The effects of temperature on egg hatching of the mayfly *Austrophlebioides marchanti* (Ephemeroptera: Leptophlebiidae)

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Abstract

Naturally fertilized eggs of *Austrophlebioides marchanti* were incubated in the laboratory, with temperatures ranging from 4°C to 22°C. Egg development was direct and strongly dependent on temperature. At 22°C, the eggs required about 10 days incubation before the onset of hatching, but at 9°C an incubation period of about 2 months was required. The relationship between incubation temperature and incubation time can be described by the power law equation: $Y = 2998.898\ X^{-1.824}$, $r^2 = 0.98$, and $p<0.01$. Hatching time and hatching success were also temperature dependent, with a large proportion of the eggs hatching at 19°C and 22°C, and the proportion decreasing as the incubation temperature was reduced. Hatching was relatively synchronous, with over 50% of the eggs incubated at 19°C and 22°C hatching on the first day, and about 90% hatching within 5 days.

Introduction

One of the main features of the life cycles of temperate Australian mayflies is the relatively asynchronous development (Campbell, 1986), resulting in the presence of large size ranges of nymphs in a population at any one time. Lack of synchrony may be caused by a number of factors including variable development during the embryonic stage (Butler, 1984).

Temperature is a principal factor influencing egg development in mayflies (Brittain, 1982), and its effects have been studied extensively in Australia (Suter & Bishop, 1990; Brittain & Campbell, 1991; Brittain, 1995) and elsewhere (Bohl, 1969; Elliott, 1978; Humpesch, 1980; Giberson & Rosenberg, 1992). Most Australian mayflies exhibit a direct relationship between temperature and incubation time; only two species of *Coloburiscoides* from Victoria and New South Wales appear to display egg diapause (Campbell, 1986).

The mayfly *Austrophlebioides* is widespread and abundant in the stony upland streams of southeastern Australia (Campbell & Suter, 1988; Brittain, 1995; Peters & Campbell, 1991), but knowledge of its biology and life history is fragmentary. It's life history varies from fairly synchronised univoltine (Campbell, et al., 1990) to asynchronous in *A. pusillus* (Campbell, et al., 1990). Both extremes are explained by a long emergence period, and delayed egg hatching. The only experimental work on temperature effects on *Austrophlebioides* egg development illustrated species-specific differences between the species at Snowy Creek and Murray River (Brittain, 1995).

The main objectives of the present work were to investigate the effects of temperature on the incubation time and the hatching success of *Austrophlebioides marchanti* (Parnrong &

Campbell, 1997) eggs. A series of constant incubation temperatures were used to determine whether extended or delayed hatching of *A. marchanti* explains the poorly synchronised development of its nymphs. This information is required in life history studies for the prediction of nymphal hatching rates based on adult emergence periods in the field.

Materials and Methods

Field site

Loch River is a fourth-order stream about 100 km east of Melbourne (37° 48' 40" S, 145° 59' 00" E). The stream is situated south of the Great Dividing Range and drains into the La Trobe River. The stream channel at the study site is about 2 m wide, and the substrate is mostly medium sized stones and cobbles, with sand comprising about 10% of the area of the stream bed.

Methods

Twenty adult females of *Austrophlebioides marchanti* were collected from light traps on the stream bank at dusk, and each female was placed in a plastic vial containing filtered stream water, and then transferred to the laboratory in an ice box filled with stream water. Eggs were laid immediately or during transportation. The number of eggs from each female ranged from less than a hundred, up to several hundreds, with part of the variability arising because some females laid their eggs before capture, or were interrupted during their transportation to the laboratory. Artificial fertilisation was unnecessary because identifiable fertilised females were readily collected in the field.

In the laboratory, eggs from each female were divided into five groups, placed in 50-ml plastic vials, and incubated at 4°C, 9°C, 14°C, 19°C, and 22°C with a range of ± 1°C. The vials were inspected daily, and the incubation time (in days) and number of hatched eggs were recorded. The relationship between incubation time and temperature was calculated using regression analysis, and the percentage of hatched eggs was also recorded.

Degree-days (i.e. incubation temperature multiplied by incubation time) required before the onset of hatching was calculated for each incubation temperature. The values were plotted against incubation temperatures, and an equation representing the relationship was established.

Results

Austrophlebioides marchanti displays direct egg development which is strongly dependent on temperature. Hatching occurred at 9°C, 14°C, 19°C and 22°C, but not at 4°C, even after the eggs were incubated for four months. However, some egg development did occur at 4° C: the embryos formed visible ocelli after three weeks of incubation.

The incubation time for eggs decreased as the temperature increased. At 22°C, eggs required about 10 days incubation before the onset of hatching, but at 9°C an incubation period of about 2 months was required. The relationship between incubation temperature and incubation time can be described by the power law equation: $Y = 2998.898\,X^{-1.824}$, where Y is incubation time (in days), and X is incubation temperature (°C), $r^2 = 0.98$, and $p < 0.01$ (Fig. 1).

Hatching time and hatching success were also temperature dependent: a large proportion of eggs hatched at 19°C and 22°C, with the proportion decreasing as the incubation temperature was reduced. Hatching was relatively synchronous, with over 50% of the eggs incubated at 19°C and 22°C hatching on the first day of hatching, and about 90% hatching within 5 days. At 9°C, only 5% of the eggs hatched in the first day of the onset of hatching, and after 5 days only 50% of the eggs had hatched (Table 1).

Approximately 440 degree-days are required for eggs to hatch at 10°C, whereas only 260 degree-days are required at 20°C. The number of degree-days for hatching is linearly related to water temperature (Fig. 2). The relationship between degree days before hatching and incubation temperature is represented by the equation: $Y = 616.549 - 17.804X$ where Y is degree-days required before hatching starts, and X is incubation temperature (°C), $r^2 = 0.99$, and $p<0.001$ (Fig. 2).

Discussion

Effect of temperature on egg hatching

The hatching of *Austrophlebioides marchanti* eggs occurred between 9°C and 22°C, when incubated at constant temperatures. This temperature range corresponds to those reported for other Australian mayflies (Suter & Bishop, 1990; Brittain & Campbell, 1991; Brittain, 1995). Generally, temperate Australian mayfly eggs hatch successfully between 9-25°C, with the only known exception being *Austrophlebioides* from the Murray River (Brittain, 1995) where egg hatching was reported at 30°C. Presumably, tropical Australian species hatch at higher temperatures. European mayfly eggs hatch successfully in an incubation temperature range between 3-25°C, a lower maximum temperature than the Australian mayflies (Elliott, 1972; Humpesch & Elliott, 1980; Humpesch, 1980, Giberson & Rosenberg, 1992). The minimum incubation temperature (3°C) was reported for *B. rhodani* from England (Elliott, 1972). The maximum temperature, such as 25 °C for *B. rhodani* (Benech, 1972), is similar to Australian species. The North American mayfly *Tricorythodes minutus* hatches at temperatures between 7.5 and 23°C (Newell & Minshall, 1978), while egg hatching for *Hexagenia rigida* occurs between 12-32°C. However, if incubation starts at a low temperature, a high temperature of 36°C is required to enforce quiescence (Friesen, et al., 1979). Eggs of the tropical African mayfly *Povilla adusta* hatch at high temperatures of up to 35°C (Ogbogu, 1999).

Incubation time decreases with an increase of temperature within a specific temperature range. An incubation time of between 10-60 days for *A. marchanti* is in the range found by previous studies of Australian mayflies. Suter & Bishop (1990) report an incubation time of between 8-72 days, at incubation temperatures between 9-24°C, for four species of South Australian mayflies. Brittain & Campbell (1991) report incubation times of between 12 and 54 days for *Coloburiscoides* from Snowy Creek, and only four days at 25°C for *Baetis* from Snowy Creek (Brittain, 1995). However, the incubation time for *A. marchanti* is slightly higher than *Austrophlebioides* from the Murray River and from Snowy Creek, where an incubation period of 7-35 days is required at 10-25°C (Brittain, 1995). A period of 10-37 days of incubation is required for *Austrophlebioides* (Brittain, 1995) and 16-55 days for *Coloburiscoides* (Brittain & Campbell, 1991) to reach 50% hatching success at the same temperature ranges. Newell & Minshall (1978) report incubation temperatures between 7.5-23°C for *Tricorythodes minutus,* with the shortest incubation time of 21 days at 23°C and the longest being 125 days at 7.5°C.

Synchronised egg hatching is characteristic of temperate Australian mayflies (Brittain, 1995). Hatching times range from a few days to less than 20 days, depending on the incubation temperature (Suter & Bishop, 1990; Brittain & Campbell, 1991; Brittain, 1995; see also Campbell, 1986). Similar characteristic have also been reported for *P. adusta* from tropical Africa, where most of the eggs hatch within 1-2 days at incubation temperatures between 15-35°C (Ogbogu, 1999). In contrast, many northern hemisphere species exhibit a longer hatching time than Australian species. A period between 26-53 days has been reported for *Tricorythodes minutus* at incubation temperatures of 21°C and 12.5°C (Newell & Minshall, 1978).

Hatching success in *A. marchanti* is high, making it similar to other Australian mayflies with reported rates of 70-93% (Suter & Bishop, 1990; Brittain & Campbell, 1991; Brittain, 1995). Northern hemisphere mayflies also generally have a high hatching success, but with greater variation, ranging from 90% in *Baetis rhodani* (Bohle, 1969; Elliott, 1972; Benech, 1972), *B. vernus* (Benech, 1972) and *Ephemerella ignita* (Bohle, 1972; Elliott, 1978) to less than 50% for *Ecdyonurus* (Humpesch, 1980). A high hatching success rate of 85% was reported for *P. adusta* at the optimum incubation temperature of 33.2°C. Although hatching success generally increases with an increase of temperature, longer storage times at low temperatures lead to an increase in hatching success in *Hexagenia limbata* (Giberson & Rosenberg, 1992).

Why are small nymphs present throughout the year?

Poorly synchronous life histories and the presence of small nymphs throughout the year are prominent characteristics of many Australian mayflies (Campbell, 1986), including *A. marchanti* (Parnrong, 1999). Campbell (1986) and Campbell et al. (1990) found more synchronised development in *Coloburiscoides* populations from higher altitude alpine or subalpine streams. However they found low synchrony in populations taken from low altitude sites with higher temperatures of taxa identified as "*Atalophlebioides*", but presumably *Austrophlebioides* .

Embryonic development could be one of the factors that influence the synchrony of later life history patterns of Australian mayflies (Marchant, et al., 1984; Campbell, 1986), arising from the underlying patterns of oviposition and embryonic development. There are three possible scenarios:

1. Eggs hatch shortly after oviposition, but oviposition occurs over a long period (i.e. there is extended adult emergence), supplying small nymphs over a long period.
2. Eggs remain unhatched for long periods (i.e. there is delayed hatching), and there is a continuous supply of small nymphs.
3. Eggs hatch over a long period of time (i.e. there is extended hatching), supplying small nymphs over a long period.

Extended emergence of adults in the field is the most probable cause for the presence of small *A. marchanti* nymphs throughout the year, because eggs hatch within a relatively short incubation time. It is most likely that long emergence periods of over 6 months (Parnrong, 1999) allow adults to deposit their eggs over a longer period of time, leading to a supply of small nymphs throughout the year. Extended emergence is common to most Australian mayflies (Campbell, et al., 1990; Campbell, 1986), and may be a cause of asynchronous development (Campbell, 1986; Brittain, 1995).

There is no evidence that extended egg hatching is the cause of asynchronous nymphal development since eggs hatch within a short time when they are incubated at a natural temperature range (Brittain, 1995). *A. marchanti* eggs display high hatching synchrony: 50% of the eggs hatch within 5 days at 9°C, and over 50% of the eggs incubated at 19°C hatch in the first day after the beginning of hatching. Previous studies of the effects of temperature on Australian mayfly egg development support the view that extended egg hatching is unlikely to cause poor synchrony (Brittain, 1995). Similar synchronised hatching was found in the Australian *Baetis,* which require only two days for 50% of the eggs to hatch (Brittain, 1995). It took about 5 days for 90% of the eggs of *B. soror* to hatch at the same temperature (Suter & Bishop, 1990). Synchronous hatching was also found in African *P. adusta* with hatching durations as short as 1-2 days (Ogbogu, 1999).

Delayed hatching of eggs was not observed in *A. marchanti*. Although eggs did not hatch at 4°C, development still occurred, and hatching was detected at the next higher temperature (9°C). This finding is similar to temperature-induced quiescence in two South Australian species (Suter & Bishop, 1990), where hatching resumed when the temperature rose above 12-15°C. Similarly, Campbell (1986) suggests an egg or early nymphal diapause in *Coloburiscoides giganteus* in Digger's Creek near Mt Kosciusko. The data on egg development from Brittain & Campbell (1991) support egg diapause or quiescence in *Coloburiscoides giganteus* because the temperature at Digger's Creek fell below the restricted temperature of 5-10°C before the development was completed (Brittain & Campbell, 1991). This type of temperature-induced quiescence has also been reported for European species, such as *B. vernus* from Germany (Bohle, 1969) and *Ephemerella ignita* from England (Bohle, 1972; see also Brittain, 1982).

Applicability of laboratory results in the field

Most egg hatching experiments have been carried out in the laboratory using a series of constant temperatures. Relationships between incubation time and temperature, and degree-day and temperature, have been used to predict egg hatching in the field. Humpesch & Elliott (1980) found agreement between their laboratory and field experiments in two out of three species, and suggested that the power equation derived in the laboratory could help predict egg hatching in the field.

The number of degree-days required for the hatching of several species of mayfly eggs has been shown to be higher at low temperatures (Suter & Bishop, 1990; Brittain & Campbell, 1991). Elliott (1978) also found a similar relationship between degree-days required for *Ephemerella ignita* eggs to hatch at different temperature. Likewise, the prediction of degree-days for *A. marchanti* eggs before hatching agrees with the results from the laboratory.

Sweeney & Schnack (1977) argue that because temperature in the field is not constant, and the rate of development is non-linear, then thermal summations (degree-hours, degree-days) should not be used. They examined the egg development of *Sigara alternata* under a fluctuating thermal regime, and found that the sum for effective temperatures between 0-12.0°C was not constant, and appeared to be delayed at high temperatures. It is possible that development might not be continuous in this temperature range, and quiescence at low temperatures could introduce errors into the degree-hours calculation. Therefore, the biological zero, or temperature threshold at which growth stops (Winberg, 1971), should be taken into account in the degree-days calculation.

A series of constant temperatures are necessary in order to identify the temperature at which growth stops (i.e. the biological zero). Subsequently, the degree-days required for eggs to hatch can be obtained, and egg hatching in the field can then be predicted because field temperatures can be measured. Alternatively, the relationship between degree-day required for hatching and water temperature can be used to predict egg hatching at a particular range of incubation temperatures.

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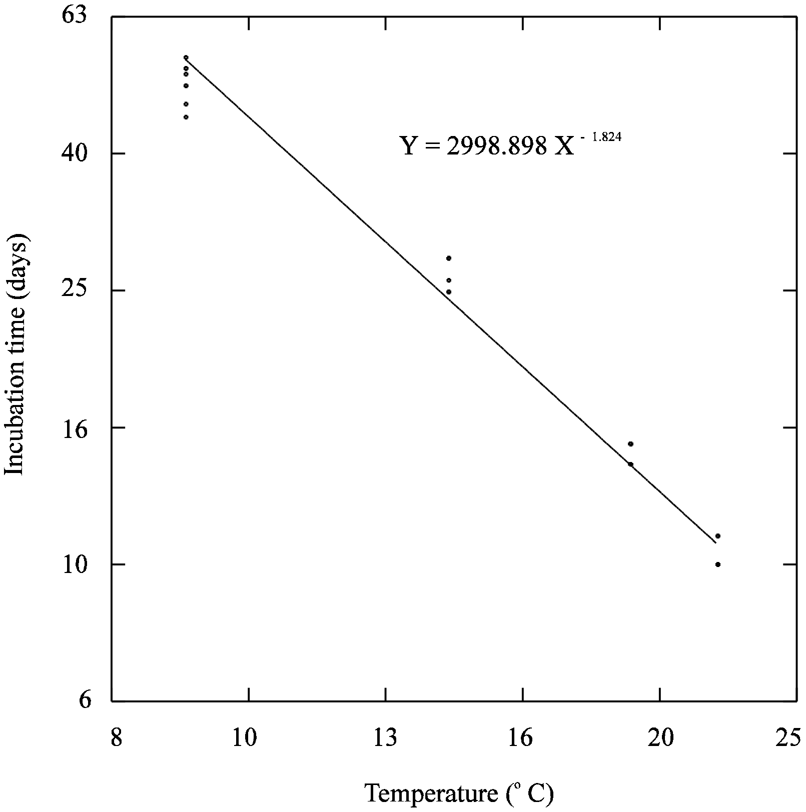
63
40
25
16
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6
8
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13
16
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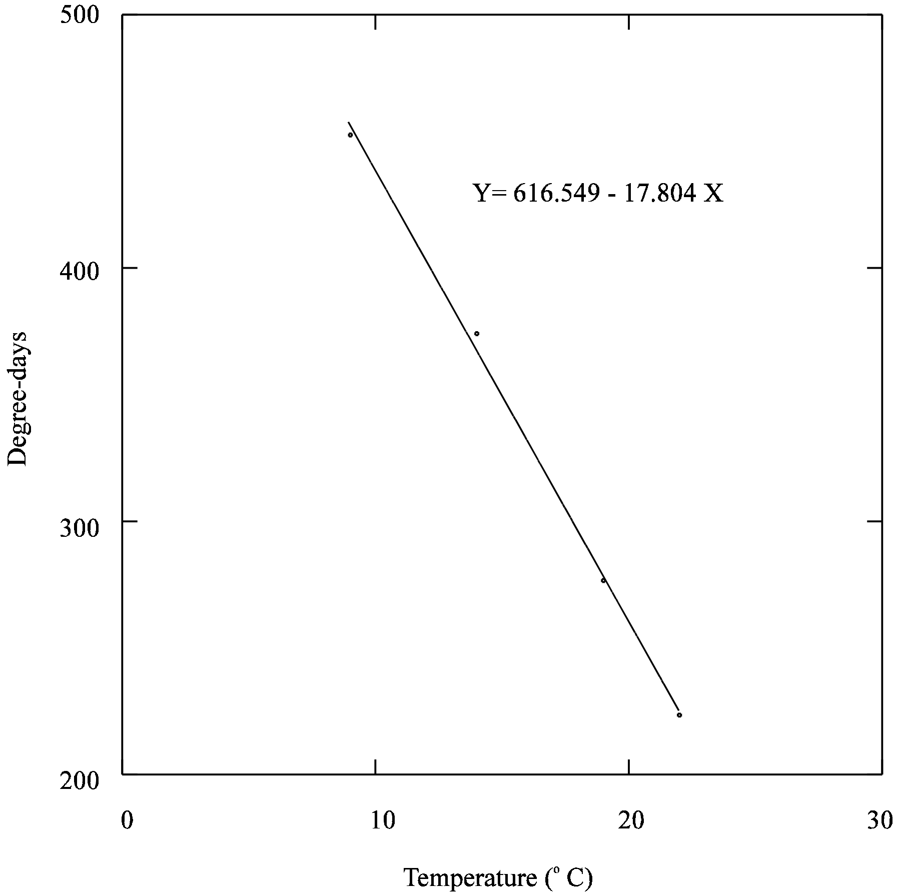
25
Incubation time (days)
Temperature (° C)
Y = 2998.898 X - 1.824


500
400
300
200
0
10
20
30
Degree-days
Temperature (° C)
Y= 616.549 - 17.804 X

Temperature (°C)	**Hatching success (%)**			
	First day	After 3 days	After 5 days	After 7 days
22	70.5 ± 9.9	79.1 ± 6.8	90.0 ± 5.8	92.3 ± 4.2
19	51.9 ± 9.7	62.8 ± 7.5	87.6 ± 3.9	94.7 ± 3.2
14	20.4 ± 11.7	62.3 ± 12.6	76.2 ± 8.0	90.6 ± 5.8
9	5.3 ± 6.9	34.5 ± 19.9	50.4 ± 12.9	54.8 ± 11.4

Fig. 1. Linear regression between incubation time (in days) and incubation temperature (°C) on logarithmic scales for *A. marchanti*, $p < 0.01$, $r^2 = 0.98$.

Fig. 2. Plot of degree-days required for *A. marchanti* eggs to hatch against temperature, $p < 0.001$, $r^2 = 0.99$.

Table 1. The average (mean ± SE) hatching success (%) of *A. marchanti* eggs, SE = standard error. Hatching success is related to incubation temperature and the time after hatching started. At 4°C, no hatching occurred.