

THE FUNCTIONS OF THE GILLS IN BURROWING MAY FLIES (*HEXAGENIA RECURVATA*)¹

(One plate)

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ALL immature May flies are thoroughly aquatic, but the burrowing ones probably remain longest in the water and have the most extensive equipments of tracheal gills. Even on slight observation, the gills of burrowers like *Hexagenia recurvata* appear to be organs of great respiratory efficiency, probably essential to life. But, the equally imposing supply of 58 tracheal gills of the caddis-fly larva *Macronema zebratum* on actual testing has appeared to be only accessory and advantageous rather than necessary to life, under ordinary conditions (Morgan and O'Neil, 1931). In view of this, these thoroughly aquatic May-fly nymphs were selected for inquiry, and the following observations were made on the functions of the gills of *Hexagenia recurvata* during the winter and early spring months.

HISTORY

The size and variety of form in the gills of May flies have attracted observation for a long time. In spite of this, few studies of their functions have been made; these are concerned both directly and indirectly with the significance of the gills in respiration. They deal with the ability of the nymphs to live without gills, and the associated question of the closed or open type of their thoracic spiracles, correlation of the gill area with the oxygen supply in the water, effects of carbon dioxide, and the influences of other physical and chemical conditions on the nymphs.

It has been shown by Cuénot (1925) and Remy (1925) that May-fly nymphs can live for 3 weeks or more without gills. In late spring (May) Cuénot removed the gills from 17 nymphs of *Cloëon dipterum*

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and 19 nymphs of *Ephemera vulgata*. One *Cloëon* nymph survived for 6 days; two *Ephemera* nymphs survived for 25 days. In late autumn (October), he took the gills from 10 more nymphs of *Cloëon*, 6 of which lived about a month and a half but were regenerating their gills within 35 days of the operation. Cuénot believed that the gill-less *Cloëon* nymphs might have secured oxygen by rising to the surface and taking air through their thoracic spiracles, which could easily have become permeable if they were not already open. He called attention to the necessity of the gills in seasons of warmth and activity and their unessential status during inactivity and in winter time (Cuénot, 1925). Commenting upon his own experiments that were similar to Cuénot's, Remy (1925) suggested that, after gill removal, nymphs might depend for at least 2 weeks upon oxygen already in their tracheae. He explained the high mortality following spring operations as due to the quicker exhaustion of the tracheal supply of oxygen. His own experiments on *Cloëon* led him to ascribe more respiratory importance to the gills than Cuénot had granted them. In experiments on gilled and gill-less nymphs briefly reported by Morgan and Porter (1920), young *Heptagenia* nymphs lived for about 5 months after gill-removal. These *Heptagenia* nymphs which were normally photo-negative became photo-positive after their gills were removed; and only 18 per cent of gill-less nymphs molted, whereas 70 per cent of the normals did so.

The question of the possible free passage of gas through the thoracic spiracles has kept reappearing. Graber (1874) reported bubbles of gas coming from the spiracles of May-fly nymphs; Heiner (1914) recorded similar bubbles escaping from thoracic spiracles of May flies when the temperature of the water was raised. No thorough study of the nymphal spiracles has yet been made, and their open or closed condition has not been actually demonstrated.

From the motion of the gills, leisurely in richly oxygenated water and vigorous where there was a poor supply, Babak and Foustka (1907) concluded them to be organs of oxygen intake. Dodds and Hisaw (1924) measured the gill area in species of May-fly nymphs living in diverse habitats, and showed that there is a definite correlation between gill area and oxygen content of the water about them. Numerous experiments were performed on normal nymphs of *Cloëon*

dipterum by Pruthi (1927) analyzing their habitats from the standpoint of respiration. His results indicated that May-fly nymphs can endure a very low concentration (1.0 cc. per liter) of oxygen, that the hydrogen-ion concentration was highly significant to them, but that the carbon-dioxide pressure was of greater importance and might be a guide to the suitability of the habitat.

MATERIAL AND METHODS

The genus *Hexagenia* contains the best known of all May flies, whose adults swarm in thousands over lakes and rivers, where, as nymphs, they but recently burrowed in the mud. *Hexagenia bilineata* is probably the most familiar and widely distributed of American May flies. The closely related *Hexagenia recurvata* of this paper is less known, for it emerges from small streams in May or earlier and does not fly in great swarms like the other species. The length of its life is not known; but fairly grown nymphs are common in October and through the winter, though they are rare in June and July. Nymphs of *Hexagenia recurvata* can be recognized by the long mandibular tusks and especially by the unbranched rudimentary first gill (Plate I, Fig. 4); the tusks of *Hexagenia bilineata* are shorter, and the rudimentary first gill is double, like a tuning-fork. Several hundred nymphs used for this and other work were collected from the same region of one stream, mainly from November to February; comparatively few were taken in March and April.

In the laboratory the nymphs were kept in running water in glass finger bowls, bedded with silt and sand from the nymphs' native stream, and tightly covered with cheesecloth. Early in the experiment crowding was found disastrous, and not more than 10 nymphs were usually housed in one bowl. In order to care for a large number of nymphs, five or six bowls were supplied from one faucet, the water being first piped into a large bottle from which it was carried to the individual bowls by glass siphon tubes. At intervals of 2 or 3 weeks scrapings of algae were stirred into the muddy beds of the bowls. The gills were usually removed from nymphs about a week after they were collected. The operated animals and their controls were then kept for varying intervals, sometimes for 5 months or more in the conditions described.

THE GILLS AND GILL MOVEMENTS

The equipment of gills in *Hexagenia recurvata* consists of 7 pairs of tracheal gills, one pair attached to each of the first 7 abdominal segments (Plate I, Fig. 1). On the first segment the gill is rudimentary, straplike, and single and can have little or no respiratory function (Plate I, Fig. 4). Those on segments 2-7 each have two leaflike divisions, the lamellae veined with tracheae and bordered by filaments which are threaded with them (Plate I, Fig. 3). Each pair of gills is jointed to a flexible eminence of the body wall, which bends with it.

Waves of motion pass backward over the gills sometimes too rapidly for the eye to follow but at other times in listless ripples. The gills may cease moving altogether, usually for a few seconds or for 1 or 2 minutes, though at times they may be quiet for half an hour. The motions vary with those of the nymph's body and also with the temperature and chemical content of the water. When resting, the gills are held almost upright or bent slightly backward (Plate I, Fig. 1). When waving, each pair bends downward and backward, then upward again. As a pair bends backward, the filaments of the opposite gills touch each other, instantly separating as the gills are pulled upward, the whole performance resembling the rapid opening and closing of a V. So far as observed, the gills move whenever the nymph is active, especially when it burrows. While digging, the nymph holds its front legs closely against its wedge-shaped head, then lunges forward, at the same time pushing these legs outward. Almost synchronously with this, its middle legs are pushed outward and backward and the hind legs backward. As its body wedges forward, the nymph fans the silt with its gills, opening and closing them in the V-shaped angle. Thus, their surfaces are cleared of the mud thrown back by the hind legs, and the water is continually circulated about them.

COMPARISON OF BEHAVIOR AND GENERAL CONDITIONS
OF NYMPHS WITH AND WITHOUT GILLS

Hexagenia nymphs which had their gills removed burrowed little or not at all. They stayed on the surface of the mud, their few spontaneous movements being slow and labored, but not otherwise

PLATE I

FIG. 1.—Dorsal view of full-grown nymph of *Hexagenia recurvata*. Living specimen. Length without setae, 25 mm.

FIG. 2.—Dorsal view of nymph showing black scars where the gills were removed. Gills were removed November 21, 1930; nymph lived till March 4, 1931. Length without setae, 17 mm.

FIG. 3.—Typical gill, with two lamellae, showing tracheae and filaments. Photographed immediately after removal.

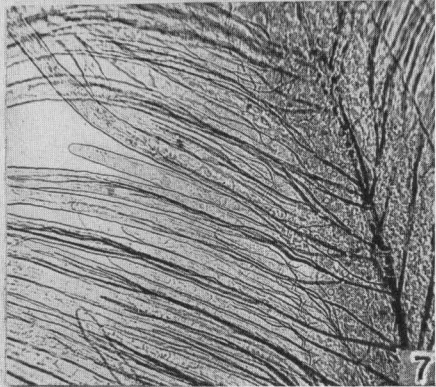
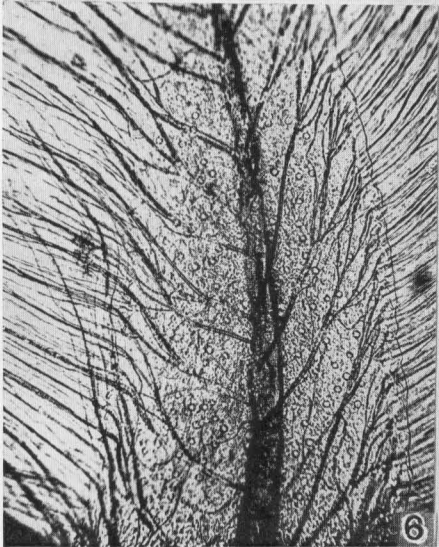
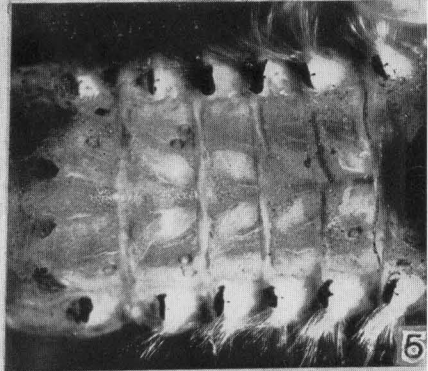
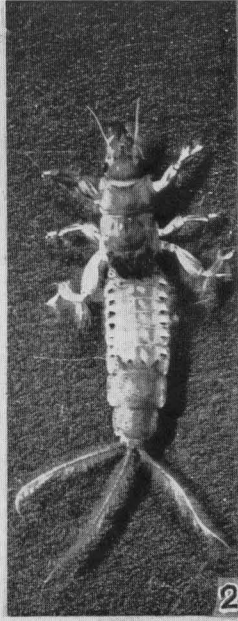
FIG. 4.—Rudimentary first gill.

FIG. 5.—Gill scars of specimen in Figure 2, showing hairs which protect the bases of the gills. The chitin overlapped by the gills is pale or transparent.

FIG. 6.—A section of the gill in Figure 3, showing the tracheal supply near the tip and in the filaments of one side.

FIG. 7.—Another section of the same gill.

PLATE I



strikingly different from those of the normal nymph. Though they were not disposed to burrow, their occasional digging showed that they were not disabled for the performance. In diffused daylight the control nymphs would dig into sand or mud rapidly, usually pausing only after the head and thorax were buried. Taken out of the light, they at once became more leisurely in their burrowing. Gill-less nymphs, on the other hand, showed little or no reaction to daylight, rested, or moved slowly over the mud surface, showing scant favor for the shaded side of the bowl where they were kept.

While gill-less nymphs showed little disposition to swim, they would do so when stimulated. When they took off from the mud surface, their postures were like those of the normal nymphs. So was their undulating progress through the water afterward. Although the difference in speed between normal and gill-less nymphs was striking, motion pictures of the two kinds of nymphs revealed little or no difference in swimming postures or in the preliminary digging of the burrow. When alighting on the mud, however, the gill-less nymphs were apt to land on one side or the other, often to roll over on their backs, showing their failure to balance themselves.

Normal nymphs lived well in the laboratory conditions earlier mentioned; and though a serious mortality often followed operations, nymphs whose gills were removed in November lived 21 weeks, while others operated upon in November and December lived 2 and 3 months. High mortality among the gill-less nymphs began in late winter and rose through February till nymphs operated in early April lived only a few hours. Even normal nymphs which had lived 3 to 4 months in the laboratory could not survive gill-removal in the spring. The greater sensitiveness was undoubtedly correlated with growth and changes preparatory to adult maturity. Normal nymphs were not easily affected by crowding, while the gill-less ones were very sensitive to it. Although no special account was taken of molting, a search for molted skins was made whenever food was put into the bowls. From early November through February, 5 skins were found among about 500 controls, but none were found in the same number of gill-less nymphs examined.

Nymphs that were collected after the middle of February had more prominent wing pads and larger reproductive organs. Such

symptoms of rising metabolism appeared not only in recently collected nymphs but to some extent in those which had been kept in the laboratory. A high mortality after operations also began to occur. Of 110 nymphs which were gill-less on February 23, only 13 were alive 9 days later, and immature nymphs were the only ones which survived at all. Freshly collected nymphs and those of long laboratory residence responded alike to the operation. On the other hand, the nymphs that had had their gills removed earlier in the winter lived on successfully and were mostly the subjects of the spring oxygen and carbon-dioxide tests (Tables I and II).

The control nymphs ate a moderate amount of food while in captivity. The stomachs of those killed from time to time through the year displayed the typical *Hexagenia* diet of diatoms, other algae, and organic débris. Microscopic sections of control nymphs which had been kept in the laboratory 5 months showed stomachs or intestines, or both, fairly filled with food débris. Examinations of gill-less nymphs usually disclosed small amounts of food, and microscopic sections of them showed the food canal only partly filled.

EXPERIMENTS TO DETERMINE THE DIFFERENCE IN OXYGEN
INTAKE BETWEEN NORMAL GILLED NYMPHS AND
OPERATED GILL-LESS ONES

These experiments were made to determine the importance of the gills in oxygen intake. In order to do this, the oxygen intake occurring when the gills were present was measured and compared with the amount consumed when the gills had been removed for a considerable time.

The oxygen determinations were made according to the Winkler method (Kemmerer *et al.*, 1923, p. 58; Shelford, 1929, p. 495; Theriault, 1925), and the measurements taken in the Thompson-Miller apparatus for the micro-determination of dissolved oxygen (Thompson and Miller, 1928; also Shelford, 1929, p. 495). The procedure with the nymphs was much like that which has been used in similar experiments upon caddis-fly larvae (Morgan and O'Neil, 1931). After its oxygen content had been measured, 200 cc. of water were poured into each of three bottles. A group of 5 normal nymphs was weighed and put into one bottle. Another group as nearly equal

TABLE I

OXYGEN CONSUMPTION OF *Hexagenia recurvata*, IN GROUPS OF 5 GILL-LESS AND 5 CONTROL NYMPHS FOR TWO SUCCESSIVE 5-HOUR PERIODS

Experiment	Date of Collection	No. of Weeks between Collection and Operation	No. of Weeks between Operation and Experiment	Temperature of Water Bath Surrounding Bottles	Weight of Groups of Nymphs in Grams	Cubic Centimeters per Liter of Oxygen in Bottles of Water at Beginning of Experiment, Blank, Control, Gill-less	Cubic Centimeters per Liter of Oxygen in Water after 5 Hours	Cubic Centimeters per Liter of Oxygen in Water after 10 Hours	Actual Oxygen (Cubic Centimeters) Consumption in 10 Hours	Oxygen (Cubic Centimeters) Consumption Calculated for Controls of Same Weight as Gill-less
I.	11/14/30	1	14	6° C.	control 0.452 gill-less 0.450	b. 9.07 c. 9.07 g. 9.07	9.07 * 6.04	9.07 5.90 7.34 3.17 1.73 3.17 1.73
II.	11/14/30	1	16	6° C.	control 0.324 gill-less 1.260	b. 7.33 c. 7.33 g. 7.33	7.33 6.90 6.76	7.33 6.20 6.76 1.13 0.57 0.91 0.57
III.	11/14/30	1	16	6.5° C.	control 0.422 gill-less 0.400	b. 8.74 c. 8.74 g. 8.74	8.74 6.48 7.45	8.74 5.64 7.19 3.10 1.55 2.94 1.55
IV.	12/1/30	8	5	7° C.	control 0.408 gill-less 0.300	b. 8.59 c. 8.59 g. 8.59	8.59 6.20 7.61	8.59 5.35 7.33 3.24 1.26 2.38 1.26
V.	11/21/30	9	15	6° C.	control 0.380 gill-less 0.280	b. 8.45 c. 8.45 g. 8.45	8.45 7.75 8.03	8.45 6.76 7.61 1.69 0.84 1.25 0.84
VI.	11/21/30	9	15	7.5° C.	control 0.390 gill-less 0.270	b. 8.59 c. 8.59 g. 8.59	8.59 7.19 8.17	8.59 5.92 7.61 2.67 0.98 1.85 0.98
VII.	12/12/30	10	2	6.5° C.	control 0.319 gill-less 0.260	b. 8.45 c. 8.45 g. 8.45	8.45 6.76 7.33	8.45 5.07 6.62 3.38 1.83 2.75 1.83

Total actual oxygen consumption (seven experiments):

	Total	Average
Control nymphs	18.38 cc.	2.63 cc.
Gill-less nymphs	8.76 cc.	1.25 cc.

Total oxygen consumption calculated for controls of the same weight as gill-less nymphs:

Control nymphs	15.25 cc.	2.18 cc.
Gill-less nymphs	8.76 cc.	1.25 cc.

* Five-hour consumption of gill-less nymphs not taken in Experiment I.

as possible in weight, consisting of 5 gill-less nymphs, was placed in a second bottle. The third bottle of water was carried as a control. A hole was made in each of the corks, and a glass tube forced through until it projected well into the water. A piece of rubber tubing was fitted to the other end and clamped tight by a pinchcock. At the beginning of the experiment, after the nymphs were in the water, a piece of wire netting was fitted rather closely down over them. Finally, a layer of paraffin oil was poured on the surface of the water

TABLE II
COMPARISON OF ACTUAL OXYGEN CONSUMPTION OF CONTROL GILLED AND
GILL-LESS NYMPHS DURING THE FIRST AND THE SECOND 5 HOURS
OF OXYGEN CONSUMPTION (SEE TABLE I)

EXPERIMENT	CONTROL NYMPHS			GILL-LESS NYMPHS		
	First 5 Hours	Second 5 Hours	Total	First 5 Hours	Second 5 Hours	Total
I.....	3.03 cc.	0.14 cc.	3.17 cc.	1.73*cc.
II.....	0.43 cc.	0.70 cc.	1.13 cc.	0.57 cc.	0.0 cc.	0.57 cc.
III.....	2.26 cc.	0.84 cc.	3.10 cc.	1.20 cc.	0.26 cc.	1.55 cc.
IV.....	2.39 cc.	0.85 cc.	3.24 cc.	0.98 cc.	0.28 cc.	1.26 cc.
V.....	0.70 cc.	0.99 cc.	1.69 cc.	0.42 cc.	0.42 cc.	0.84 cc.
VI.....	1.40 cc.	1.27 cc.	2.67 cc.	0.42 cc.	0.56 cc.	0.98 cc.
VII.....	1.69 cc.	1.69 cc.	3.38 cc.	1.12 cc.	0.71 cc.	1.83 cc.
Total (Exps. II-VII).....	8.87 cc.	6.34 cc.	15.21 cc.	4.80 cc.	2.23 cc.	7.03 cc.
Average.....	1.48 cc.	1.06 cc.	2.54 cc.	0.80 cc.	0.37 cc.	1.17 cc.

* Five-hour consumption of gill-less nymphs not taken in Experiment I.

in each bottle, to keep oxygen from entering or escaping. The corks and tubing connections were then sealed over with paraffin. All the bottles were kept in a water bath during the experiment (Table I, temperature of water bath).

Samples of water for determination were taken at the beginning and after the fifth and tenth hours of the experiment. Air was prevented from entering by technique of manipulation possible after practice with the Thompson-Miller apparatus.

The amounts of oxygen consumed by nymphs in seven experiments, each dealing with 5 control gilled and 5 gill-less nymphs, are given in Table I.

Most of the gill-less nymphs used in the experiments (Table I)

had had their gills removed 14 or 16 weeks before; 10 nymphs had their gills removed 2 and 5 weeks before. No regeneration occurred. *Hexagenia* nymphs are naturally vigorous, sometimes jerky, in their motions including those of the gills. Since all movements are obviously correlated with the amount of oxygen consumed, it was necessary to take care to have the nymphs as quiet as possible during the experiments.

In every experiment the oxygen consumption of the normal nymphs for the 10 hours greatly exceeded that of the gill-less ones (Table I). In the first 5 hours the total oxygen consumed by the gilled nymphs was almost twice that of the gill-less ones; in the second 5 hours it was nearly three times as great. The oxygen consumption of the gill-less nymphs was only one-half as much in the second as in the first 5 hours (0.8 cc. in first 5 hours to 0.4 in second 5 hours). The oxygen consumption of the gilled nymphs was slightly less in the second than in the first 5 hours (1.5 cc. in first 5 hours to 1.1 in second 5 hours) (Table II).

In considering the unevennesses of oxygen consumption in individual experiments, it should be remembered that some movement of the nymphs was unavoidable. The length of time between gill-removal and experiment seemed to make no difference in the amount of oxygen that the nymphs consumed. Compare experiments III and IV, VI and VII (Table I). A few nymphs which were tested in the spring, a day or two after gill removal, consumed the same amount of oxygen as those that had lived several weeks after the operation. The series of tests showed that, although *Hexagenia* nymphs can live through the winter season without gills, their oxygen consumption is cut down about one-half.

INDICATOR TESTS TO COMPARE THE RATE OF CARBON-DIOXIDE ELIMINATION IN NORMAL AND GILL-LESS NYMPHS

These tests were made to find out whether the gills were relatively as important in carbon-dioxide elimination as in oxygen intake. They were performed by Fox's (1921) indicator method.¹ Three

¹ Enough potassium hydroxide was added to a weak solution of aqueous alkaline haematoxylin to change the color of the solution from pink to bluish pink. As carbon dioxide is mixed with this non-toxic solution, its color changes from bluish pink to orange and finally to a pale yellow.

vials, 40 mm. long and 25 mm. in diameter, were filled with very dilute alkaline haematoxylin solution. A normal nymph was placed

TABLE III

RESULTS OF INDICATOR TESTS SHOWING THE COMPARATIVE RATE OF CARBON-DIOXIDE ELIMINATION IN NORMAL AND GILL-LESS NYMPHS*

In each experiment one vial contained a control nymph and one a gill-less nymph. For the third vial, carried as a control, no record is shown here.

EXP.	DATE OF COLLECTION	NO. OF WEEKS BETWEEN COLLECTION AND OPERATION	NO. OF WEEKS BETWEEN OPERATION AND EXPERIMENT	MINUTES REQUIRED TO BLEACH INDICATOR SOLUTION		RATIO OF TIME OF GILL-LESS TO CONTROL
				Control	Gill-less	
I.....	II/10/30	1	23	6	15	2.5
II.....	II/14/30	1	23	8	16	2.0
IV.....	II/14/30	1	23	5	18	3.6
V.....	II/14/30	1	23	6	15	2.5
VI.....	II/14/30	1	23	7	17	2.4
Total for experiments.				32	81	
Average.				6.4	16.2	

SAME TESTS MADE UPON NYMPHS COLLECTED IN SPRING AND RECENTLY DEPRIVED OF GILLS

EXP.	DATE OF COLLECTION	NO. OF DAYS BETWEEN COLLECTION AND OPERATION	NO. OF DAYS BETWEEN OPERATION AND EXPERIMENT	MINUTES REQUIRED TO BLEACH INDICATOR SOLUTION		RATIO OF TIME OF GILL-LESS TO CONTROL
				Control	Gill-less	
VIII.....	4/18/31	12	1	4.0	17.0	4.3
IX.....	4/18/31	12	1	3.5	17.5	5.0
X.....	4/18/31	12	1	4.5	16.0	3.5
XI.....	4/18/31	12	1	4.0	16.0	4.0
XII.....	4/18/31	12	1	3.0	15.0	5.0
Total for experiments.				19.0	81.5	
Average.				3.8	16.3	

* Two experiments made upon nymphs with the same history as in Experiment II but immersed in more dilute indicator solutions required, respectively, 13 and 10 minutes for the normals and 28 and 28 minutes for the gill-less nymphs to "bleach" them. The ratio of time taken, gill-less to control, was 2.2 and 2.8.

in one vial, a gill-less one of the same weight in another, and the third was carried as a blank control. The corks were sealed with

paraffin, and observations were made immediately. The vials were placed against white paper, and observations continued until the solutions were "bleached" to pale yellow. In the first set of tests made upon nymphs that had lived for 6 months in the laboratory, the normal animal bleached the solution two to three times faster than the gill-less ones. In the spring the same tests were repeated upon nymphs whose gills had been cut off the day before (Table III, Exps. VIII-XII). Although gilled and gill-less nymphs of the same weight were selected as before, the gilled nymphs bleached the solution about four times faster than the operated ones. In neither series of tests was there any indication of "bleaching" localized about the spiracles.

DISCUSSION

The observations on the gill-less *Hexagenia* nymphs indicated that their gills were, first of all, important respiratory organs whose absence was responsible for the marked decrease in oxygen consumption and carbon-dioxide elimination shown in the tests. This was further apparent in their consistently slow motions and general inactivity.

Some of their motions, especially swimming, were hampered by the lack of mechanical aid from the gills; but even these were usually executed in good form. But their sluggishness and inertia were evidently expressive of the changed metabolism resulting from the decreased exchange of gases. It seemed altogether likely that the gill-less nymphs' positive reaction to light was correlated with the excess carbon dioxide accumulated within them (Allee and Stein, 1918). It is true that these nymphs ate less than normals and that a hungry condition has more than once been the apparent cause of reversed phototropism in other animals. Stier (1926) found this in the newt *Triturus viridescens*. But it is also true that, almost immediately after their gills had been removed, these nymphs changed from photo-negative to photo-positive even when they had but recently taken food and still had it in their stomachs. In experiments which Wodsedalek (1911) performed on the May fly *Heptagenia interpunctata*, he found that the normal nymphs were practically all negatively phototropic. But as soon as carbon dioxide was bubbled through the water, a large percentage of them became positively phototropic.

Natural reversal of phototropism preceding emergence is common in May flies and in other aquatic insects. Nymphs of the May flies *Epeorus* and *Iron* live on the under sides of rocks, ordinarily never coming to the lighted side. But, just before their emergence, they move onto the lighted side by hundreds. Their nymphal covers are thick then, the tracheae of the gills are beginning to be cut off from those of the body, and the gill is losing its function. It seems probable that their condition is similar to that of the gill-less *Hexagenia* nymphs, and their reaction the same.

Various suggestions have been made concerning the passageways of gases in nymphs that are living without their gills. Cuénot (1925) believed it possible that his nymphs of *Cloëon dipterum* went to the surface of the water and took air through their thoracic spiracles. Remy (1925) thought that May fly nymphs, when deprived of their gills, depended for some time on the supply of air that had been in their tracheae before the operation. He cited examples of aërial insects which were able to survive 4 to 5 days' immersion in water by so doing. He further explained that, in summer, May flies survived gill amputation but a short time because they exhausted their tracheal air supply so much more quickly than in winter. He felt it unnecessary to ascribe tegumentary or intestinal respiration to them.

The gill-less *Hexagenia recurvata* nymphs were never observed coming to the surface for air or in any other way relying upon their spiracles. As already mentioned, during the carbon-dioxide experiments the solution about the spiracle region was bleached no more rapidly than elsewhere. Sections of *Hexagenia* nymphs that have thus far been made indicate that the spiracles are closed. However, this preliminary impression should in no way be regarded as a conclusion. The open or closed nature of the spiracles can only be decided by thorough morphological work. Nevertheless, in the present studies the behavior of the gill-less nymphs, as well as the results of the carbon-dioxide experiments, indicated that exchange of gases took place through the body wall.

No regeneration was observed among the approximately 500 gill-less nymphs under observation at some time during the experiments (Plate I, Figs. 2, 5). Cuénot (1925) found that nymphs of *Cloëon dipterum* regenerated their gills freely, and several other investiga-

tors have reported the same thing in various species of May flies. In collected material it is not uncommon to find regenerating gills on nymphs of various species. Such specimens of *Hexagenia recurvata* were collected during the present study. The failure of *Hexagenia* to regenerate in the laboratory could not have been associated with the age of the nymphs, for several sizes were used and many were but half-grown when their gills were removed. (Full-size nymphs are 24-28 mm. long, without caudal setae.) This may have been due to the thoroughness with which the base of the gill was removed.

Hexagenia nymphs lived for a long time without their gills if they were removed in the early winter season of lowered activity. Nymphs whose gills were removed in November lived about 5 months; those that underwent the same operation in early spring lived but a week or two, and in late spring only 2 or 3 days or even less. Those that lost their gills in November lived through the season of low activity very easily, but in the spring they suddenly began to die in large numbers. The fate of these nymphs and the swift mortality that followed the spring operations showed that gills are essential to the full activity of life in these May flies. Their dependence on their gills is in striking contrast to the caddis fly, *Macronema zebrastratum*, whose larvae not only lived months after their gills were removed but pupated and emerged more or less successfully (Morgan and O'Neil, 1931). This difference in the importance of the gills of *Hexagenia* and *Macronema* is also suggested by the ratios of oxygen intake of gilled and gill-less animals in the two forms. Results of a series of tests on *Macronema* showed no significant difference between the average total oxygen consumption of normal and gill-less larvae (Morgan and O'Neil, 1931). On the other hand, control *Hexagenia* nymphs consumed twice as much oxygen as gill-less ones (Table I).

SUMMARY

1. In *Hexagenia recurvata* the tracheal gills are respiratory organs that are essential to life. Nymphs that lived without gills through the winter period of lowered activity were unable to survive through the spring. Nymphs whose gills were removed in spring died very soon, often within a few hours.

2. The oxygen intake of normal nymphs was approximately twice as much as that of gill-less ones.

3. Normal nymphs eliminated carbon dioxide more than twice as fast as gill-less ones.

4. In gill-less nymphs, exchange of gases appeared to occur through the body wall. There was no physiological evidence of open spiracles.

5. Normal nymphs are generally photo-negative except before emergence. After gill-removal they became photo-positive, the condition appearing to correlate with their content of carbon dioxide.

6. Nymphs that lived through the winter without gills were inactive, made few spontaneous movements, and rested upon the surface of the mud, whereas their controls were active and burrowed freely. The swimming and burrowing movements of gill-less nymphs were slow but not different in form from those of normal nymphs.

7. From November to March control nymphs molted very rarely; gill-less ones, not at all.

8. No regeneration of the gills was observed on the experimental animals, though a few specimens with regenerating gills were found in collected material.

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