Lake	23.8	22.2	1,938	2,140	4,078

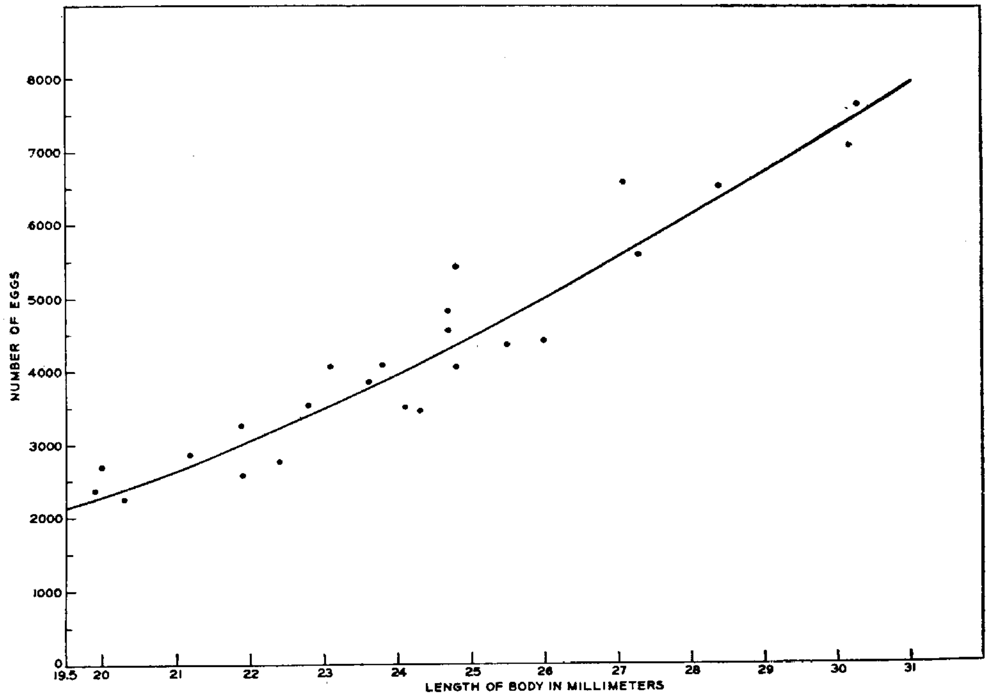

Fig. 1.—Correlation between length of body and number of eggs produced by *H. limbata* female imagoes.

indicates clearly that the relationship approaches that of a straight line. Examination of the figure shows that an average size female will produce about 4,000 eggs. Although no egg counts were obtained from females collected on the Au Sable River, there is no reason to believe the results would vary from the above.

EGG DEPOSITION

Mating activities of *Hexagenia* and many other species of mayflies have been adequately described by Morgan (1913), Needham (1927), Neave (1932), Needham, Traver and Hsu (1935), Spieth (1940), and others. However, the deposition of eggs, the number laid, percentage fertilized, and place and time of incubation have received little attention. Mating and ovipositing flights of *H. limbata,* which take place in May, June and July in Michigan, ordinarily occur at dusk or shortly after dark. Extensive night observations by means of a powerful spotlight supported published statements that females lay their eggs on the surface of open water in both lakes and streams. Eggs were deposited in the water in three different ways. The method employed most frequently was as follows: Once the females had copulated in the mating dance, which invariably occurred in the air along the margin of a lake and above a stream, they left the swarm and flew out over the water. After flying rapidly back and forth 10-20 feet above it for a few seconds to several minutes, the females simply plummeted erratically to the water surface. As they lay fluttering on the surface, the last two abdominal segments were raised sharply upward and the two egg packets quickly extruded by rhythmical contractions of the abdomen. Females picked up within 10-15 seconds after striking the surface had invariably discharged most of their eggs. Those which had thus "crash landed" usually drowned, for seldom were they able to right themselves and fly from the water. The second method, used by comparatively few individuals, was to light upright upon the surface, remain quiet for a few seconds, discharge a few eggs, then fly up again to repeat the performance. Some females which came to collecting lights during ovipositing flights were only partially spent. Since they easily discharged the remaining eggs when placed in contact with water, it is obvious they had been merely dipping to the surface. In the third method, observed only a few times, the female extruded the eggs while flying above the water and dropped the packets from a height of 10-15 feet. On several occasions females were seen carrying partially extruded egg packets as they plunged to the water surface. Upon contact with water the eggs separated and began to sink immediately.

Examination of various objects and material from the lake bottom showed that eggs found lodgment on aquatic vegetation, pieces of wood and small stones. Surface mud samples, obtained by utilizing a plastic tube ½ inch in diameter and 5 feet long, also contained numerous eggs, indicating that they were spread over the entire shoal area. Examination of sand from the water's edge during the height of the ovipositing season resulted in finding only 2 eggs, and suggests that few of them are washed ashore and lost. No attempt was made to obtain eggs at depths greater than 5 feet and nothing is known of the fate of those which sank into deep water. The fate of eggs released by ovipositing females in the Au Sable River was not determined.

Collection of eggs, deposited in Big Silver Lake, by means of submerged glass plates revealed that distribution of eggs on the lake bottom was very patchy. Four glass plates (total area 370 square inches), mounted individually in wooden frames with a stabilizing weight underneath, were placed at random on the bottom in an area about 100 feet square covered with water about 3 feet deep. Eggs which settled on the glass soon became firmly attached and the plates could be raised and handled without losing them. These plates were in operation from June 20-27, 1947, during the period of *limbata* emergence and mating activities. Each day they were raised and all adhering eggs deposited the previous night removed with a small brush. The residue was examined under a microscope and the eggs counted. Average number secured was 336 per square foot of surface for the 8-night period. Quantity of eggs reaching the plates each night varied greatly, the smallest number during any night being 25 and the largest 310. Great variation was also noted in the number of eggs adhering to individual plates on any night. The indication was that eggs tended to reach bottom in clumps and were not distributed evenly over the bottom even in a comparatively small area.

TABLE 2.—TIME REQUIRED FOR EGGS OF *H. limbata* TO SINK TO BOTTOM OF A COLUMN OF STILL WATER 25 INCHES DEEP. WATER TEMPERATURE 76°F.

Trial	Approximate number of eggs	Time of sinking in seconds		
		First egg	Last egg	Median egg
1	9	152	164	160
2	20–30	150	192	155
3	50–70	150	180	160
4	20	170	190	177
5	60	175	192	180
6	100	165	194	176
7	40	160	177	165
8	50	162	177	166
9	50	150	187	178
10	75	160	197	176
11	50	152	177	157
12	50	150	175	160
13	50	153	177	157
14	200	155	192	160
15	100	157	184	174
Avg. time to sink 1 in.		6.3	7.4	6.7
Avg. time to sink 1 ft.		76.1	88.6	80.0

To determine the rate of sinking, a 1,000 cc. graduated cylinder was filled with lake water to a depth of 25 inches and allowed to stand for 2 hours. A black background and a strong light were so arranged that sinking eggs were clearly visible. Using a spatula, small lots of eggs, taken from the body of a newlyemerged imago, were placed on the water with a minimum amount of agitation. It was noticed that the surface disturbance influenced the start of sinking of individual eggs, but after sinking began it proceeded at a uniform rate. Time required for each lot of eggs to reach bottom was recorded (Table 2). Since eggs of each lot were scattered somewhat when placed on the water, they sank in a rather scattered group. Time required for approximately one-half the eggs to reach bottom is recorded in the table under the heading "Median egg." It was found that individual eggs sank at an average rate of 1 foot in 80 seconds, but small clumps of eggs settled at a faster rate, approximately 1 foot in 60 seconds. Four to 6 minutes were required for eggs to settle to the bottom after the water was stirred vigorously.

RATE OF FERTILIZATION

Eggs obtained from a number of female imagoes captured during ovipositing flights were incubated in aerated jars to determine the efficiency of natural fertilization. Eggs in various stages are readily recognized. Those which have hatched appear as empty shells; live embryos are translucent and the embryo is visible; dead embryos are usually brown or reddish in color; and eggs which apparently were not fertilized are dark or black. A count made after hatching had been completed (Table 3) showed that in all cases more than 91 per cent of the eggs hatched (avg. 96.3 per cent). Since some embryos died during incubation, the initial fertilization rate was slightly higher. The high fertility and the large number of eggs produced by individual females point to a high reproductive potential. In nature, however, the loss of eggs and very young nymphs must be enormous.

Unfertilized eggs obtained from 7 reared virgin female imagoes were incubated for a long period. Some embryonic development occurred in a few eggs but no nymphs hatched, implying that parthenogenesis does not take place.

Artificial insemination of eggs is easily accomplished by applying macerated sexual elements of a male to the exposed egg mass of a female (Neave, 1932; Needham, Traver, Hsu, 1935). The author resorted to artificial insemination 27 different times to secure fertilized eggs for various purposes. In some cases the female could be induced to discharge her eggs by gently stroking the ventral side of the abdomen with

TABLE 3.—EFFICIENCY OF NATURAL FERTILIZATION OF *H. limbata* EGGS AS SHOWN BY HATCHING EXPERIMENTS.

Origin of females	Lot number	Number females stripped	Total eggs examined	Number eggs hatched	Per cent eggs hatched
Pine Lake	1	1	209	200	95.7
Pine Lake	2	1	389	379	97.4
Pine Lake	3	1	517	510	98.6
Pine Lake	4	1	421	419	99.5
Pine Lake	5	12	761	742	97.5
Pine Lake	6	1	851	842	98.9
Pine Lake	7	1	926	905	97.7
Gun Lake	8	1	248	238	96.0
Gun Lake	9	1	686	626	91.2
Gun Lake	10	1	234	226	96.6
Gun Lake	11	50	1,621	1,511	93.2
Au Sable River ..	12	1	351	338	96.3
Au Sable River ..	13	15	436	427	97.9
Au Sable River ..	14	22	457	442	96.7
Au Sable River ..	15	1	269	265	98.5
Total		110	8,376	8,070	96.3

a dissecting needle. In most instances, however, it was necessary to dissect the ovaries. The macerated sexual organs of the male were then mixed with the eggs and allowed to stand for 1-2 minutes, after which the eggs were placed in water in glass containers of various kinds. About 45 seconds after coming in contact with water, they became sticky and began to adhere to the glass. At the end of 3 minutes all were firmly attached and remained so during incubation and hatching. This adhesive property made it very convenient to handle them since they remained in place at all times.

Results of artificial insemination and incubation varied greatly, for the percentage of eggs which hatched ranged between 2.7 and 88.6. Poor fertilization resulted when adults used were old and dying. *Imagoes and subimagoes proved to be equally fertile, showing that ova and spermatozoa are fully mature when the winged fly first emerges.* One attempt to fertilize eggs from a female subimago with the sexual elements of a last instar male nymph whose wing pads were quite dark was unsuccessful.

INCUBATION

Published accounts of the incubation period of *Hexagenia* vary greatly. No temperature records were given by Clemens (1913, 1915, 1922) when he stated that in two instances eggs of "*H. bilineata*" hatched in 36 days and in a third from 29-40 days. Eggs of *H. l. occulta* from Lake Winnipeg hatched in 17-19 days at temperatures ranging from 18°-23° C. (Neave, 1932). Spieth (1938) succeeded in hatching eggs of *H. occulta* in the laboratory in 14 days and in a nearby stream in 20 days.

Length of incubation is important since a short one means a longer growth period for newly-hatched nymphs during the remainder of the summer. Temperature is an important factor influencing rate of embryonic development. In the laboratory at temperatures ranging from 75°-95° F., hatching began in 11-14 days. Eggs incubated under temperatures ranging between 67° and 81° F. began hatching at the end of 18-22 days. Where eggs were scattered on the bottom of containers, hatching of all eggs was virtually completed within 4 days after it started. In other instances, to be discussed later, where masses of eggs had clumped together, newly-hatched nymphs continued to appear for many weeks. Eggs placed in 2-quart jars and submerged in the East Branch of the Au Sable River began hatching after 20-26 days at temperatures ranging from 62°-73° F.

Effect of temperature was determined more specifically in the following experiment. A portion of the eggs obtained from naturally fertilized females (Lots 2, 5, 10, Table 3) was placed in quart jars in a refrigerator (36°-40° F.) 8 days after incubation began. Jars were removed from the refrigerator 70 days later and the eggs allowed to incubate at room temperature (68°-80° F.). Hatching began 16 days later and was completed in about 4 days, total elapsed time since fertilization being 94 days. The total 26-day incubation period at room temperature was slightly greater than that for the controls (20 days) and the percentage of successful hatching was nearly the same. It can be concluded that embryonic development was extremely slow or that a state of dormancy existed while the eggs remained at 36°-40° F., and that no ill effect resulted from exposure to low temperatures. It is quite possible that eggs deposited in late summer in natural waters may remain alive over winter and hatch successfully when water temperatures rise in the spring. It is also probable that eggs which settle in deep water below the thermocline, providing sufficient oxygen is present, may require several months for embryos to mature.

A greatly prolonged incubation period of *H. limbata* eggs occurred in several instances under similar circumstances. Eggs stripped from 50 females captured at Gun Lake, July 1, 1947, were all placed in a 2-quart jar and incubated in the laboratory (Lot No. 11, Table 3). Hatching first began July 19, after 18 days of incubation. Within a week more than half of the eggs had hatched but a diminishing number of nymphs continued to appear until November 6, 1947, 98 days after the start of incubation. Circumstances surrounding the position of eggs in the incubation jar, later duplicated with similar results, seemed to influence drastically the rate of embryonic development. Examination revealed that in some areas on the bottom of the container, eggs were piled on top of each other 10-20 layers deep and solidly attached together by the naturally adhesive material around them. Those on the periphery of these masses, and those located singly or in layers no more than 3 eggs deep, hatched within 3 weeks. Embryos within these masses, however, developed very slowly, those nearest the center hatching last of all. Reasons for this delayed development are not known, but the conclusion must be reached that some condition resulting from the crowding or smothering of these eggs retarded their development. Even though these embryos developed slowly, most of them eventually hatched (Lot No. 11, Table 3). Similar results were obtained by covering eggs firmly attached to the bottom of jars with 2-3 inches of stream silt. In these cases development of embryos was greatly retarded so that hatching did not occur until 2-3 weeks after uncovered eggs in jars in the same stream had produced nymphs. These experiments suggest that eggs covered by silt in natural waters take much longer to hatch than those exposed, and that embryos may be killed from being buried. Prolonged hatching has been observed among eggs of other mayflies but details of the conditions of incubation were not given. Ide (1935) found that eggs of *Stenonema canadense,* incubated in a glass container, hatched over a period of 6 weeks and that there was evidence to indicate that eggs of *Iron pleuralis* remain in an unhatched condition in the stream for at least 4 months.

Effect of desiccation on fertilized eggs was determined in 9 cases by dividing eggs both artificially and naturally fertilized into two groups. One group, serving as a control, was placed in water immediately and the other dried at air temperature for periods ranging from 4-72 hours before being placed in water and incubated. In all cases, nymphs hatched from the control eggs but none were produced from, nor did any embryonic development occur in, the desiccated eggs.

SUMMARY

1. Examination of the ovaries of 24 female imagoes revealed that the number of eggs produced ranged from 2,260 to 7,684. A positive correlation exists between body length and number of eggs produced. An average size female produces about 4,000 eggs.

2. Eggs were deposited on the water surface in three ways. In most instances ovipositing females plunged erratically to the water surface, then discharged their eggs within a few seconds and drowned. Occasionally females alighted upright on the water, discharged a few eggs, took wing and repeated the procedure. In a few cases females extruded their eggs in mid-air and dropped the egg packets from a height of 10 to 20 feet.

3. Eggs deposited in lakes either become attached to aquatic vegetation and other solid objects or come to rest on the mud surface. Eggs sink in still lake water at a rate of 1 foot in 80 seconds.

4. Eggs obtained from 110 naturally mated females were incubated in the laboratory. The percentage of eggs which hatched ranged from 91.2 to 99.5 (average 96.3).

5. The incubation period is greatly influenced by water temperature. Eggs hatched within 11 to 14 days when water temperatures ranged from 75° to 95° F., and within 20-26 days at a temperature range of 62° to 73° F. Eggs kept at 36° to 40° F. for 70 days apparently lay dormant while refrigerated, but later hatched when returned to room temperature.

6. Eggs which were air dried for 4 hours or longer before incubation began failed to hatch.

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