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Effect of Temperature on the Egg Hatching of *Potamanthus formosus* (Ephemeroptera: Potamanthidae)

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Abstract

Eggs of *Potamanthus formosus* were kept at constant temperature (range 4.8-30°C) in the laboratory. The percentage of eggs hatched was 34-53% over a mean temperature range of 15-30°C, peaking at *ca.* 20°C, but dropping abruptly below 15°C to almost zero at *ca.* 13°C. The developmental time (Y) was inversely related to the water temperature, and the relationship was well described by a hyperbolic equation. The threshold temperature for egg development was calculated to be 10.5°C for 50% hatch based on the relationship between the rate of development ($1/Y$) and temperature. This suggests that the threshold temperature for egg development may differ from that for egg hatching. The calculated values of the summation of effective degree-days were 222.2 above 10.5°C for 50% hatch. Extrapolating the results to a natural stream, eggs must be laid until late in September to hatch by the time water temperature decreases to 15°C. The above estimation suggests that eggs laid late in the emergence period from June to October would not hatch before winter. These eggs may overwinter and hatch in spring. This conjecture is supported by the fact that the number of tiny nymphs evidently increased in April and May.

Key words: mayfly, egg hatching, temperature, Japanese stream

1. Introduction

There are many mayfly species whose tiny nymphs are found long after their emergence period. This phenomenon has been attributed by many authors to delayed hatching (e.g. MACAN, 1957; HYNES, 1970). On the other hand, ELLIOTT and HUMPESCH (1980) believed instead that the prolonged occurrence of tiny nymphs is due to the very slow growth of some nymphs after hatching, since experiments proved that the eggs of many mayflies hatched within a remarkably short period. However, these experiments also showed that the period required for egg-hatching generally lengthened at lower temperatures, although the eggs hatched synchronously at a particular constant temperature (ELLIOTT, 1972; FRIESEN *et al.*, 1979; HUMPESCH, 1978, 1980a, 1980b; HUMPESCH and ELLIOTT, 1980; NEWELL and MINSHALL, 1978; SUTER and BISHOP, 1990). This suggests that in autumn when the temperature gradually decreases, minor differences in the start of egg development may result in major discrepancies in hatching time.

WATANABE (1988) reported that, on the basis of seasonal change in nymphal size, *Potamanthodes kamonis* (IMANISHI) (=*Potamanthus formosus* EATON; synonymized by UÉNO, 1969 and combination revalidated by BAE and Mc CAFFERTY, 1991) had three cohorts a year which emerged one after another from June to early October, and that the tiny nymphs found in winter and spring composed a cohort which emerged intermediately. He suggested the possibility that the eggs laid in the late emergence period overwintered and hatched in spring since the number of tiny nymphs evidently increased in April and May in the size distributions.

In the present study, I examine the relationship between water temperature and the hatching time of eggs, and discuss the possibility mentioned above.

2. Materials and methods

The eggs were obtained from a population of *P. formosus* in the upper reach of the Kohto River, Kagawa Prefecture (34°10′N, 134°05′E). This site is 140 km distant from Hatsukagawa Creek where their life history was studied by WATANABE (1988). Annual regimes of water temperature did not differ appreciably between both streams (see Fig. 3).

Winged adults of *P. formosus* were collected with a light trap on 15 and 20 August 1990. All adults collected were subimagos, and moulted to imagos in the laboratory on the next day. The eggs were fertilized artificially. An egg mass extracted with forceps from a female imago was divided into 3-5 batches, each of which was put into a glass Petri dish with a drop of a linger solution (NaCl 0.9%, KCl 0.02%, $CaCl_2$ 0.02%, $NaHCO_3$ 0.002%). Then the tip of the abdomen of the male imago was crushed, and the sperm was transferred to and mixed with the eggs in the dish with a preparation needle. After fertilization, the dish was filled with dechlorinated water to a depth of 4-5 mm. The eggs shortly became attached to the bottom of the dishes which were then placed under different experimental temperatures, using a temperature gradient chamber (Type TG-100-AD; Nippon Medical & Chemical Instruments). The water was changed every three days, and there was no forced aeration in the dishes. The temperatures were measured randomly day and night over the whole experimental period. The photoperiod in the chamber was 12L-12D throughout the experiments with artificial light. Four and seven replicates at each experimental temperature were started on 16 and 21 August, respectively, but the data of both replicated series were combined because no difference in hatching time was detected between them.

When hatching commenced, the newly-hatched larvae were removed and counted daily under a binocular microscope. On the 180th day after fertilization, the experiments were terminated, and the number of hatched and unhatched eggs in each dish was counted with a pen-type colony counter.

3. Results and discussion

Eggs hatched in all dishes at 19.5-30.0°C experiments. But no eggs hatched in one dish at 15.1°C, and in eight dishes at 13°C. Hatching failed to occur in any dishes at temperatures below 11°C (Table 1).

The mean percentage of eggs hatched was the highest at 19.5°C, and slightly lower at higher temperatures. It abruptly dropped at temperatures below 15.1°C (Fig. 1).

Developmental time (the number of days required for hatching) for 5%, 50% and 95% of the total number of hatched eggs of each dish and the length of the hatching period (5-95% of eggs hatched) were shown in Table 2. Developmental time was inversely related to water temperature. The hatching period for 5-50% eggs was much shorter than that for

Table 1. Incubation temperature, number of eggs per dish, and number of successful experiments (some eggs hatched).

Temperature (°C)									
mean	30.0	27.8	25.1	19.5	15.1	13.0	10.8	7.7	4.8
range	29.7-30.3	27.5-28.7	24.8-25.7	18.8-20.2	14.9-16.8	12.6-14.3	10.1-12.0	7.2-8.4	4.2-5.5
No. dishes	11	11	11	11	11	11	11	11	11
No. eggs/dish									
mean	329	419	367	371	322	366	395	432	469
range	133-575	234-661	196-493	223-611	189-426	174-587	239-724	262-572	254-657
No. successful experiments	11	11	11	11	10	3	0	0	0

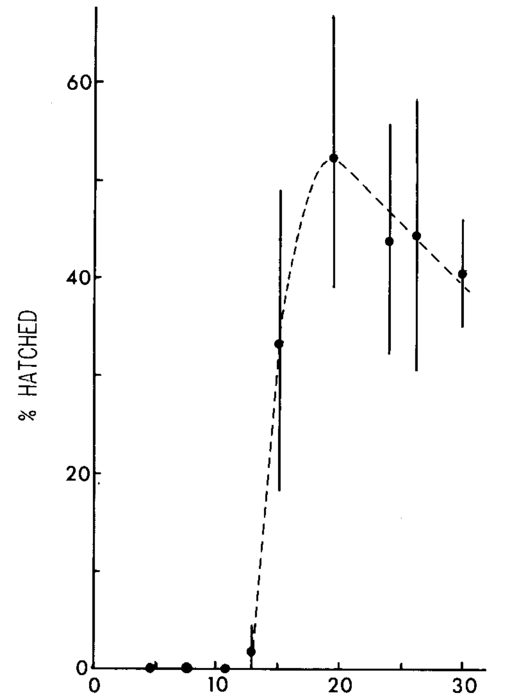

Fig. 1. Percentage of eggs hatched per dish at each average temperature. Each value is the mean with 95% confidence limits. Curve was fitted visually.

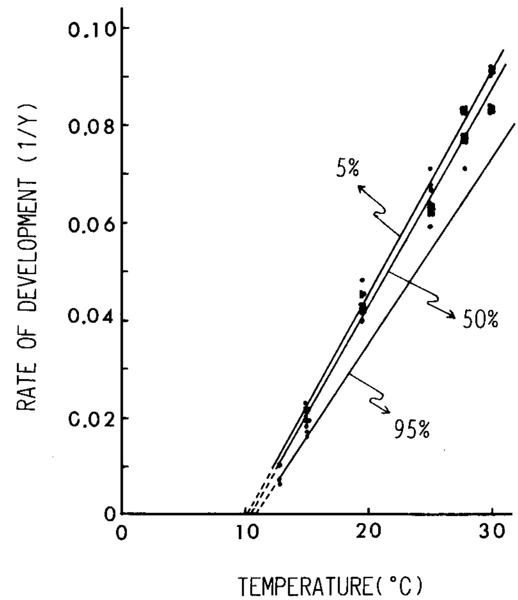

Fig. 2. Relationship between rate of development for 5%, 50% and 95% of eggs hatched (1/Ydays) and temperature(T°C) in the laboratory. Values for 50% hatch only are dotted.
Regression equation: 5% hatch, $1/Y = 4.6\times10^{-3}T - 4.6\times10^{-2}$ (r=0.993; $P<0.01$); 50 % hatch, $1/Y = 4.5\times10^{-3}T - 4.7\times10^{-2}$ (r=0.988; $P<0.01$); 95 % hatch, $1/Y = 3.8\times10^{-3}T - 4.2\times10^{-2}$ (r=0.967; $P<0.01$).

50-95% eggs hatched. This means that the majority of the eggs synchronously hatched at the beginning of the hatching period. Most of the eggs hatched within a week at temperatures above 19.5°C, but the hatching period was remarkably extended at 15.1°C.

The relationship between the rate of development (a reciprocal of the developmental time; $1/Y$) and water temperature over the range of 13-30°C was linear (Fig. 2). Threshold temperatures for egg development (the temperatures at which $1/Y$ was zero) were 10.1°C for

Table 2. Developmental time for 5%, 50% and 95% of eggs to hatch, and hatching period (5-95% of eggs hatched), at different constant temperatures. Values are indicated by the mean ±95% confidence limits at 15.1°C-30°C and ranges at 13°C. Hatching period at 13.0°C is not shown because only three experiments were successful and hatching rate in each experiment was very low.

Mean temperature (°C)	30.0	27.8	25.1	19.5	15.1	13.0
Developmental time (days)						
5 %	11.1±0.2	12.5±0.4	14.9±0.2	21.8±0.8	46.1± 2.4	87−167
50 %	11.7±0.6	12.7±0.4	15.6±0.6	23.1±0.8	52.3± 4.2	98−167
95 %	14.2±1.5	15.8±0.9	18.8±0.8	27.5±1.8	84.5±20.1	137−179
Hatching period (days)	4.1±1.4	4.3±0.8	4.9±0.8	6.6±1.8	39.2±19.0	—

5% hatch, 10.5°C for 50% hatch, and 11.0°C for 95% hatch. This suggests that the threshold temperature for egg development may differ from that for egg hatching since only a few eggs hatched at 13.0°C (Fig. 1). The relationship between the developmental time (Y) and water temperature (T) was given by the hyperbolic equation:

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

where D is the total number of degree-days required for hatching, and t is the threshold temperature for egg development (ANDREWARTHA and BIRCH, 1954). The mean numbers of degree-days above the threshold temperatures were calculated from equation (1) and the regression equations in Figure 2 to be 217.4 above 10.1°C, 222.2 above 10.5°C, and 263.2 above 11.0°C for 5%, 50% and 95% hatch, respectively.

The data obtained by the constant-temperature experiments cannot be simply applied to the egg development at fluctuating temperature regimes in the field (ANDREWARTHA and BIRCH, 1954; SWEENEY and SCHNACK, 1977). HUMPESCH (1978) reported that the developmental time of some heptageniid eggs in the fluctuating temperature regime was shorter than under constant temperature conditions. SWEENEY (1978) also mentioned that the rate of development of mayfly eggs was correlated positively with increased magnitude of the diel temperature pulse. However, his interpretation of the results seems to admit of argument. On the other hand, HUMPESCH (1982) reported, contrary to his preliminary experiment mentioned above, that the effect of temperature on the rate of change in hatching time and the rate of develop-

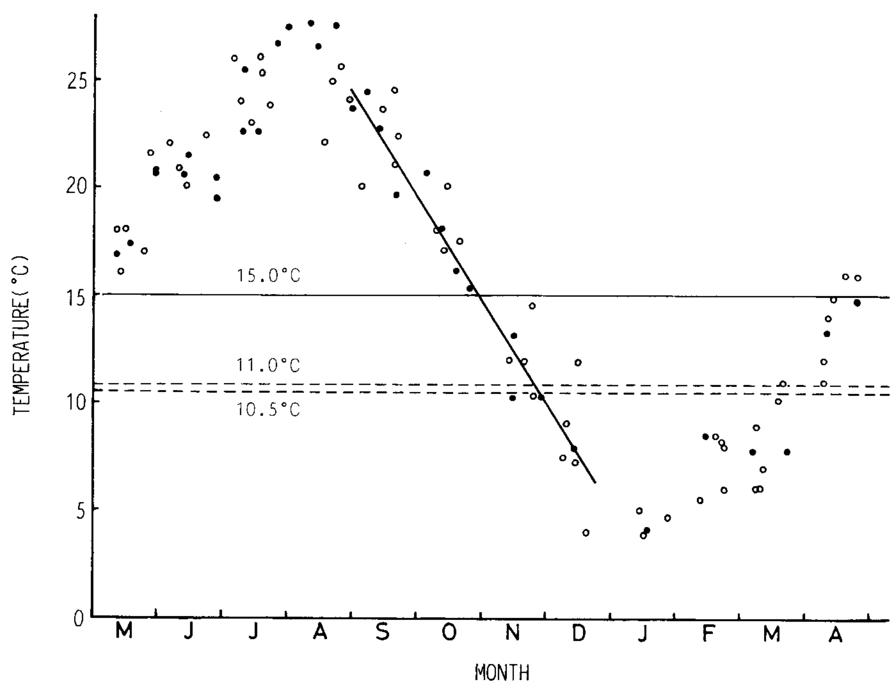

Fig. 3. Seasonal change in water temperature at 15 : 00-15 : 30 in 1978 and 1979 in Hatsukagawa Creek (solid circles). Regression line was fitted to temperature values from 29 Aug. to 13 Dec. in Hatsukagawa Creek. Horizontal solid line shows the temperature (15°C) below which hatchability abruptly dropped in laboratory. Broken lines show threshold temperatures for egg development for 50% and 95% hatch. See text for details. Open circles are water temperature at 13 : 30-14 : 30 in 1983-1988 at upper reach of Kohto River where adults were collected. Data were obtained from Annual Report of Water Quality published by Kagawa Prefectural Government.

ment of heptageniid eggs is similar for both constant and fluctuating temperatures. In addition, it has been reported that in some other mayflies the rate of development obtained at constant temperatures agrees well with that at fluctuating temperatures in natural streams (ELLIOTT, 1972, 1978; HUMPESCH, 1980a; HUMPESCH and ELLIOTT, 1980). Therefore, although the effect of temperature fluctuation on the egg development of mayflies is somewhat uncertain at present, it is well worth estimating the hatching time of eggs in the field using the regression equations calculated from the results at constant temperatures.

Figure 3 shows the temperatures in Hatsukagawa Creek at 15 : 00 to 15 : 30 of the days when the larvae were sampled in 1978 and 1979 (WATANABE, 1988). The temperature gradually decreased in autumn, and the relationship between the temperature (Th) and the number of days from 1 September (L) is described by the following linear equation from 29 August to 13 December:

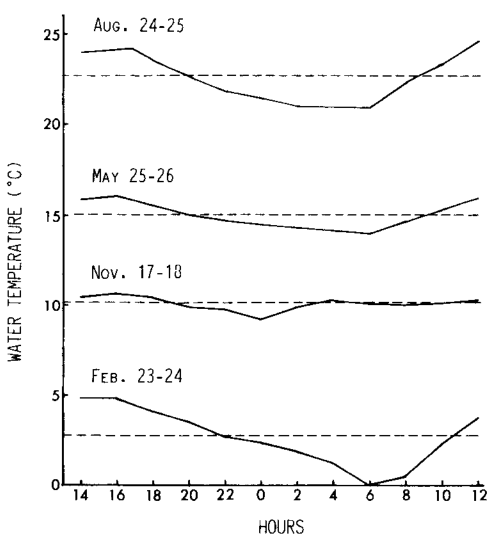

Fig. 4. Daily change in water temperature on four days in 1976 in Hatsukagawa Creek. Broken lines are daily mean temperatures. Good weather on 24-25 August and 23-24 February, cloudy on 25-26 May, rainy on 17-18 November.

$$\mathrm{Th}=24.2-0.16L \quad (r=0.980,\ P<0.01) \qquad (2)$$

From the equation, the water temperature decreased on 29 October to 15°C, below which point the hatchability abruptly dropped (Table 1, Fig. 1). The effective summation of the degree-days for egg hatching can be obtained by the integration along the line of equation (2) above the threshold temperature between the dates of oviposition and hatching. As before, the summations of the degree-days for hatching were 222.2 above 10.5°C and 263.2 above 11.0°C for 50% and 95% eggs, respectively. To achieve the temperature summation and hatch before 29 October, the eggs must be laid by 27 September for 50% hatch, and by 21 September for 95% hatch.

However, the number of days required for hatching calculated above from the temperature summation at 15 : 00-15 : 30 may be underestimated. Daily change of water temperature in Hatsukagawa Creek on four days in 1976 is shown in Figure 4. The daily maximum temperature was between 14 : 00 and 16 : 00 on all days studied, and its deviation from the mean temperature was over 1°C except on 17-18 November which were rainy days. Assuming that the daily mean temperature is 1°C lower than the values recorded at 15 : 00-15 : 30, the mean temperature decreased to 15°C on 23 October by the calculation from equation (2) with the intercept of 23.2 instead of 24.2. In which case, the eggs need to be laid by 21 September and 15 September for 50% and 95% eggs, respectively, to hatch before 23 October.

The emergence of *P. formosus* continued until early October (WATANABE, 1989). Therefore, according to the above estimation, the eggs oviposited in the late emergence period cannot hatch within the year. These eggs could overwinter, and hatch in spring when the water temperature exceeds 15°C. This conjecture is supported by the fact that the number of tiny nymphs evidently increased in April and May (WATANABE, 1988).

On the other hand, even in winter when the eggs cannot hatch according to the above estimation, tiny nymphs were collected

(WATANABE, 1988). It would seem that the growth of some nymphs which hatched in early autumn was retarded due to the low temperature.

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摘　　要

キイロカワカゲロウの孵化におよぼす水温の影響

香川県香東川から採集されたキイロカワカゲロウの卵を，温度範囲 4.8°C〜30°C の 9 段階の恒温条件で飼育し，孵化におよぼす水温の影響を調べた。温度範囲 15〜30°C における孵化率は 34〜53 %であり，約 20°C で最も高かった。しかし，15°C 以下では急激に孵化率が低下し，13°C ではほとんど孵化しなかった。孵化に要する日数（Y）と水温との間には逆の相関が認められ，この関係は双曲線式によって近似された。卵発生速度（$1/Y$）と水温との関係から計算された，50 %の卵が孵化するための卵発生の臨界温度は 10.5°C となった。このことは，卵発生の臨界温度と孵化の臨界温度とが異なることを示唆するものである。また，50 %の卵が孵化するのに要する有効積算温量は 10.5°C 以上の積算で 222.2 日度と計算された。キイロカワカゲロウの生活環が調べられている兵庫県羽束川の水温データに上の実験結果を当てはめると，水温が 15°C に低下する以前に孵化するためには，9 月下旬までに産卵されなければならないことになる。実際の羽化期は 6 月から 10 月まで続くので，羽化期終盤に生み出された卵は秋の間には孵化出来ないはずである。これらの卵は越冬して翌年の春に孵化する可能性がある。この推測は 4 月・5 月には小型個体が明らかに増加する事実と一致している。

References

ANDREWARTHA, H. G. and L. C. BIRCH (1954): *The Distribution and Abundance of Animals*. Univ. Chicago Press.

BAE, Y. J. and W. P. MCCAFFERTY (1991): Phylogenetic systematics of the Potamanthidae (Ephemeroptera). Trans. Amer. Entomol. Soc., *117*: 1-143.

ELLIOTT, J. M. (1972): Effect of temperature on the time of hatching in *Baetis rhodani* (Ephemeroptera: Baetidae). Oecologia (Berl.), *9*: 47-51.

ELLIOTT, J. M. (1978): Effect of temperature on the hatching time of eggs of *Ephemerella ignita* (PODA) (Ephemeroptera: Ephemerellidae). Freshwat. Biol., *8*: 51-58.

ELLIOTT, J. M. and U. HUMPESCH (1980): Eggs of Ephemeroptera. FBA Annual Report, *48*: 41-52.

FRIESEN, M. K., J. F. FLANNAGAN and S. G. LAWRENCE (1979): Effects of temperature and cold storage on development time and viability of eggs of the burrowing mayfly *Hexagenia regida* (Ephemeroptera: Ephemeridae). Can. Ent., *111*: 665-673.

HUMPESCH, U. H. (1978): Preliminary notes on the effect of temperature and light-condition on the time of hatching in some Heptageniidae (Ephemeroptera). Verh. Internat. Verein. Limnol., *20*: 2605-2611.

HUMPESCH, U. H. (1980a): Effect of temperature on the hatching time of eggs of five *Ecdyonurus* spp. (Ephemeroptera) from Austrian streams and English streams, rivers and lakes. J. Anim. Ecol., *49*: 317-333.

HUMPESCH, U. H. (1980b): Effect of temperature on the hatching time of parthenogenetic eggs of five *Ecdyonurus* spp. and two *Rhithrogena* spp. (Ephemeroptera) from Austrian streams and English rivers and lakes. J. Anim. Ecol., *49*: 927-937.

HUMPESCH, U. H. (1982): Effect of fluctuating temperature on the duration of embryonic development in two *Ecdyonurus* spp. and *Rhithrogena* cf. *hybrida* (Ephemeroptera) from Austrian streams. Oecologia (Berl.), *55*: 285-288.

HUMPESCH, U. H. and J. M. ELLIOTT (1980): Effect of temperature on the hatching time of eggs of three *Rhithrogena* spp. (Ephemeroptera) from Austrian streams and English stream and river. J. Anim. Ecol., *49*: 643-661.

HYNES, H. B. N. (1970): *The Ecology of Running Waters*. Univ. Toronto Press.

MACAN, T. T. (1957): The life histories and migrations of the Ephemeroptera in a stony stream. Trans. Soc. Brit. Ent., *12*: 129-156.

NEWELL, R. L. and G. W. MINSHALL (1978): Effect of temperature on the hatching time of *Tricorythodes minutus* (Ephemeroptera: Trichorythidae). J. Kansas Entomol. Soc., *51*: 504-506.

SUTER, P. J. and J. E. BISHOP (1990): Post-oviposition development of eggs of South Australian mayflies, p. 85-94. In I. C. CAMPBELL (ed.), *Mayflies and Stoneflies: Life Histories and Biology*. Kluwer Academic Publ.

SWEENEY, B. W. (1978): Bioenergetic and developmental response of a mayfly to thermal variation. Limnol. Oceanogr., *23*: 461-477.

SWEENEY, B. W. and J. A. SCHNACK (1977): Egg development, growth, and metabolism of *Sigara alternata* (SAY) (Hemiptera: Corixidae) in fluctuating thermal environments. Ecology, *58*: 265-277.

UÉNO, M. (1969): Mayflies (Ephemeroptera) from various regions of Southeast Asia. Oriental Insects, *3*: 221-238.

WATANABE, N. C. (1988): Life history of *Potamanthodes kamonis* in a stream of central Japan (Ephemeroptera: Potamanthidae). Verh. Internat. Verein. Limnol., *23*: 2118-2125.

WATANABE, N. C. (1989): Seasonal and diurnal changes in emergence of *Potamanthodes kamonis* in a stream of central Japan (Ephemeroptera: Potamanthidae). Jpn. J. Limnol., *50*: 157-161.

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