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Experimental studies on nymphal growth in *Leptophlebia vespertina* (L.) (Ephemeroptera)

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Summary

The mayfly, Leptophlebia vespertina (L.) was reared at field temperatures in the laboratory. The egg incubation period was 21–26 days. There were seventeen to nineteen nymphal instars. Growth was generally rapid during the first 3½ months and slow thereafter. This change occurred at the same time in both the laboratory and the field population. Temperature is considered to be the major factor regulating this and other changes in growth rate.

Introduction

The mayfly, *Leptophlebia vespertina* (L.) is one of the most widespread and abundant European species, occurring in a range of both lotic and lentic habitats. The first instar nymph of *L. vespertina* was described and figured by Degrange (1960), but no study has been made of its whole nymphal development. The aim of the present investigation was to rear *L. vespertina* in the laboratory through its life cycle from egg to imago. A number of life cycle studies have been made in the field (e.g. Moon, 1938; Macan, 1965; Kjellberg, 1972), but no attempt has been made to combine laboratory and field studies. In this paper nymphal growth in the laboratory is compared with field data (Brittain, 1972) from Llyn Dinas, an oligotrophic lake situated at 54 m a.s.l. in the mountains of North Wales. Llyn Dinas has an area of 0·31 km² and a maximum depth of 9 m. The littoral temperature regime is considered in detail in Brittain (1976). The successful rearing of *L. vespertina* under laboratory conditions also makes it possible in the future to investigate the responses of this important freshwater insect to various environmental parameters.

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Methods

A single female imago, taken near Llyn Dinas, oviposited while being transported to the laboratory and thus provided the batch of eggs. The eggs were kept in small Petri dishes half filled with lake water. They were maintained in a temperature room where the temperature was regulated approximately weekly to the mean littoral temperature prevailing in Dinas (Fig. 1 and Brittain, 1976). The laboratory temperature varied 1°C either side of the set value, so temperatures were generally more constant than Dinas, especially during the summer. The light regime was a constant 12 h day.

After hatching, ten nymphs were reared individually in Petri dishes. Mortality was high (60–70%) during the first 2 months of nymphal life, and while stocks lasted (July and August), dead nymphs were replaced by nymphs of known instar taken from the same egg batch. Nine nymphs were measured in September, seven in October and six in subsequent months. Food was provided in the form of small *Littorella* plants together with their associated detritus and epiphytes collected from Dinas. A fresh supply of food was added each week when the water was changed. The body length of each nymph, excluding antennae and cerci, was measured weekly and the presence of exuviae, indicating an instar change, was noted.

Results

In the laboratory the eggs of *L. vespertina* began to hatch on 30 June after 21 days. Although about 90% hatched during days 21 and 22, sporadic hatching

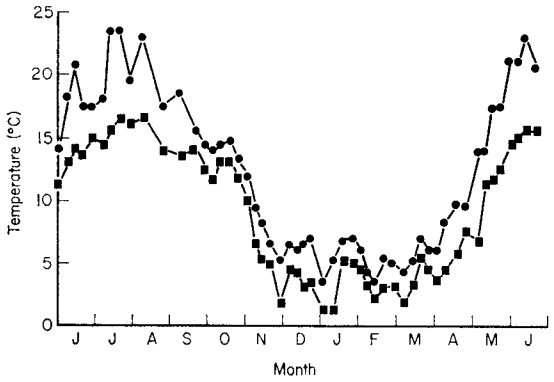

Fig. 1. Maximum and minimum temperatures in the littoral zone of Llyn Dinas from June 1969 to June 1970.

occurred until day 26. Temperatures during this period ranged from 14·5 to 20·5°C.

The growth of nymphs of *L. vespertina* reared in the laboratory was compared with that of the littoral population of Llyn Dinas (Fig. 2). The growth curves were divided into periods during which exponential growth was constant, the semi-logarithmic plot transforming the exponential curves into straight lines and stabilizing the variances. The relationship between nymphal body length (Y) and the number of days after hatching (X) was defined by the regression equation,

$$\log_e Y = \log_e A + RX, \text{ or } Y = Ae^{RX}$$

where A and R are constants. The proportion of the variance of Y attributable to its regression on X is given approximately by the square of the correlation coefficient (r) (Snedecor & Cochran, 1967, p. 176). Values of A, R, and r^2 are given in Table 1. For the laboratory population, values of A, R and r^2 were calculated both for the whole of the period from hatching to 106 days and for the constituent periods, 17–60 days and 60–106 days. Although the regression equation for the whole period 0–106 days gave narrow limits for R and a high value of r^2 (Table 1), it did not fit the individual points as well as the three separate periods. In addition, calculation of regression values for 60–106 days enabled comparison with field data from the same period, the first field data being from day 61. As the regression equations for growth in the laboratory during the periods 106–300 days and 300–342 days were not significantly different ($P > 0{\cdot}05$), the data were combined to give a single regression equation. If growth was assumed to be exponential during the first 17 days in the laboratory and from 290 to 317 days in the field, the respective relative growth rates (R) were 0·0343 and 0·0082.

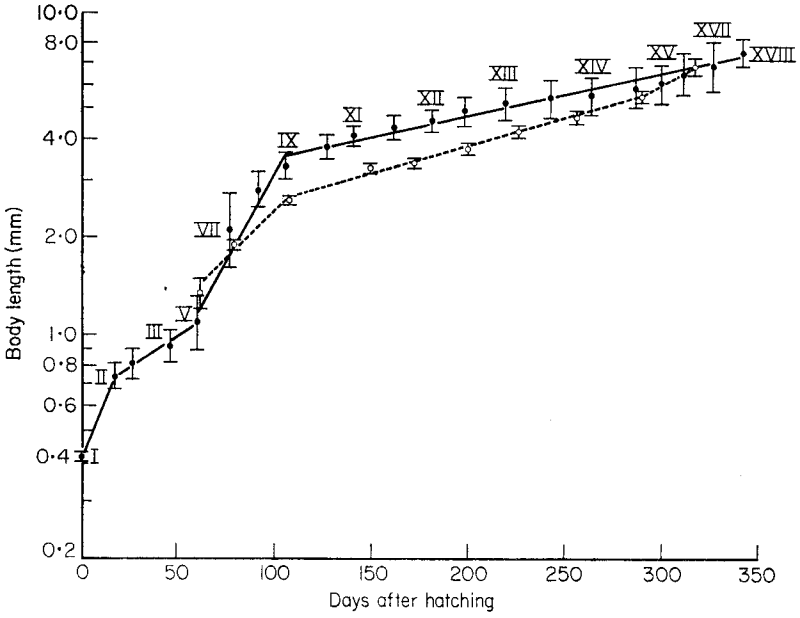

Fig. 2. Growth of *Leptophlebia vespertina*, 1969–70, in the laboratory (●) and in Llyn Dinas (○). Mean body lengths are given ±95% confidence limits. The predominant instar in the laboratory population is indicated by Roman numerals. The growth curves are divided into periods of exponential growth.

Table 1. Values of the constants A and R (±95% confidence limits) for each growth period of *Leptophlebia vespertina* in the laboratory and the field (Llyn Dinas). The proportion of the variance of Y due to the regression (r^2) is also given for each growth period, together with values for the residual variance (RMS). F from the variance ratio, degrees of freedom and significance level

	Time period								
	Dates	Days after hatching	A	R (±95%C.L.)	r^2	RMS	F	d.f.	P
Lab.	30.6.69–13.10.69	0–106	0·448	0·0186±0·0020	0·89	0·053	567	1 : 72	<0·001
Lab.	16.7.69–28.8.69	17–60	0·639	0·0085±0·0073	0·40	0·033	25·1	1 : 38	<0·001
Lab.	28.8.69–13.10.69	60–106	0·263	0·0249±0·0112	0·75	0·065	93·8	1 : 32	<0·001
Field	29.8.69–14.10.69	61–107	0·617	0·0136±0·0064	0·84	0·012	674	1 : 126	<0·001
Lab.	13.10.69–4.6.70	106–342	2·659	0·0029±0·004	0·79	0·013	327	1 : 85	<0·001
Field	14.10.69–13.4.70	107–290	1·756	0·0039±0·0003	0·73	0·019	926	1 : 348	<0·001

On account of the change in population composition, no mean length values are given after emergence had commenced. The first point on the field curve is at the end of August, 61 days after hatching. This is because of the problems of sampling small nymphs and the difficulty of separating early instar nymphs of *L. vespertina* from those of *L. marginata* (L.). A sample of *Leptophlebia* was in fact taken on 18 August (day 50) and the geometric mean length was 1·14 (95% confidence limits 1·10–1·19), which fits well into the Dinas regression for the period 61–107 days, despite the possible presence of nymphs of *L. marginata*.

During the first 3½ months (days 0–106/7) the growth rate was high, although somewhat retarded from mid-July to the end of August (days 17–60). From mid-October (days 106/7) until emergence the growth rate was low apart from a slight increase in the field population in the month prior to emergence. Thus the major change in growth rate, both in the laboratory and the field, occurred during the middle of October, approximately 110 days after hatching. In the laboratory the mean relative growth rate (R) changed markedly from 0·0186 to 0·0029, while a similar decrease occurred in Llyn Dinas (Table 1). Most nymphs in the laboratory had reached instar 9, half the total number of instars, after 110 days or about one third of the nymphal generation time.

The number of instars varied from seventeen to nineteen, although three out of the six that reached the final instar had eighteen instars (Table 2). From a total of twenty-one nymphs used in the laboratory study, two emerged successfully, thus giving an overall mortality of 90%. Mortality occurred mainly in the early instars and in the final nymphal instar.

Table 2. Relationship between body length and instar for a laboratory population of *Leptophlebia vespertina* reared at field temperatures. Means and confidence limits are arithmetic

Instar no.	Mean body length (mm) ±95% confidence limits	No. of nymphs measured
I	0·41±0·01	10
II	0·74±0·07	10
III	0·93±0·07	10
IV	1·20	4
V	1·60±0·19	5
VI	1·92±0·19	5
VII	2·36±0·18	7
VIII	2·63±0·20	8
IX	3·14±0·25	8
X	3·51±0·26	7
XI	4·03±0·24	6
XII	4·52±0·27	6
XIII	5·23±0·21	6
XIV	5·78±0·27	6
XV	6·43±0·24	6
XVI	6·97±0·36	6
XVII	7·35±0·44	6
XVIII	7·95	4
XIX	8·25	1

Discussion

In studies of egg development in *Baëtis rhodani* (Pictet) (Bohle, 1969; Benech, 1972; Elliott, 1972) egg incubation period and duration of hatching decreased with increased temperature. The present data for *L. vespertina* are similar to those obtained in the more comprehensive studies of *B. rhodani*.

The major change in growth rate occurred at the same time in the field and in the laboratory and so is likely to be caused by a factor common to both. Possible factors include temperature, nutrition and photoperiod. Temperature has been shown to be a

major regulatory factor in the life cycle of *L. vespertina*. Macan & Maudsley (1966) demonstrated that the onset of emergence was related to the date on which water temperatures reached 11°C, while Brittain (1976) showed advancement and retardation of emergence by alteration of the temperature. Both the major and minor changes in growth rate can be explained in terms of water temperatures (Fig. 1).

The major change in growth rate occurred when temperatures were falling rapidly from 10 to 15°C to around 5°C. The growth rate was also lower during the later part of July and the month of August (days 17–60), a period in which temperatures were high in Dinas, with maximum temperatures often exceeding 20°C and minimum temperatures generally over 15°C (Fig. 1). In the field population the growth rate increased at the same time as temperatures began to rise in April. If temperature is the cause of these changes in growth rate it appears that *L. vespertina* has an optimum temperature range, about 10–15°C, which may also vary with different developmental stages as Heiman & Knight (1975) found with the stonefly, *Acroneuria californica* Banks.

Other possible factors influencing the growth rate are nutrition and photoperiod. As the laboratory studies were carried out in a constant photoperiod this would not have provided the necessary signal and since changes in growth rate occurred at the same time in both populations, any influence of photoperiod is unlikely.

Nutritional factors could influence the growth rate. As the laboratory population was given excess food it is unlikely that the changes in growth rate were brought about by changes in available food. However, nutritional factors could modify the growth rate. The greater mean rate of the laboratory population from 60 to 106/7 days could be the result of food shortage in the field. It is interesting, however, that the field population reduced this deficit in the spring, and in fact the emergence of the two adults in the laboratory coincided with the emergence of adults at Llyn Dinas. This was due to a short burst of rapid growth prior to emergence in the field population, which could be caused by the greater variability of field temperatures resulting in temperatures reaching the critical point for initiation of rapid growth earlier. A similar reason could explain the slightly higher growth rate in the field during the winter months. Despite these minor differences the growth rates in the field and the laboratory were remarkably similar. From mid-July to mid-October the values of R were not significantly different ($P > 0{\cdot}05$), and although significantly different ($P < 0{\cdot}05$) from October until emergence the difference was small (Table 1).

Mortality during the early instars and the pre-emergence period may be a laboratory artefact, although Kjellberg (1972) in a field study of *L. vespertina* found the highest nymphal mortality in these stages. All the instars could not be distinguished on the basis of body length. In half the instars, the 95% confidence limits of two subsequent instars were greater than the differences between their mean values. The sexes were not separated in this study and thus some of the variation in nymphal size, particularly in the later instars, may be due to differences between the males and females.

The number of nymphal instars has not previously been determined for a member of the Leptophlebiidae. Other Ephemeropteran families have between twelve and twenty-nine nymphal instars (Murphy, 1922; Grandi, 1941; Degrange, 1959; Landa, 1969; Pescador & Peters, 1974), although Ide (1935) estimated between thirty and forty-five instars for *Stenonema canadense* Walker. In some cases the number of instars was constant and in others variable. Degrange (1959) found twenty to twenty-nine nymphal instars in *Cloëon simile* Eaton hatched from the same female and Cianciaria (in press) has recently shown that the number of instars in *Cloëon dipterum* (L.) can be varied by providing different types of food.

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