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Effect of temperature on the hatching time of eggs of *Ephemerella ignita* (Poda) (Ephemeroptera : Ephemerellidae)

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SUMMARY. Eggs of *Ephemerella ignita* (Poda) were kept at eight constant temperatures (range 5.9—19.8°C) in the laboratory. Over 85% of the eggs hatched in the temperature range 10.0—14.2°C but the percentage decreased markedly to 39% at 5.9°C and 42% at 19.8°C. Hatching time (days after oviposition) decreased with increasing water temperature over the range 5.9—14.2°C and the relationship between the two variables was well described by a hyperbola. Therefore, the time taken for development was expressed in units of degree-days above a threshold temperature. Mean values (with 95% CL) were 552 (534—573) degree-days above 4.25°C for 10% of the eggs hatched, 862 (725—1064) degree-days above 3.57°C for 50% hatched and 1383 (1294—1486) degree-days above 3.14°C for 90% hatched. These values can be used to predict hatching times at temperatures below 14.68°C for 10% hatched, 14.54°C for 50% hatched and 14.45°C for 90% hatched. At higher temperatures, the hatching time and the number of degree-days required for development both increased with increasing temperature. Equations were developed to estimate the number of degree-days required for development at these higher temperatures.

Eggs were also placed in the Wilfin Beck, a small stony stream in the English Lake District. Maximum and minimum water temperatures were recorded in each week and the summation of degree-days was used to predict the dates on which 10%, 50% and 90% of the eggs should have hatched. There was good agreement between these estimates and the actual hatching times. Only 10—15% of the eggs hatched between October and late February with most of the eggs hatching in March, April and May. Nymphs hatching in October and November probably did not survive the winter.

Introduction

Nymphs of *Ephemerella ignita* (Poda) occur in small stony streams, in rivers and very rarely on lake shores (Macan, 1970). They are often abundant where there are higher plants or moss. The life-cycle in upland streams and rivers in Europe usually includes a long overwintering period in the egg, with nymphs present from March or April to September and a flight

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period from late June to September. This life-cycle has been recorded in Austria (Pleskot, 1958, 1961), Czechoslovakia (Petr, 1961), England (Butcher, Longwell & Pentelow, 1937; Macan, 1957; Elliott, 1967), Germany (Dürken, 1923; Dittmar, 1955; Müller-Liebenau, 1960), Scotland (Maitland, 1965; Egglishaw & Mackay, 1967), and Wales (Badcock, 1949; Hynes, 1961). In some of these studies, small nymphs were occasionally found in October and November after the flight period had ended, but they did not survive the winter (Pleskot, 1958; Hynes,

1961; Elliott, 1967). The life-cycle is markedly different in some lowland streams and rivers; the flight period is extended to almost the whole year in southern England (Sawyer, 1953; Kite, 1962), and nymphs have been recorded throughout the winter in Denmark (Jensen, 1956), southern France (Guillouzic, 1965; Thibault, 1971), and England (Percival & Whitehead, 1930; Crisp & Gledhill, 1970; Langford, 1971; Bass, 1976).

Some workers have suggested that the chief reason for this marked difference in the life-cycle is that the eggs develop quicker in the warmer lowland streams (Jensen, 1956; Thibault, 1971; Bass, 1976), but there is little detailed information on the hatching time of the eggs. Dürken (1923) notes that it took about nine months from June to March for eggs to hatch, and Thibault (1969) found that the hatching time varied between 101 and 339 days at $10°C$ and between 41 and 293 days at $15°C$ with very few eggs hatching at temperatures above $20°C$. The most detailed study of eggs of Ephemerella ignita is that of Bohle (1972) who concluded that there is an obligatory diapause in the tenth stage of the embryo and that the termination of the diapause within a year requires a water temperature below $16°C$, preferably below $13.3°C$. Different results were obtained in the present study which examined the hatching of eggs of E. ignita at different temperatures in the stream and the laboratory. Other studies of hatching in Ephemeroptera are limited to Baëtis spp. (Bohle, 1969; Elliott, 1972; Benech, 1972).

Materials and methods

The eggs of Ephemerella ignita have been described in detail by Degrange (1960). Oviposition was observed in the present study and has been described by Percival & Whitehead (1928) and Harris (1952). Swarming for oviposition usually occurs in the late afternoon and evening. The egg mass forms a spherical greenish ball that is carried at the genital aperture with the posterior abdominal segments curved downwards and round the ball to hold it in position. Eggs are usually laid in fast flowing and turbulent water, usually where moss is present. The female flies up-

stream and descends to the water surface, releasing the egg ball on contact with the water. After oviposition is finished, the female usually falls on to the water surface and is carried downstream, but occasionally she is able to fly to the bank. The egg mass separates immediately on entering the water and each egg has a polar anchoring mechanism (Degrange, 1960) that attaches the egg firmly to the substratum.

Eggs were obtained from females that were about to oviposit in the Wilfin Beck, a small stony stream on the west shore of Windermere in the English Lake District. The stream has been described by Elliott (1973). Sawyer (1950) and Harris (1952) record that females of E. ignita fly upstream before oviposition but this was unlikely in the Wilfin Beck because most of the eggs were laid in the section about 200 m from the mouth of the stream. Thirty-eight females were caught in the last two weeks of July and the first week of August. Each egg mass was placed on a glass slide under water and immediately the eggs separated to form roughly a monolayer of attached eggs. Six slides were placed in small plastic tubes that were closed at each end with nylon sifting cloth (aperture $75 \mu m$). Each plastic tube was placed in a heavy metal tube that was wedged between large stones in a swift flowing section of the stream. The eggs were inspected at monthly intervals and then at intervals of fourteen days when they started to hatch. A maximum and minimum thermometer was placed near the six tubes and was read and reset under water in each week. Oxygen concentration was measured with a Mackereth (1964) meter and was always over 85% saturation.

The laboratory experiments were performed in tanks originally used for fish studies and described in detail by Swift (1961). Each tank contained about $100 \, dm^3$ of water that was stirred and aerated by compressed air, and maintained within $\pm 0.1 - 0.2°C$ of a constant temperature. Oxygen concentration was always over 85% saturation. The tanks were covered with transparent polyethylene so that the eggs were illuminated by daylight. As there was only one window in the tank room, the maximum light intensity at the water surface in each tank was about 100 lux. Four slides, each covered by one egg

TABLE 1. Water temperature, number of eggs used in each experiment, and percentage of eggs that did not develop or died in the embryo stage

Temperature (°C) (mean and range)	5.9 ±0.2	8.2 ±0.2	10.0 ±0.1	12.1 ±0.1	14.2 ±0.1	15.8 ±0.1	18.2 ±0.2	19.8 ±0.3
No. eggs/egg mass (mean and range)	299 182–462	333 282–401	340 156–603	307 162–402	288 186–383	381 228–588	360 148–556	274 192–386
Undeveloped eggs (mean % with 95% CL)	60.1 ±8.3	24.2 ±9.6	10.1 ±4.3	11.4 ±5.3	13.5 ±8.6	17.0 ±6.2	23.8 ±12.4	45.1 ±18.5
Dead embryos (mean % with 95% CL)	1.4 ±0.7	0.6 ±0.5	0.4 ±0.1	0.4 ±0.2	0.8 ±0.5	4.5 ±1.2	10.7 ±4.7	13.2 ±3.1
Hatching period (days after oviposition)	308–588	112–392	56–266	56–224	56–196	56–294	126–420	140–532

mass, were placed in transparent plastic tubes closed at each end with nylon sifting cloth (aperture 75 μm), and the four tubes were placed in a tank. The eggs were examined at intervals of 14 days. When hatching commenced, the newly hatched nymphs were removed and counted. When hatching had apparently ceased, the slides were examined for a further 28 days and finally the dead embryos were counted. The water temperatures and number of eggs used in each experiment are given in Table 1.

Results

The number of eggs in each egg mass varied considerably with an overall range of 148–603 (Table 1). Less than 1% of the eggs died in the embryo stage at temperatures ≤ 14.2°C, but this percentage increased with higher temperatures to a maximum value of 13.2% at 19.8°C. A high proportion of the eggs did not even reach the embryo stage at both high and low temperatures (undeveloped eggs in Table 1). Therefore, the percentage of eggs hatching varied considerably with temperature (Fig. 1). Over 85% hatched in the temperature range 10.0–14.2°C but the percentage decreased markedly to 39% at 5.9°C and 42% at 19.8°C.

As there was a large variation in the number of eggs hatching at each temperature, the counts of newly hatched nymphs were expressed as a cumulative percentage of the total number of eggs that hatched at each temperature. When these cumulative percentages were plotted against time (days after oviposition), they followed a sigmoid curve (Fig.

2). As the eggs were examined every 14 days and there was some variation between the four egg batches in each experiment, it was impossible to estimate accurately the hatching times near the start and finish of the hatching period. Therefore the times at which 10%, 50% and 90% of the eggs had hatched were used in all subsequent analyses.

The relationship between hatching time and water temperature was well described by a hyperbola over the temperature range 5.9–14.2°C, and therefore there was a linear
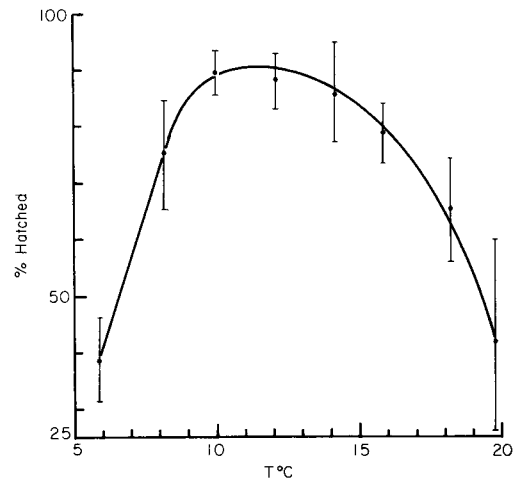

FIG. 1. Percentage of eggs hatching at each temperature; each value is a mean (with 95% confidence limits) for the four egg masses in each experiment.

relationship between rate of development (reciprocal of hatching time) and temperature over the same temperature range (Fig. 3). When data follow this relationship, the time taken for development can be expressed in
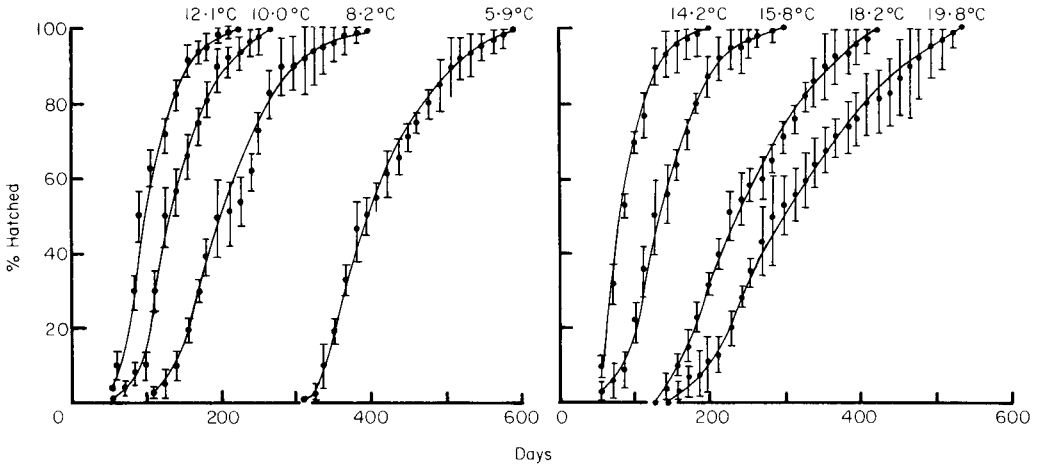
FIG. 2. Time required for eggs to hatch at different temperatures. Abscissa: number of days after oviposition. Ordinate: cumulative percentage of total number of eggs hatched in each experiment (each point is the mean with 95% confidence limits for the four egg masses).
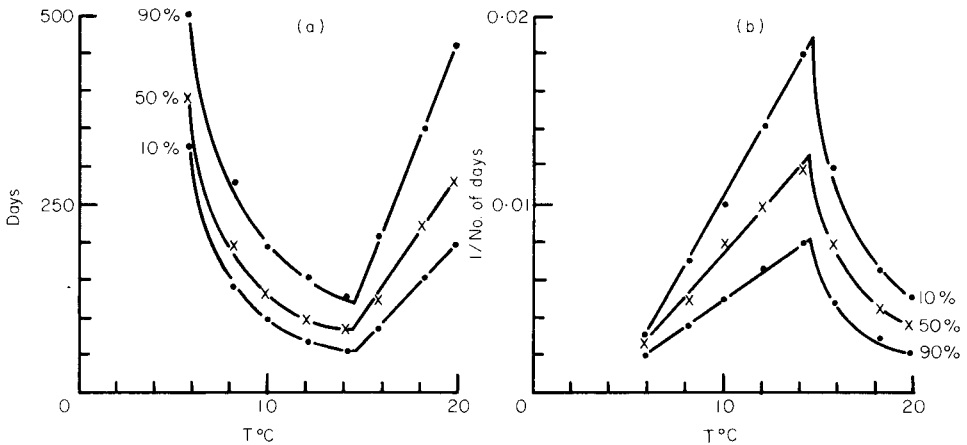

FIG. 3. Relationship between: (a) hatching time (days after oviposition) and temperature; (b) rate of development and temperature for 10%, 50%, and 90% of the eggs hatched.

units of degree-days above a threshold temperature. A linear regression line is calculated for the relationship between rate of development and temperature, the reciprocal of the regression coefficient is the total number of degree-days for development to be completed and the threshold temperature is obtained from the regression line as the temperature at which the development rate is zero (for a full description and discussion of this method, see Andrewartha & Birch, 1954). As the threshold temperature is usually obtained by extrapolation of the regression

line, it cannot be regarded as a true biological threshold. This was certainly the case in the present study and slightly different threshold temperatures were obtained for 10%, 50% and 90% of the eggs hatched. The final values for the mean number (with 95% CL) of degree-days are 552 (534–573) degree-days above $4.25 \pm 0.0004^{\circ}C$ for 10% hatched, 862 (752–1064) degree-days above $3.57 \pm 0.0016^{\circ}C$ for 50% hatched, and 1383 (1294–1486) degree-days above $3.14 \pm 0.0003^{\circ}C$ for 90% hatched. The three regression lines and therefore the three hyperbolic curves are highly significant

($P < 0.01$) and their excellent agreement with the data is illustrated in Fig. 3. The relationship between hatching time (d days after oviposition) and temperature ($T°C$) is therefore given by;

$$d = D/(T - t) \qquad (1)$$

where D is the total number of degree-days required for hatching and $t°C$ is the threshold temperature.

The hyperbolic relationship between hatching time and temperature ended at a temperature just above $14.2°C$ (Fig. 3). At higher temperatures, there was a marked increase in hatching time with increasing temperature and the relationship between the two variables is given by the regression equation:

$$d = b(T - T_0) \qquad (2)$$

where b is the regression coefficient and $T_0 °C$ is the temperature at which the value of d is zero (i.e. the value of T at the intercept of the regression line and the abscissa). Mean values of b and T_0 are 28.09 and $12.78°C$ for 10% hatched, 38.68 and $12.51°C$ for 50% hatched, and 62.63 and $12.49°C$ for 90% hatched. As there are only three pairs of values and one degree of freedom for each regression line, it is not possible to give confidence limits for b and T_0. The three regression lines were highly significant ($P < 0.01$).

An iterative process was used to calculate the following temperatures at which the curves given by equation (1) meet the regression lines given by equation (2): $14.68°C$ for 10% hatched, $14.54°C$ for 50% hatched, and $14.45°C$ for 90% hatched. These temperatures are the upper limit for the use of a constant number of degree-days in the estimation of hatching time. The number of degree-days is obtained by integrating temperature over time. For example, if temperatures remain below $14.54°C$, then about 50% of the eggs should have hatched when the total area under the curve relating temperature to days after oviposition is equal to 862 degree-days above $3.57°C$.

It is obvious from Fig. 3 and a comparison of equations (1) and (2) that the effect of higher temperatures is to increase the number of degree-days required for hatching. For example, the number of degree-days for 50% hatched is constant at 862 for temperatures

less than $14.54°C$, but increases to 1541 at $15.8°C$, 3277 at $18.2°C$, and 4544 at $19.8°C$. The following method was used to derive a general equation to estimate the total degree-days required for hatching. The highest temperature and minimum number of days required for 862 degree-days are $14.54°C$ and 78.58 days (from equation (1)). If temperatures ($T°C$) are higher than $14.54°C$ and the total time (d days) over which temperature exceeds $14.54°C$ is longer than 78.58 days, then the total number of degree-days (D) required for 50% hatched is given by:

$$
\begin{aligned}
D &= [78.58 + (d - 78.58)] \\
&\quad \times [(14.54 - 3.57)(T - 14.54)] \\
&= 862 + d(T - 14.54) + 10.97(d - 78.58)
\end{aligned}
$$
$$(3a)$$

where D is the number of degree-days above $3.57°C$.
If $T > 14.54°C$, but $d < 78.58$ days, then:

$$D = 862 + d(T - 14.54) \qquad (3b)$$

The equivalent equations for 10% hatched are:

$$D = 552 + d(T - 14.68) + 10.43(d - 52.92)$$
$$(4a)$$

where $T > 14.68$, $d > 52.92$ days with temperatures $> 14.68°C$.

$$D = 552 + d(T - 14.68) \qquad (4b)$$

where $T > 4.68$, $d < 52.92$ days.
The equivalent equations for 90% hatched are:

$$
\begin{aligned}
D &= 1383 + d(T - 14.45) \\
&\quad + 11.31(d - 122.28)
\end{aligned}
\qquad (5a)
$$

where $T > 14.45$, $d > 122.28$ days with temperatures $> 14.45°C$.

$$D = 1383 + d(T - 14.45) \qquad (5b)$$

where $T > 14.45$, $d < 122.28$ days.

Equations (3a), (4a), (5a) provide values for D that are identical to those obtained directly from the experiments, e.g. estimated values for 50% hatched are 1541 at $15.8°C$, 3277 at $18.2°C$ and 4544 at $19.8°C$. The adequacy of these equations for fluctuating temperatures in the stream could not be tested because water temperatures were rarely greater than $14.5°C$ during the period when eggs were present. It was possible, however, to examine the suitability of units of

degree-days for the prediction of hatching times in the stream.

The six egg masses placed in the stream were obtained from six females captured on 7 August 1966. This was therefore assumed to be the date of oviposition. The number of eggs in each mass varied from 164 to 403 and the mean percentage of eggs that hatched in each egg mass was $86.83 \pm 3.66\%$, a value comparable to the highest values obtained in the laboratory experiments (cf. Fig. 1). Mean

temperatures were obtained from the maximum and minimum values recorded in each week, and were plotted against time (Fig. 4). The area under the temperature curve was used to estimate the following dates on which the required numbers of degree-days were attained: 20 October 1966 for 10% hatched (552 degree-days above $4.25°C$), 24 February 1967 for 50% hatched (862 degree-days above $3.57°C$), 22 May 1967 for 90% hatched (1383 degree-days above $3.14°C$). There was good
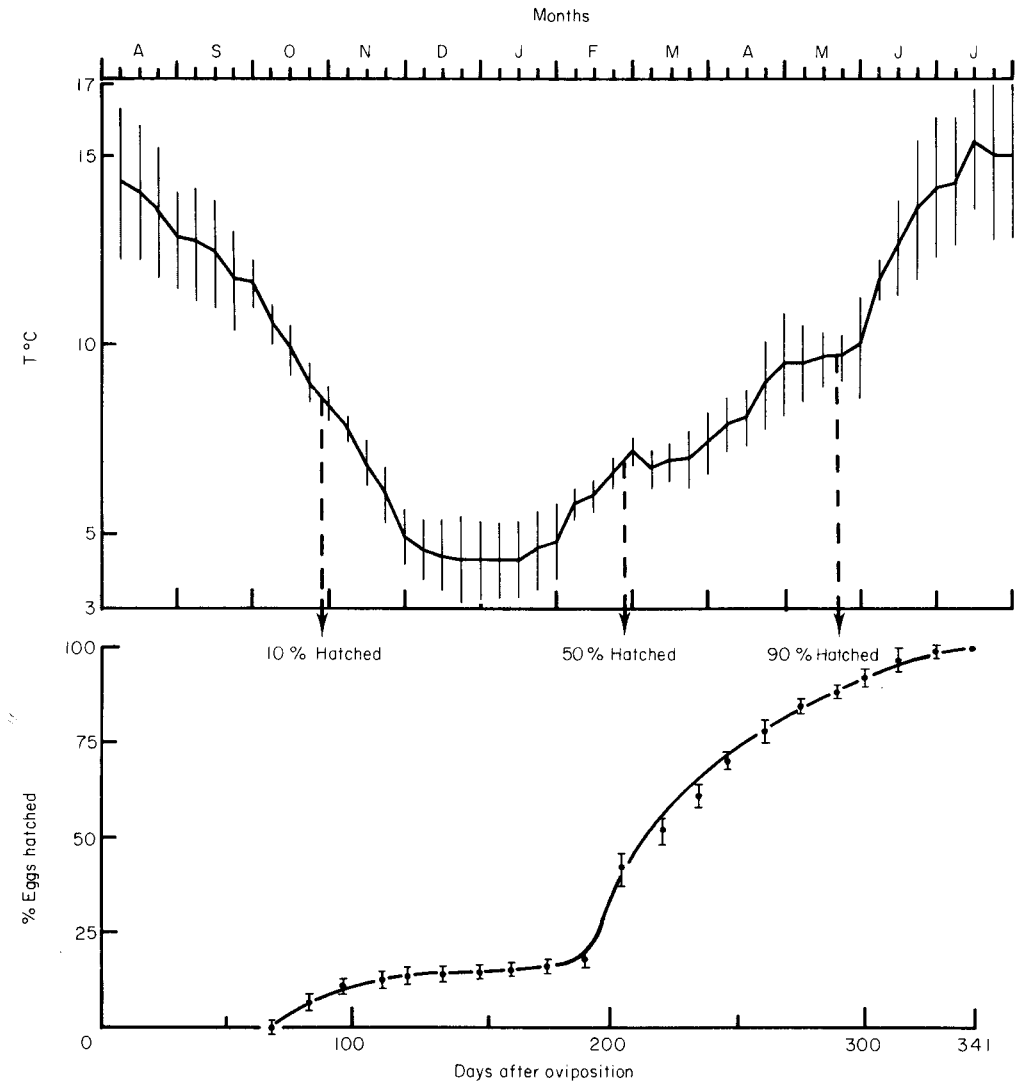

FIG. 4. Mean, maximum and minimum water temperatures in the Wilfin Beck with the predicted dates on which 10%, 50%, and 90% of the eggs should have hatched, and the cumulative percentage of the total number of eggs hatched in the stream (each point is the mean with 95% confidence limits for the six egg masses).

agreement between these estimates and the actual hatching times (Fig. 4). The latter values showed that only 10–15% of the eggs hatched between October and late February with most of the eggs hatching in March, April and May. The hatching of eggs in October and November explains the presence of a small number of tiny nymphs in the benthos in these months. As these nymphs were absent from benthic samples taken in December and January, they probably did not survive the winter in the Wilfin Beck.

Discussion

The only other detailed study of eggs of *E. ignita* is that of Bohle (1972) but as he found an obligatory diapause in the embryo, his hatching times are very different from those given in the present study. There was no evidence for an obligatory diapause in eggs of *E. ignita* from the Wilfin Beck. The simplest explanation for the differences between the two studies is that some populations of *E. ignita* have eggs that go into diapause whilst others do not, and Degrange (1960) has shown that some eggs may even develop parthenogenetically. Another possible explanation is that different photoperiods were used in the two studies. The daily light—dark alternation was constant at 12:12 h in Bohle's study, whereas natural illumination was used in the present study and therefore the photoperiod was not constant. In his limited study of eggs of *E. ignita* from a Pyrenean stream, Thibault (1969) also found that there was no diapause in the egg, and his hatching times of 101–339 days at 10°C and 41–293 days at 15°C are fairly comparable to those obtained in the present study (cf. Table 1).

Several workers have found small nymphs of *E. ignita* in October and November, long after the flight period, but have concluded that these nymphs did not survive the winter in cold upland streams (Pleskot, 1958; Hynes, 1961; Elliott, 1967). These workers have suggested that the nymphs were slow-growing stragglers from cohorts that hatched in summer, but the present study indicates that they were probably recently hatched nymphs.

Nymphs are found throughout the year in warm lowland streams. The higher temperatures may be partially responsible for the survival of the small nymphs in winter, and some workers have suggested that the eggs develop at a faster rate because of these higher temperatures (Jensen, 1956; Thibault, 1971; Bass, 1976). The present study has shown that this hypothesis is only partially correct. Higher temperatures in autumn and winter will shorten the hatching time but temperatures above about 14.5°C in spring and summer will lengthen it. The general effect of higher temperatures is thus to extend the hatching period to several months. It would be interesting to discover if the equations developed in the present study are applicable to these warmer lowland streams.

Acknowledgment

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