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THE OXYGEN CONSUMPTION OF HEXAGENIA
RECURVATA DURING THE WINTER
AND EARLY SPRING

(Three figures)

ANN H. MORGAN AND JANET F. WILDER
Department of Zoölogy, Mount Holyoke College

LIKE other May flies, the large burrower, *Hexagenia recurvata*, lives in the water during its immature or nymphal stage, which in this species lasts for about two years. These nymphs live in ponds and slow streams, tunneling their way through the mud or creeping over the bottoms. Where they occur at all, they are usually abundant and obtainable throughout the winter in goodly numbers. The nymphs used in these experiments were taken from one region of a spring-fed brook near South Hadley, Massachusetts, where *H. recurvata* nymphs were also collected for an earlier study of the functions of their gills (Morgan and Grierson, 1932).

MATERIAL AND METHODS

Material.—Eighteen collecting trips were made to Lithia Springs Brook during the fall and winter (September 19, 1934—March 15, 1935) and one trip on May 3. Observations of weather conditions, temperature of the water, and activities and abundance of the nymphs were made on each trip. Nineteen hundred and eighty-five nymphs, ranging from 10 to 30 mm. in length, were collected.

Immediately after the field trips the nymphs were measured and caged in groups according to size. Two kinds of cages were used. The stock cages were 1 foot in diameter and had sides and bottom of copper-wire screen. These were set in galvanized iron trays 2 inches deep, each having a capacity of 150 nymphs. The small cages holding from one to seven nymphs were used for those which had been chosen for observation. Each of these was a 3×4-inch compartment separated off by wire screening in a multiple-unit tray of forty such cages. All cages were supplied with running water and diatom-saturated ooze from the nymphs' own habitat. Fresh mud was

added from time to time, and in February filamentous algae were stirred in with it.

About once a week the small cages were examined for molted skins and dead nymphs. The stock cages were examined once a month. Four other cylindrical screen cages, containing a total of about twenty nymphs, were kept in Lithia Springs Brook as a check on the condition of the laboratory-kept nymphs.

During the spring and fall the death-rate was high. Through the winter, however, probably as a result of the lowered metabolism accompanying winter conditions, the death-rate was lowered to about one out of eight nymphs per week.

Organization of the experiments.—Twelve experiments were conducted from October 27 to March 17, but the first three of these were discarded because of faults in technique. Of the remaining nine, three were performed in December (December 1, 15, 29), three in January (January 2, 12, 29), two in February (February 9, 23), and one in March (March 16).

Each experiment was set up as follows: three 500-cc. bottles each containing 250 cc. of distilled water and three nymphs and one bottle with the same water content but no nymphs (the blank) were set in each of three water baths, whose average temperature was respectively 1.0° , 6.4° , and 12.3° C. Thus, twelve bottles were used. Of the nine containing nymphs, usually two out of the three in each temperature held recently collected nymphs, while the others held nymphs that had been kept in the laboratory from 10 days to 2 months.

The experiments lasted 25 hours. Five determinations of the oxygen content of the water in the bottles were made, namely, at the start and at the fifth, tenth, fifteenth, and twenty-fifth hours.

Care of nymphs before the experiments.—After the nymphs had been brought to the laboratory, they were at once sorted and grouped according to size. Those which were to be kept in the laboratory were placed either in the stock or unit cages before described. The largest nymphs available (approximately 27 mm.) were used in the experiments. Sometimes these could not be secured and it was necessary to use 24–25-mm. lengths. They were acclimated by being placed in bottles set in water baths maintained at the temperatures

at which the tests were to be made. It was hoped that the period of acclimatization would provide time for the digestion of food already consumed, as well as accustom the nymphs to the temperature at which they were to be tested. The 500-cc. bottles used for acclimatization were the same ones in which the nymphs were later tested. Wire mesh was placed in the bottom of each bottle as a foothold for the nymphs.

Maintenance of constant temperature.—The lowest temperature, 1° C. used in the experiments was obtained by the use of an electrically controlled constant-temperature bath. The medium temperature was maintained by an even flow of water into a museum jar containing the experimental bottles. This temperature remained constant $\pm 1^\circ$ through an experiment, but varied from month to month, showing a slow decline from 7.0° C. on December 1 to 5° C. on March 15, with a seasonal average of 6.4° C. The highest average temperature, 12.3° C. (average of the highest temperatures in each of nine experiments) was obtained by running tap water through a 1-liter Erlenmeyer flask heated by the coils of an electric unit. After some experimentation this temperature could be kept constant $\pm 3^\circ$ during an experiment, but it varied slightly from experiment to experiment.

Oxygen determinations.—Following the practice of Hiestand (1931), during experimentation all nymphs were kept in distilled water, which appeared to have no harmful effect upon them. Since the determinations were made by the unmodified Winkler method, it was thought that by the use of the distilled water errors due to impurities in the water could be avoided. The distilled water was brought to the right temperature and, just before using, was shaken up with air in order to oxygenate it.

The experiments were started in the morning following a collection of the nymphs. Bottle A, containing nymphs collected the day before and acclimated at 1.0° C. through the night, was taken from the water bath. The acclimatization water was poured out. The bottle was then rinsed with distilled water of the same temperature, and finally 250 cc. of distilled water was siphoned into the bottle. A deep layer of motor oil was then poured onto the water. The cork was pressed in and oil added through a small hole in the cork till the

bottle was completely full. The hole was then plugged, and the whole top coated with melted paraffin. The bottle was then completely submerged in the water bath. In the earlier experiments the glass bend of the exit siphon was left above water, but bubbles of gas collected there; so in later experiments it was submerged. Fifteen minutes after the procedure with bottle A, the same thing was repeated for bottle B; 15 minutes after that, for C; and so on. Thus, each of the twelve bottles was started in sequence 15 minutes apart. The determinations were made at the fifth, tenth, fifteenth, and twenty-fifth hours and each took 15 minutes to perform; thus the contents of each bottle could "run" the same length of time. Before each experiment determinations were made of the oxygen content of the water to be used in the test bottles.

The determinations were made in a Thompson-Miller apparatus for the micro-Winkler method, 5.5 cc. capacity. Before each test this was filled to capacity with water from the experimental bottle, then emptied, and refilled to capacity for the test. Thus, equal amounts of water were always removed from all bottles in each series of determinations. After each test the apparatus was rinsed, first with tap water and then with distilled water. Directions for the use of the apparatus are given by Thompson and Miller (1928); and notes on its accuracy, by Snoke (1929) and Allee and Oesting (1934). As soon as the sample had been drawn from the bottle, more oil was added in order to bring the oil surface to the level of the cork again. The top was then resealed with paraffin. The condition of the nymphs was always noted during these operations and before the bottle was replaced in the bath.

The water sample was treated according to the method of Kremmerer, Bovard, and Boorman (1923) and modified to suit the Thompson-Miller micro-apparatus. The sodium thiosulphate was standardized against N/100 potassium permanganate instead of against potassium bichromate. The equation used was that given by these workers for finding the amount of oxygen in cubic centimeters per liter. This was changed to milligrams, and the milligrams per gram body weight computed.

The use of motor oil as a seal.—Motor oil was used to cover the water during the tests in all the experiments. It was finally adopted

after considerable experimentation and practice. In preliminary experiments the water was covered with paraffin oil (Amberson, Mayerson, and Scott, 1924; Morgan and O'Neil, 1931; and others). It is well known that atmospheric oxygen will penetrate through paraffin oil, especially after periods longer than 10 hours. These experiments on *Hexagenia* lasted 25 hours. The 500-cc. bottles were chosen in order to allow for a deep layer of paraffin oil over the water. Even with this precaution some leakage was detected by a rise of oxygen in the water of the blank bottles.

By the use of luminescent bacteria Hill (1928) showed that oxygen would go through paraffin oil and into water within 6 hours but that it would not go through motor oil. In the practice experiments with the motor oil it was discovered that with this oil oxygen passed from the water into the oil. There was an apparently greater oxygen consumption by the nymphs in water covered by motor oil than there had been by those in water covered by paraffin oil, and the oxygen content of the blank bottles fell in proportion to the temperature. No deduction for the error inherent in the motor oil was made in the calculations of the oxygen consumption. However, the average loss from the water into the oil at three temperatures was measured and is presented in Table I.

In other experiments in this laboratory paraffin oil has served as well as motor oil when the test bottles were totally submerged and duplicate determinations averaged. Either motor or paraffin oil gave only comparative results. These, however, are satisfactory for the biological questions at hand. For absolute measurements, bottles should be sealed with mercury or inclosed in a nitrogen chamber.

OBSERVATIONS

At a given temperature the average oxygen consumption of *Hexagenia* nymphs was nearly the same through the four succeeding 5-hour intervals from one determination to another (Table II). But variation in the rate of consumption occurred in individual sets of animals. Such variation of individual oxygen consumption from hour to hour is not uncommon amongst invertebrates (Dakin and Dakin, 1925, p. 315).

At the lower temperatures 1.0° C. and 6.4° C. the consumption of the nymphs was unaffected by the decreasing oxygen tension. At

TABLE I

AMOUNTS OF OXYGEN THAT ESCAPED FROM DISTILLED WATER INTO A SEAL OF MOTOR OIL IN EXPOSURES MADE AT THREE TEMPERATURES

Amounts given are the averages of determinations made on the "blank bottles" of five experiments (January 1 through March 16), given in milligrams of oxygen per liter of water.

TEMPERATURE DEGREES C.	OXYGEN CONTENT AT START	OXYGEN LOSS AT—			
		5 Hours	10 Hours	15 Hours	25 Hours
1.0.....	9.603	0.552	0.737	1.313	2.189
5.64.....	8.619	0.476	0.745	1.465	2.356
11.88.....	7.491	1.349	2.158	2.856	4.130

TABLE II

THE COMPARATIVE OXYGEN CONSUMPTION OF RECENTLY COLLECTED (REC. COL.) AND LABORATORY-KEPT (LAB. KEPT) NYMPHS OF *Hexagenia recurvata* DURING 5, 10, 15, AND 25 HOURS AT THREE TEMPERATURES

Average of twenty-fifth hour determinations at 12.3° C. based on January-March only. An average of nine experiments. Amounts of oxygen in milligrams per gram body weight.

Hours of Consumption	Nymphs	1.0° C.	6.4° C.	12.3° C
5.....	Rec. col.	2.305	2.982	5.510
	Lab. kept	1.444	2.503	4.460
10.....	Rec. col.	3.582	6.058	9.387
	Lab. kept	1.960	4.592	8.229
15.....	Rec. col.	5.006	8.598	12.174
	Lab. kept	3.359	6.885	11.558
25.....	Rec. col.	7.584	11.950	8.66
	Lab. kept	5.203	10.692	7.89

12.3° C. the consumption decreased between the fifteenth and twenty-fifth hour, but this was probably due to the effect of accumulating carbon dioxide.

Singh-Pruthi (1927) found that May-fly nymphs could remain in water with oxygen tensions as low as 0.3-0.2 cc. per liter without ap-

parently being affected, provided the pH and the tension of carbon dioxide were right. Many *Hexagenia recurvata* nymphs died after 15 hours at 12.3° C., and it is believed that carbon dioxide narcosis may have been responsible.

The oxygen consumption of recently collected and laboratory-kept nymphs.—The average oxygen consumption of nymphs kept in the laboratory 10 days or more is less than that of nymphs collected within 24 hours.

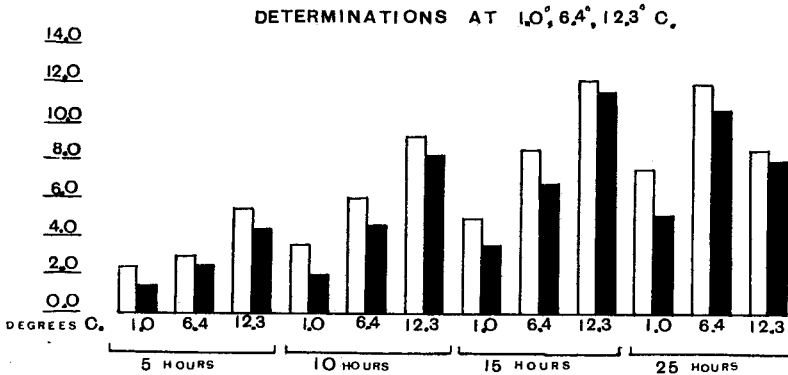


FIG. 1.—The comparative oxygen consumption of recently collected (white) and laboratory-kept (black) nymphs of *Hexagenia recurvata* at three temperatures for 5, 10, 15, and 25 hours. Averages of results of nine experiments are represented covering a period from December 1 through March 17. December–January determinations lacking for twenty-fifth hour at 12.3° C. (Fig. 2, 12.3° C.); hence, average for whole year reduced out of proportion to other columns. Ordinates, milligrams of oxygen per gram of body weight.

Averages of the results of nine experiments each involving an average of 22.8 nymphs revealed that laboratory-kept nymphs showed a consistently lower oxygen consumption than recently collected ones (Table II and Fig. 1).

The exception indicated in Figure 2 in the fifteenth-hour determinations (December 1—January 13) at 12.3° C. is only an apparent one. Several of the recently collected nymphs involved in these 12.3° C. tests showed a lessened resistance toward the end of the experiments, which allowed the laboratory-kept nymphs to overtake them in oxygen consumption. In several cases the nymphs died by the fifteenth hour. From December 1 to January 13 no consumption record was used for the twenty-fifth hour at 12.3° C., because one or

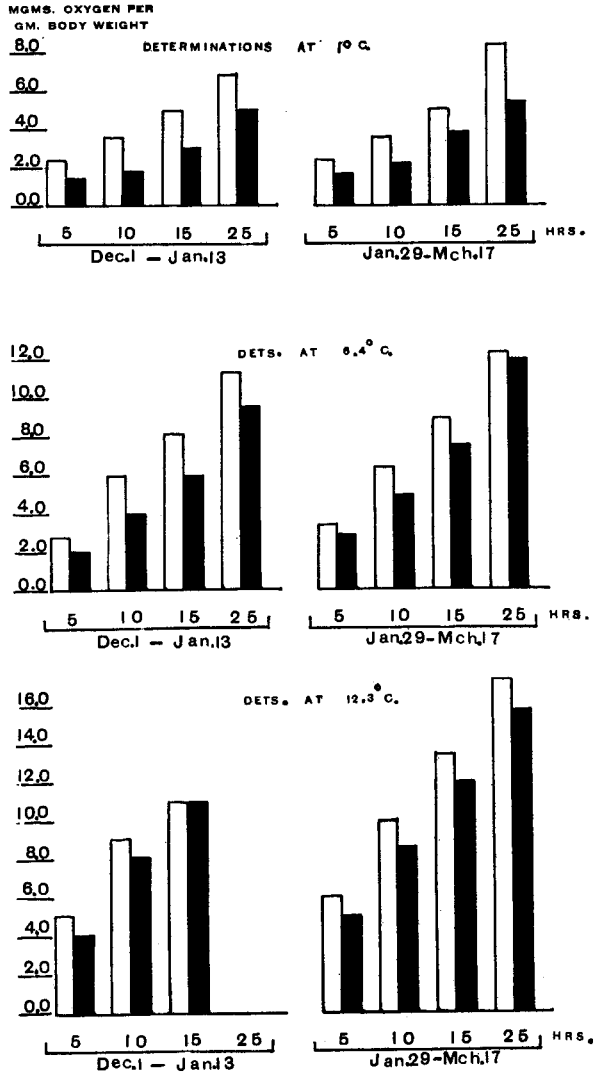


FIG. 2.—The average oxygen consumption of recently collected (white) and laboratory-kept (black) nymphs of *Hexagenia recurvata*, showing (1) the early-winter period of low oxygen consumption followed by the late-winter and spring rise, and (2) the higher consumption of the recently collected nymphs (for explanation of exception in fifteenth-hour determination at 12.3° C. see p. 159). Abscissa, intervals at which determinations were taken; ordinate, milligrams of oxygen per gram of body weight. Averages based on five experiments for the first and four for the second periods.

two out of the three nymphs were dead before this hour of this series. Such records were discounted (twenty-fifth hour, 12.3°, Table III).

TABLE III

THE AVERAGE OXYGEN CONSUMPTION OF RECENTLY COLLECTED AND LABORATORY-KEPT NYMPHS OF *Hexagenia recurvata* FOR THE EARLY-WINTER (DECEMBER 1—JANUARY 13) AND THE LATE-WINTER PERIOD (JANUARY 29—MARCH 17)

Tests made at three temperatures. Amounts of oxygen in milligrams per gram of body weight. Average based on nine experiments each involving an average of 22.8 nymphs.

Hours of Consumption	Season	1.0° C.	6.4° C.	12.3° C.
Recently collected nymphs				
5.....	Dec.-Jan. Jan.-March	2.265 2.346	2.260 3.305	4.998 6.021
10.....	Dec.-Jan. Jan.-March	3.508 3.657	5.774 6.343	8.927 9.847
15.....	Dec.-Jan. Jan.-March	4.948 5.063	8.241 8.955	10.936 13.411
25.....	Dec.-Jan. Jan.-March	6.805 8.362	11.479 12.420 17.327
Laboratory-kept nymphs				
5.....	Dec.-Jan. Jan.-March	1.304 1.585	2.079 2.927	3.970 4.950
10.....	Dec.-Jan. Jan.-March	1.709 2.210	4.024 5.160	7.804 8.654
15.....	Dec.-Jan. Jan.-March	2.944 3.774	6.036 7.734	10.995 12.122
25.....	Dec.-Jan. Jan.-March	5.016 5.390	9.503 11.801 15.784

Correlated with the higher metabolism indicated by the respiration of the recently collected nymphs was the fact that between March 15 and May 3, 78.4 per cent of them had molted, while between April 15 and April 29 only 5.3 per cent of the laboratory-kept nymphs had done so.

As evidenced by the oxygen consumption just mentioned, it appears that the metabolism of the nymphs was lowered by laboratory conditions, even though the temperature of the water in which they were kept was approximately 5° higher than that of their own habitat. This emphasizes the fact that in physiological studies it cannot be taken for granted that animals are living at their normal rate. Dreyer (1932) calls attention to the importance of the imitation of natural conditions for animals that are being used for physiological experiments. He was able to imitate the ant's normal habitat and found that the respiratory behavior of laboratory colonies was "similar to that of animals brought directly from the field nests." The laboratory conditions in which *Hexagenia recurvata* lived were certainly as good as those of most aquatic animals in the laboratory. These nymphs were in constantly flowing water of high oxygen content and a pH of 6.8, only two-tenths less than that of their own habitat, Lithia Springs Brook. Lack of space seems unlikely to have hurt them, since a group of fifteen nymphs tried out in a 3×4 -inch cage apparently lived as well as did one to five nymphs in cages of the same size. Metabolic products could not have been an influence, since currents of water constantly cleared these away. However, there were obviously unavoidable differences between the laboratory and native environments, such as those incident to depth of water and to materials in solution.

Oxygen consumption as affected by season.—The oxygen consumption of all nymphs was higher in February and March than it had been earlier in the winter. When the consumptions in five early winter experiments (December 1 through January 3) were averaged and compared with the four late winter ones (January 29 through March 17), the results showed that the late ones were greater in every case (Table III and Fig. 2).

This seasonal rise involves the problem in much greater complexity. If the metabolism of the nymphs were dependent upon the temperature of the environment, as Dreyer (1932) has found for the ant, a constant temperature would lead one to expect a steadily maintained metabolism, and a falling temperature a lowered metabolism. But the rise in oxygen consumption was not correlated with a rise in habitat temperature in either freshly collected or in labora-

tory-kept nymphs, as records of habitat temperature and oxygen consumption show (Fig. 3, graphs A, B, and C). The January rise

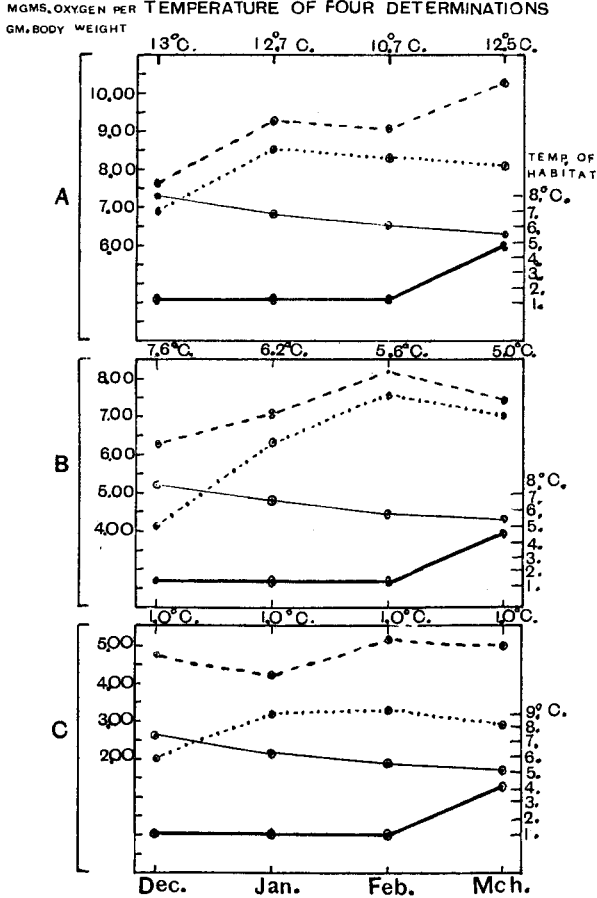


FIG. 3.—The average oxygen consumption of recently collected (heavy dash line) and laboratory-kept (light dot-and-dash line) nymphs of *Hexagenia recurvata*, December 1 through March 17, showing monthly fluctuations and the temperature of their habitats: temperature of native stream (heavy continuous line), temperature of tap water (light continuous line). Abscissa, months when determinations were made; ordinate, milligrams of oxygen per gram of body weight.

occurred, without exception, in both types of nymphs, except that in those tested at 1.0° C. it was not found until February (Fig. 3, graph C).

In the laboratory-kept nymphs and in the recently collected ones tested at a temperature higher than that at which they had been living the late-winter rise was first noticed in January (Fig. 3, graph A). In recently collected nymphs tested at the temperature of their habitat (1.0° C.) the rise occurred in February (Fig. 3, graph C). None of these rises can be correlated with temperature, since in Lithia Springs Brook the temperature was about the same as on earlier winter dates (December, 1.5° ; January, 1° ; February, 1° C.) and in water at the laboratory it was lower (Fig. 3, graphs A, B, C, temperature of habitat). A correlation of increased body weight and proportionately lowered oxygen consumption was observed in the March determinations.

The fall in the March consumption of recently collected nymphs shown in Figure 3, graph B, may be correlated with the fact that these nymphs were the heaviest ones collected. In individual experiments it was found that a heavy nymph consumed proportionately less oxygen than a lighter one. The converse is shown in graph A of the same figure, where the March rise is in the oxygen consumption of lighter-weight nymphs. At this season the greater growth activity of these smaller nymphs created a greater oxygen demand, in addition to that resulting from the greater proportion of surface to the volume of their bodies.

Contrary to these observations, Dreyer (1932), as already mentioned, found that the oxygen consumption and respiratory exchange in the ant paralleled the temperature, whether natural or experimental. He kept ants, *Formica ulkei*, in a hibernating condition throughout the year by placing them in a low temperature, and in an active condition by keeping them in a high temperature, and determined that their respiratory exchange closely followed their surrounding temperature throughout the year.

He has thus shown that the rate of metabolism in the ant is generally dependent upon environmental temperature. When the temperature was varied experimentally, the oxygen consumption of nymphs of *Hexagenia recurvata* was also affected by the temperature (Tables II and III; Fig. 1). On the other hand, in January and February their oxygen consumption rose in spite of consistently low temperatures (Fig. 3). Thus it becomes apparent that, while these

nymphs may be affected experimentally by temperature, their metabolism is nevertheless, to a considerable degree, independent of it. It is likely that there is a similar situation in the winter stone flies, as their winter behavior demonstrates. *Capnia* and *Taeniopteryx*, which are active, grow rapidly and even emerge into the air as adults as early as January and February when the temperature of their habitat is as low or lower than it has been earlier in the winter.

The oxygen consumption as affected by experimentally varied temperature.—In these experiments, as already stated, three temperatures (1.0° , 6.4° , and 12.3° C.) were used, and three levels of oxygen consumption resulted (Tables II and III, and Figs. 1 and 2). The objection may be raised that the absorption of oxygen by the motor oil might account for these levels. This objection has been considered. The loss of oxygen from the water into a seal of motor oil was determined for three temperatures at 5-, 10-, 15-, and 25-hour intervals (Table I). The loss of oxygen in the oil at 25 hours at 1.0° C. was, on the average, 2.189 mg. of oxygen per liter of water; at 5.6° C. it was 2.356 mg.; and at 11.88° C. it was 4.130 mg. The increase in loss of oxygen between 1.0° C. and 5.6° C. is not proportionate to the much greater loss, owing to its consumption by the nymphs (cf. Tables I and II). With the experimentally varied temperatures the average increase in oxygen consumption with a rise of 6° was 1.26 times that at 1.0° C.; and with a rise of 12° , 2.77 times that at 1.0° C. When these figures were calculated on the basis of the Q_{10} of van't Hoff (Rogers, 1929), a Q_{10} value of 3.319 was obtained from the temperature rise of 1.0° – 6.4° C., and 2.645 from the rise of 6.4° – 12.3° C. The smaller Q_{10} for the rise from 6.4° to 12.3° C. indicates that at the higher temperatures factors such as carbon dioxide accumulation (Fig. 2, December 1—January 13, 15-hour determination, and death of nymphs before 25 hours) were probably operating on the nymphs to reduce their metabolism.

DISCUSSION

The fact that *Hexagenia recurvata* consumed less oxygen during the first part of the winter indicates its depressed metabolism in that season. Possibly in November, certainly in December and the first

part of January, occurs its winter "low." Near the end of January, and in February and March, there is a definite rise in oxygen consumption, evidently correlated with the quickened growth and activity characteristic of May flies at this season.

Does other behavior of the nymphs in any way express their "low" winter condition? When its light response changes from positive to negative, the metabolism of the May-fly nymph *Epeorus* is lowered (Allee and Stein, 1918). In a series of preliminary tests made upon the light responses of the *Hexagenia recurvata* nymphs under various conditions, there was some evidence that, on the average, they were more negative to light in October and February than in March and May. A possible correlation is thus indicated between their phototropism and respiratory metabolism. A relationship of this kind has been suggested by Allee and Stein (1918) in nymphs of the May flies *Epeorus* and *Leptophlebia*. Using potassium cyanide as an index of metabolism, they demonstrated a correlation between phototactic reaction and metabolic condition. In their normal behavior Holmquist (1928) found that, in the cold, muscid flies were inactive and had a negative reaction to light; whereas in the warmth, they were more active and were positive to light. So far as they have been studied here, the responses of *Hexagenia recurvata* nymphs also indicate that they manifest their lower metabolism by more negative reactions to light during the winter and their higher metabolism by more positive ones in the spring.

Absence of molting may be taken as a manifestation of low metabolism in nymphs of *Hexagenia recurvata*, as it has been shown to be in the dragon fly, *Anax junius*, by Calvert (1929) and as discussed for insects by Singh-Pruthi (1925). No nymphs molted in the laboratory cages from December 3 to April 29. Neither were there any recently molted nymphs among those collected from Lithia Springs Brook between November 15 and March 15. On the other hand, of those taken on May 3, 17 per cent were recently molted ones. The oxygen consumption of the nymphs showed that their metabolism started to rise in the latter part of January. Its continued rise is indicated by the nymphs' greater positiveness to light in March and the frequent appearance of molted skins after April 29.

All nymphs of *Hexagenia recurvata* showed greater resistance to laboratory conditions between November and February, their period of lowered oxygen consumption, than they did before and after those months. In the examinations of the cages from November 12 to January 8, 4.96 was the highest percentage of nymphs found dead. But beginning on January 21 the mortality rose from 11.45 per cent to 38.15 per cent on April 15.

Experiments and natural behavior have shown that sluggish animals can endure an environment which would be fatal to them if their metabolism were more rapid. For example, animals with a higher metabolism are more susceptible to a strong solution of potassium cyanide than those with a lower one (Allee and Stein, 1918; Child, 1919; Hyman, 1919). It seems therefore that in *Hexagenia recurvata* the earlier resistance, and later lack of resistance, to unfavorable conditions of the laboratory is also a manifestation of their lowered metabolism during the winter and its rise toward spring.

It has already been shown that there is a metabolic rhythm in these nymphs which is, to a considerable degree, independent of temperature. Annual metabolic cycles are more evident in other insects undergoing a diapause, a period of low metabolism resulting in complete quiescence occurring independently of temperature. Nymphs of *Hexagenia recurvata* have no diapause, nor can they be said to be intermittently inactive. Yet, their period of low oxygen consumption, even though occurring during their winter of more or less continuous activity, is suggestive of the more striking hibernation phases of other insects.

SUMMARY

1. Nineteen hundred and eighty-five nymphs of *Hexagenia recurvata* were collected from a spring-fed brook in eighteen collections, made mainly in the winter and early spring (September 19 to May 3). Observations were made upon their behavior in their habitat and under laboratory conditions in which they were kept from 10 days to 2 months.

2. Experimental tests of the oxygen consumption of a total of 205 laboratory-kept and recently collected nymphs were conducted from

October 27 through March 17. Three tests made in October and November were counted as preliminary; and the results of nine, performed in December, January, February, and March, are reported here.

3. The oxygen consumption of nymphs kept in the laboratory from 10 days to 2 months was less than that of nymphs collected from their own habitat and tested within 24 hours.

4. Between December 1 and January 13 the oxygen consumption of all nymphs was lowered; between January 29 and March 17 it rose. The nymphs thus exhibit a late-winter rise in oxygen consumption.

5. In all laboratory-kept nymphs and in recently collected ones tested at temperatures higher than that of their own habitat, a "pickup" in oxygen consumption occurred in the last part of January. In recently collected nymphs tested at the temperature of their own habitat, it occurred in February.

6. In experimentally varied temperatures the oxygen consumption was directly proportionate to the temperature, a Q_{10} of from 2.6 to 3.3 being obtained.

7. In their rate of oxygen consumption nymphs of *Hexagenia recurvata* are independent of the oxygen tension from 9.75 to 2.34 cc. of oxygen per liter of water, despite accumulating carbon dioxide. They may survive when there is but 0.50 cc. of oxygen per liter of water.

8. Among other evidences of low metabolism was an absence of molting from December 3 to the end of April. Molting was frequent, however, during May and October.

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