POST-OVIPOSITION DEVELOPMENT OF EGGS OF SOUTH AUSTRALIAN MAYFLIES

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ABSTRACT

Eggs of *Atalophlebia australis*, *Nousia inconspicua*, *N. fuscula* and *Baetis soror* from South Australia were incubated under constant temperature conditions in the laboratory (Range 4-24 °C). For each species the relationship between length of incubation period (Y) and incubation temperature (T) for the initial hatch (Hi) and mean egg hatch (Hx) was given by the equation $Y = aT^{-b}$. The rate of development (V) was given by the equation $V = bT + a$. Photoperiod length had no effect on the incubation period.

Embryonic development occurred at all temperatures, but hatching did not occur below 12 °C for *A. australis*, below 15 °C for *N. inconspicua* and below 5 °C for *N. fuscula* and *B. soror*.

Two strategies of egg development for the South Australian mayflies are proposed on the basis of these experiments and field life cycle studies: (1) a direct relationship between temperature and incubation period (*N. fuscula* and *B. soror*) and (2) a direct relationship between temperature and incubation period with a temperature enforced quiescence (*A. australis* and *N. inconspicua*).

INTRODUCTION

During the study of life cycles of some South Australian mayflies the seasonal occurrence of first instar nymphs suggested at least two distinct egg development relationships. First instar nymphs of *Atalophlebia australasica* and *Tasmanocoenis tillyardi* were never recorded during the cool winter months but were found in early Spring, before any emergence of adults had taken place. In contrast, first instar nymphs of *Baetis soror* were recorded in the winter months, and an emergence period preceded each recruitment period.

The differences observed in the presence of the first instar nymphs of *A. australasica* and *T. tillyardi* could be accounted for by two alternatives:

1. that the nymphs may have lived for several months without growing, but were inadequately collected; or
2. they remained as eggs, unhatched, over the winter months.

The sampling technique effectively collected first instar nymphs during the Spring, Summer, and Autumn, and for *B. soror*, first instar nymphs were recorded throughout the year, suggesting that the absence of observations was not an artifact of the sampling technique, unless the early instars of *T. tillyardi* and *A. australasica* entered the hyporheos immediately after hatching, (in which case they would not have been collected), remaining there until Spring.

Relationships between egg development time

I.C. Campbell (ed.) Mayflies and Stoneflies, 85–94.

Table 1. Incubation temperature, number of eggs, minimum incubation period (until first egg hatched), mean incubation period (50%), total duration of hatching and hatching success for eggs of four South Australian mayflies

Species	Temperature (°C)	No. of eggs	Minimum incubation period (d)	Mean incubation period (d)	Duration of hatch (d)	Hatch success (%)
Atalophlebia	24	25	9.0	12.5	3.5	56.0
australis	19	68	13.0	14.7	1.7	79.4
	12	25	?	?	?	?
	15*	25	8*	?	–	16.0
Nousia	24	53	10.0	10.3	1.8	79.3
inconspicua	22	262	13.0	14.1	3.8	82.8
	20	24	15.0	17.2	4.3	83.3
	20	50	15.0	16.8	5.8	82.0
	18	115	16.3	17.0	4.6	87.0
	18	29	18.2	19.7	5.9	69.0
	15	63	–	–	–	–
	12	26	–	–	–	–
	11	23	–	–	–	–
Nousia	24	74	13.9	14.3	5.0	93.2
fuscula	19	265	17.9	20.0	5.0	76.9
	19	86	17.9	20.3	5.0	72.1
	14	55	30.9	35.5	5.0	52.7
	14	118	30.9	36.9	7.0	52.5
	12.5	54	34.9	36.0	5.1	88.9
	12.5	68	35.2	36.2	4.1	92.7
	12.5	90	35.4	37.5	4.7	67.8
	9	82	71.9	73.0	14.0	69.5
	9	85	71.9	73.2	7.7	72.9
	9	93	72.0	74.4	6.0	55.9
	4	111	–	–	–	–
	4	78	–	–	–	–
	4	63	–	–	–	–
Baetis	24	86	7.8	8.4	3.0	87.2
soror	19	20	10.9	11.6	4.0	90.0
	19	10	10.9	11.9	2.0	50.0
	14	12	21.9	22.5	2.0	91.7
	14	12	21.9	22.5	2.0	66.7
	12.5	24	26.0	26.7	1.9	87.5
	12.5	22	26.0	27.7	1.9	68.2
	9	50	62.1	–	18.8	34.0
	4	16	–	–	–	–

* Eggs incubated as 12 °C for 120 days were transferred to 15 °C. Hatching occurred after eight days at the new temperature.

and water temperature have been widely demonstrated in the Ephemeroptera (e.g. Humpesch 1980a, 1980b, Humpesch and Elliott 1980) with temperature thresholds for hatching in some species (e.g. Bohle 1972, Friesen *et al.* 1979). Delayed hatching and/or migration into the hyporheos could lead to errors in the interpretation of life histories, growth rates, and production estimates. Therefore a series of egg development experiments at constant temperature, as suggested by Andrewartha and Birch (1954), were designed to determine if the observed phenomena could be explained by delayed hatching.

Environmental parameters, other than temperature, were also considered in the developmental experiments. Elliott (1978) suggested that the differences between his developmental data and those presented by Bohle (1972) for *Ephemerella ignita* might be explained by photo-period differences. In the present study eggs at different temperatures were allowed to develop in total darkness and in normal summer photo-period of long day: short night (16 hours: 8 hours).

MATERIALS AND METHODS

Artificial insemination with crushed male reproductive tissue (Pescador and Peters 1974) failed to fertilize any of the eggs stripped from female imagos reared in the laboratory, therefore eggs which had been fertilized naturally had to be obtained. All species examined, *Baetis soror, Nousia fuscula, N. inconspicua* and *Atalophlebia australis* oviposited by dipping the abdomen in slow flowing reaches of streams or in stationary pools or lakes. The eggs, once liberated, sank individually towards the bottom, and therefore no convenient egg mass could be collected from the substrate. Females in the process of ovipositing were collected and allowed to continue laying into a clear plastic vial of stream water. The vials of collected eggs were maintained at or near stream temperature and were immediately returned to the laboratory.

A series of 5 cm diameter plastic petri dishes containing stream water 4-5 mm deep were prepared and approximately 100 eggs were transferred to each dish. The petri dishes were then placed under different experimental conditions.

a. In constant temperature cabinets at 24 °C, 22 or 20 °C, 19 or 18 °C, 14 °C, 12.5 °C and 11 °C. A further series of 9 °C, 5 °C and 4 °C were added for *Nousia fuscula* and *Baetis soror.*

b. Under dark conditions at 24 °C, 20 °C and 18 or 19 °C constant temperature.

In all experiments replicates of 2-3 petri dishes were used. At temperatures below 24 °C the temperature variation was ± 1 °C, but at 24 °C the range was ± 2 °C.

The petri dishes were examined daily and development recorded. After eyes became apparent, the dishes were examined every three to four hours, and when hatching commenced the nymphs were counted and removed from the petri dish using a fine pipette. The volume of water in all experimental dishes was maintained every 3-4 days, or as required. When hatching ceased, the petri dishes were maintained and examined daily for a further 21 days, and the number of undeveloped eggs and dead embryos counted.

RESULTS

The incubation period, percentage egg hatch and duration of eclosion for each species are shown in Table 1. Two incubation periods were determined; a minimum time, taken as the number of days from oviposition to the initial egg hatch, determined by regular observation, and a mean time as the number of days from oviposition to the day on which 50% of the eggs ultimately hatching successfully had hatched. Both values were used in the calculation of subsequent regression. The mean value is probably more relevant to the population, especially if used as a predictive tool, as it overcomes the extreme cases of early and late hatchings that occur under normal circumstances, but comparisons between the minimum and mean values were considered valuable.

In the following discussion each species is considered individually, but the data presentation is consistent throughout, and is therefore discussed separately.

The number of eggs that hatched and the total hatching success was variable with temperature, and therefore counts of newly hatched nymphs were expressed as cumulative percentages of the total number of eggs that hatched at each temperature. These data were plotted against time and the resulting sigmoid curves allowed determination of the mean incubation period for each temperature. The relationship between the incubation period and water temperature was linear on a logarithmic scale, and is given by the regression equation $Y = a T^{-b}$ where Y = incubation period (days), T = temperature (°C) and a and b are constants.

The relationship between the rate of development V (V = the reciprocal of the duration of the hatching) and temperature is also given by a linear equation $V = bT + a$. When this is the case, the regression line may be extrapolated to determine the temperature at which $V = 0$, where theoretically no development occurs, i.e. the "threshold temperature" (Andrewartha and Birch, 1954). Andrewartha and Birch (1984) and Elliott (1978) both emphasised that this threshold temperature may not be a biological threshold, as development may continue below this temperature.

If the regression lines represent the relationship between hatching time and temperature the number of degree days required by the eggs to hatch can be calculated using the equation $D = d (T - t)$, where D = total number of days required for hatching, d = days after oviposition, T = incubation temperature and t = threshold temperature.

Atalophlebia australis

Part of the total egg compliment from one *A. australis* female imago was obtained and eggs were allowed to develop at 24°C, 19°C and 12°C.

Eggs hatched at the higher temperatures, none at 12°C. The duration of hatch curves for *A. australis* are shown in Fig. 1. With only two values determined, no regressions were calculated and consequently a threshold value could not be determined.

After four months at 12°C the eggs were transferred to 15°C, where hatching commenced within eight days, and continued for three days. A reduced rate of hatching success was observed, with only 24% of the eggs hatching at the elevated temperature. The requirement of only eight elapsed days to hatch at 15°C compared with ten days at 25°C and 13 days at 19°C suggests that much of the embryonic development had occurred at 12°C. The three ocelli of the developing nymph could be clearly seen in the egg after 18 days, but no further development was apparent at 12°C. On dissection of some eggs, the embryos were found to have differentiated cephalic, thoracic and abdominal regions, and the thoracic appendages were distinguishable. This study follows Wigglesworth (1972) in defining this type of arrested development controlled directly by external conditions and ending when conditions are again favourable as *quiescence*, distinguishing it from an arrest of development persisting when environmental conditions are favourable which he termed diapause.

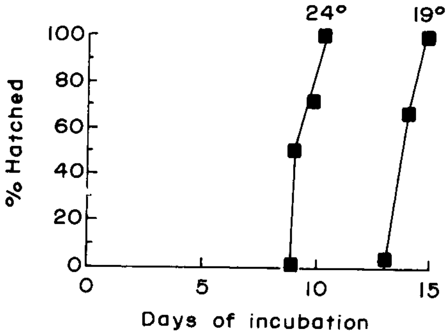


Fig. 1. Duration of incubation of eggs of *Atalophlebia australis* at 19 and 24°C.

Eggs of *A. australis* enter a period of quiescence when water temperatures fall below a temperature between 12°C and 15°C. More accurate determination of the quiescent initiating temperature was not possible due to shortage of fertilised eggs.

Nousia inconspicua

A greater number of experimental temperatures ranging from 11°C to 24°C were included for eggs of *N. inconspicua*, but once again no hatching occurred at the lower temperatures.

The duration of hatch curves, from which the mean incubation period was determined, are shown in Fig. 2. The length of the minimum incubation period varied between 10.0d and 18.2d and was linearly related to water temperature on a

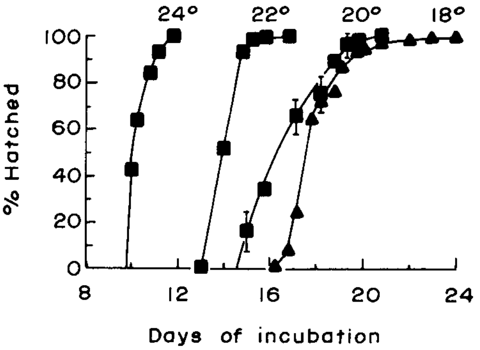


Fig. 2. Duration of incubation of eggs of *Nousia inconspicua* at 18, 20, 22 and 24°C.

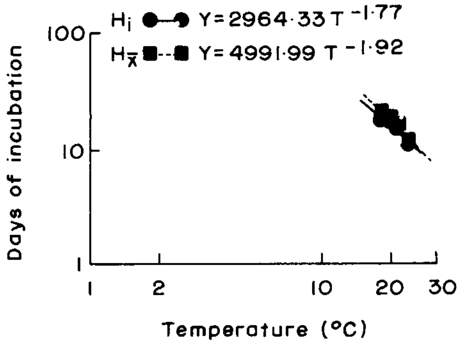


Fig. 3. Relationship between the time required for hatching (Days of incubation) and water temperature (°C) for initial hatch (Hi) and mean hatch (Hx) of *Nousia inconspicua.*

logarithmic plot (Fig. 3). The regression constants are tabulated in Table 2.

The duration of the initial and mean incubation periods were correlated with temperature and a linear relationship was found when the rate of development (V) was plotted against temperature (T). The values of these regressions for the minimum and mean incubation periods are given in Table 3.

An iterative process was used to calculate the value of the threshold temperature from the re-

Table 2. The relationship between length of incubation period (Y) and incubation temperature (T) for intitial (H_i) and mean egg hatch ($H\bar{x}$), where $Y = aT^{-b}$

Species		a	b	r^2	95% confidence limits of b	Probability
Nousia inconspicua	H_1	2964.33	1.77	0.93	0.47	**
	$H\bar{x}$	4991.99	1.92	0.89	0.67	**
Nousia fuscula	H_1	3136.66	1.75	0.99	0.14	**
	$H\bar{x}$	2835.92	1.69	0.99	0.12	**
Baetis soror	H_1	5904.09	2.12	0.99	0.20	***
	$H\bar{x}$	5485.61	2.08	0.99	0.24	***

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 3. The relationship between rate of development (Y) and temperature (T) for intitial (H_i) and mean egg hatch ($H\bar{x}$), where $V = bT + a$

Species		a	b	r^2	95% confidence limits of b	Probability
Nousia inconspicua	H_1	0.0065	−0.061	0.93	±0.0018	**
	$H\bar{x}$	0.0069	−0.073	0.88	±0.0026	**
Nousia fuscula	H_1	0.0040	−0.022	0.99	±0.0002	**
	$H\bar{x}$	0.0037	−0.020	0.99	±0.0002	**
Baetis soror	H_1	0.0078	−0.059	0.99	±0.0006	***
	$H\bar{x}$	0.0072	−0.052	0.99	±0.0005	***

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

gression equation. The threshold temperatures of the minimum and mean incubation period were 9.34°C (± 0.0018°C) and 10.63°C (± 0.0026°C) respectively. The values of the mean number of degree-days, with 95% confidence limits are 155 (145–165) degree-days above 9.34°C for the first hatching and 148 (136–158) degree-days above 10.63°C for 50% hatch. Clearly this cannot be a realistic situation with the mean number of hatchings occurring before the initial hatch. However, the values are really only applicable for development above 15°C. The threshold temperatures calculated above bear little resemblance to the observed hatching data obtained, because no hatching occurred below 15°C. The eggs were observed to stop developing at a similar stage to that observed for *Atalophlebia australis* and a similar development mechanism is suggested for both these species.

Eggs maintained at 20°C and 18°C, and in total darkness, hatched at the same time as those in the normal summer photoperiod, suggesting that light and/or photperiod were of minor importance in determining the incubation period of *Nousia inconspicua*.

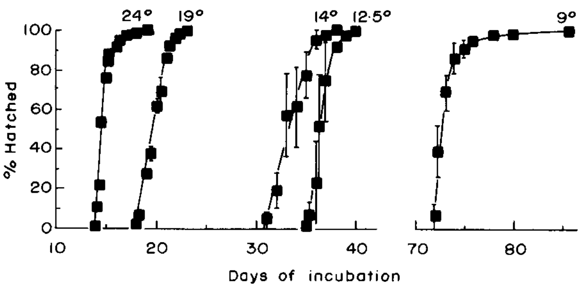
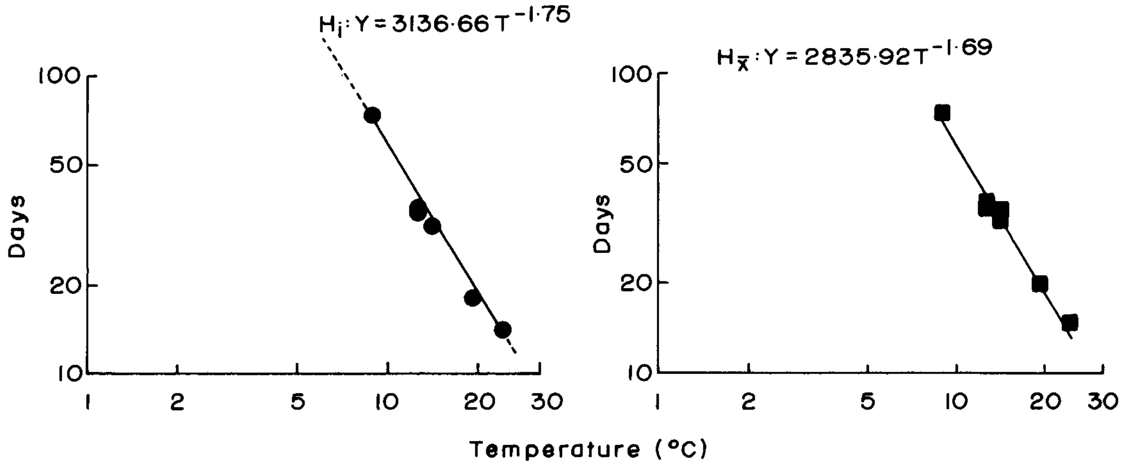


Fig. 4. Duration of incubation of eggs of *Nousia fuscula* at 9, 12.5, 14, 19 and 24°C.

Nousia fuscula

In contrast to the previous species, eggs of *N. fuscula* were observed to develop and hatch at temperatures from 9°C to 24°C. Eggs at 5°C did not hatch.

The duration of hatch curves are shown in Fig. 4. The minimum incubation period varied from 13.9d to 72.0d depending on the water temperature. The relationship of incubation time to temperature was linear on logarithmic plot (Fig. 5 and 6) and the regressions for the minimum and



Figs 5 & 6. Relationships between the time required for hatching (days of incubation) and water temperature (°C) for (5) initial hatch (H_i), and (6) mean hatch ($H_{\bar{x}}$) of *Nousia fuscula*.

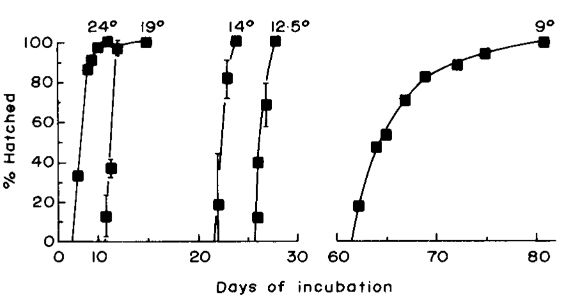


Fig. 7. Duration of incubation of eggs of *Baetis soror* at 9, 12.5, 14, 19 and 24°C.

mean incubation periods were significant (Table 2).

The relationship between rate of development (V) and temperature was linear, and the regressions for both initial and mean hatch were also significant (Table 3).

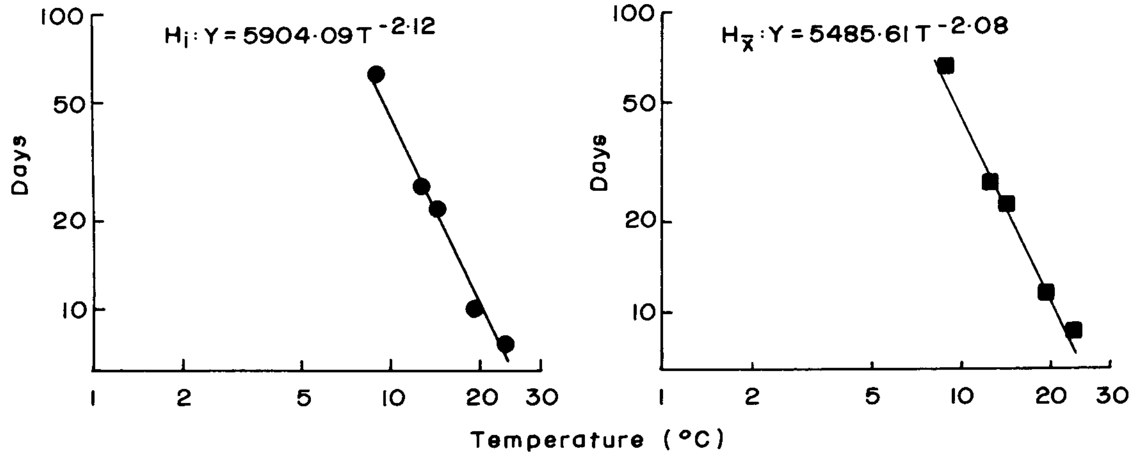
Using an iterative process with each regression the threshold temperature for *N. fuscula* was determined, being 5.40°C (± 0.0002°C) and 5.38°C (± 0.0002°C) for the initial and mean hatch respectively. The values of the mean number of degree-days with 95% confidence limits were 255 (245–265) degree-days above 5.40°C for the initial hatch, and 267 (257–277) degree-days above 5.38°C for 50% hatch.

The calculated degree-day values at each temperature were very similar for both the initial and mean incubation periods of the eggs of *N. fuscula*, and therefore they may be useful in predicting the hatching times of field populations. The adequacy of these values was not tested for the type of fluctuating temperature regime experienced in streams. However, a different mechanism of development to that observed in *Atalophlebia australis* and *Nousia inconspicua* is indicated.

The extrapolated threshold value of approximately 5.4°C was not a biological zero temperature because development did occur in eggs maintained at 5°C. The ocelli of the developing nymphs were visible after five months at the experimental temperature, but no hatching was observed. The calculated threshold temperature may therefore be close to the minimum hatching temperatures rather than the temperature of zero development.

Eggs maintained at 24°C and 19°C and in total darkness hatched at the same time as those in the normal summer photoperiod, suggesting that light and/or photoperiod were not important in the determination of the incubation period of *N. fuscula*.

Drying of *N. fuscula* eggs was also performed at



Figs 8 & 9. Relationships between the time required for hatching (days of incubation) and water temperature (°C) for (8) initial hatch (H_i), and (9) mean hatch (H_x) of *Baetos soror*.

24 °C to determine if eggs were capable of entering diapause, because Williams and Hynes (1976, 1977) postulated the presence of resistant eggs in mayflies from temporary stream habitats. Two batches of 48 and 60 eggs were allowed to dry at 24 °C. Drying was gradual, and took eight days, a process similar to drying pools in temporary stream beds. Some eggs were observed to collapse, but many maintained their shape. After 28d at 24 °C the dry eggs were re-wetted with freshwater (K_{18} = 311 μS) and subsequently observed daily for a further 28 days. No eggs hatched, and no development took place indicating that eggs of *N. fuscula* were unable to survive periods of drying.

Baetis soror

The eggs of *B. soror* also hatched at temperatures between 9 °C and 24 °C, with minimum incubation periods ranging from 7.8d to 62.1d respectively. At 5 °C no hatching occurred.

The curves for duration of egg hatch are shown in Fig. 7. The relationship between incubation period and temperature was linear on logarithmic plot, (Figs 8, 9) and the regressions for the minimum and mean incubation periods are given in Table 2. The linear regressions of development rate versus temperature were both highly significant and regression values are given in Table 3.

The threshold temperatures were calculated using an iterative process and were 7.55 °C (± 0.0006 °C) and 7.25 °C (± 0.0005 °C) for the initial and mean hatch respectively. Using these values the number of degree-days required for initial and mean hatching with 95% confidence limits were calculated, 126 (188–134) degree-days above 7.55 °C and 137 (129–145) degree-days above 7.25 °C respectively.

As for *Nousia fuscula*, the calculated number of degree-days required for egg development and hatching shows good correspondance for all the experimental water temperatures used. This agreement with the concept of thermal summation and degree-days may enable prediction of egg hatches from field observation of adult emergence periods, if mating and oviposition occur immediately.

Development was not tested under fluctuating temperature regimes, and therefore the applicability of the above data to field conditions is still unknown. An attempt was made, however, to assess these mean hatching data by using the observed field emergence times and calculating the data at which the first instar nymphs should have been collected. The date at which collections of adults were first made was taken as the zero time for each emergence period and it was assumed that eggs were oviposited on that date. Mean temperatures were obtained by taking the average of maximum and minimum temperatures recorded between collecting dates. The area under the temperature curve was used to calculate the number of degree-days experienced between collecting dates, and therefore estimates of the earliest date at which hatching should occur were obtained. Although this technique is crude, close correspondence between the predicted and observed dates was obtained.

For eggs laid on 8 October 1976, initial and mean hatching should have occurred by 10 November 1976. Similarly, for eggs laid on 6 January 1977 hatching should have occurred by 20 January 1977; for eggs laid 1 June 1977 hatching should have been evident by 31 August 1977 and for eggs laid on 21 August 1977 hatching should have been evident by 12 October 1977. From the life cycle of *B. soror*, (Suter 1980) there is some agreement to these calculated dates, if the presence of second or third instar nymphs is considered evidence in hatching. The 31 August 1977 sample did not include early instar nymphs, and in fact they were not recorded until 24 September 1977. This suggests that development under fluctuating stream temperatures may not be as simple a relationship as indicated under constant conditions. Alternatively, early instar *B. soror* may enter hyporheos during cooler, high discharge periods.

Twenty-six eggs of *B. soror* were allowed to dry as described for *N. fuscula*, at 24 °C. The results were similar to those observed for *N. fuscula*, with no eggs hatching after drying and no microscopic evidence of any development. Thus *B. soror* would appear unable to survive in temporary streams as dry, resistant eggs.

DISCUSSION

There are four published types of egg development for the Ephemeroptera:

a. a direct relationship between temperature and incubation period (e.g. Bohle 1969, Elliott 1978 in part; Friesen *et al.* 1979, Humpesch 1979, 1980b).
b. a direct relationship between temperature and incubation period between a low and intermediate temperature above which hatching time increases with increasing temperature (Elliott 1978, Humpesch and Elliott 1980).
c. development with an obligatory diapause (Bohle 1969, 1972).
d. a direct relationship between temperature and incubation period with a temperature enforced quiescence (Tsui and Peters 1974).

These types of egg development allow different mechanisms of regulation of life cycle in the egg stage. The direct relationship is the least effective of the four. The obligate diapause and development quiescence strategies of egg development allow the animal to persist in the egg stage during periods of unfavourable environmental conditions (i.e. low stream temperatures, high stream flow, low algal biomass, reduced organic detrital material) and synchronises hatching when the conditions become favourable. This facilitates recolonisation during conditions conducive to survival of the nymph. Hatching from the obligate diapause stage is a more complex mechanism than from simple quiescence, requiring very specific conditions to break the diapause. This strategy therefore synchronises the hatching of eggs more precisely than the quiescent mechanism. However, as Brittain (1982) commented egg diapause should not be used as an explanation of small nymphs in field collections.

In this study direct development occurred in *B. soror* and *N. fuscula*, but with higher threshold temperatures than those recorded for Northern Hemisphere species, and quiescent development occurred in *A. australis* and *N. inconspicua*. In *N. inconspicua* quiescence occurred at temperatures between 15°C and 18°C, while *Atalophlebia australis* was quiescent at temperatures below 15°C. *B. soror* had continuous egg development between 9°C and 24°C. Campbell (1986) considered the presence of first instar nymphs in early spring in the life cycle described by Suter and Bishop (1980) and Suter (1980) as evidence of a diapause, but this is not the case as the observed hatchings showed close correspondence with the predicted dates of hatching calculated using the concept of thermal summation.

Measuring rates of egg development under constant conditions in the laboratory inevitably imposes reservations on any extrapolation back to field conditions. Sweeney and Schnack (1977) and Sweeney (1978) considered that a fluctuating experimental regime should be used to determine egg development rates. They concluded that "thermal summation" should be abandoned because "the relationship between developmental rate and temperature is not linear". However, if egg development is not continuous over the range of the temperature fluctuations, and a quiescence occurs at lower temperatures, then fluctuating regimes may be introducing a considerable error in the degree-hours calculated. Sweeney and Schnack observed an inverse relationship between development time of the eggs of the corixid *Sigara alternata* (Say) and accumulated degree-hours with thermal increments above 15°C. This suggests that development may be retarded at temperatures below 15°C thus introducing error into their degree-hour calculation.

Because of the problems inherent in either technique of recording egg development, where possible both should be used. The rates at constant temperature should be determined first and once these have been established, studies in fluctating temperature regimes would be useful to determine their validity in the field situation.

In the present study two other factors which could influence egg development were investigated. Day length or light appeared to be of minor importance in regulation of the life cycle, with water temperature explaining the duration of incubation for all species. Williams (1968) discussed the existence of mayflies in temporary streams in

Australia, and suggesteded that the eggs of these had a diapause. Williams and Hynes (1977) also claimed that Ephemeroptera survived in temporary stream habitats with resistant eggs, but presented no direct evidence. Since temporary streams dominate the lotic habitats in South Australia, the mechanism of survival during the summer months is of prime importance. Non-viability of dried eggs of *B. soror* and *N. fuscula* in this study and the absence of records of resistant eggs from Ephemeroptera elsewhere (e.g. Edmunds *et al.* 1956) suggests that survival of mayflies in temporary habitats is by alternative mechanisms.

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