CHANGE IN THE EFFECT OF TEMPERATURE ON EGG HATCHING OF *POTAMANTHUS FORMOSUS* DURING A LONG EMERGENCE PERIOD (EPHEMEROPTERA: POTAMANTHIDAE)

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Potamanthus formosus has a long emergence period from June to October. The eggs obtained from a population at different dates during the emergence period were kept at seven different temperatures ranging from ca. 7 °C to ca. 30 °C after artificial insemination. The relationship between developmental time and water temperature changed seasonally within a population. The eggs in the later emergence period stopped hatching or required a longer developmental time at the temperatures where the eggs in the early emergence period hatch in relatively short period. The eggs deposited in the stream after early September are unlikely to hatch before winter. They probably overwinter and hatch in the next spring.

INTRODUCTION

The developmental time of eggs, or the period required for hatching, is inversely related to water temperature in many aquatic insects (ELLIOTT & HUMPESCH, 1980). The length of the developmental time at a given temperature differs among species. It is often different for different populations of the same species (BOHLE, 1972; ELLIOTT, 1978; HUMPESCH, 1980). For the species with a long reproductive period, the eggs in early and late reproductive period develop under different temperature regimes in temperate regions. In these cases the eggs deposited in different seasons may respond differently to temperature because the species have to adapt their life cycle to seasonal fluctuations of climate.

Potamanthus formosus, an inhabitant of slow-current riffles or shallow pools in streams, is distributed in South-eastern Asia, China, Korea and Japan (BAE & MCCAFFERTY, 1991). WATANABE (1988, 1989) reported that *Potamanthodes kamonis* (= *Potamanthus formosus*) had a long emergence period from June to October when the adults from three cohorts emerged one after another. In this study, we compare the effect of temperature on the hatching of the eggs of *P. formosus* obtained from adults in different seasons.

MATERIALS AND METHODS

The eggs were obtained from a population in the upper reach of the Koto River, Kagawa Prefecture (34°10' N; 134°05' E). Winged adults of *P. formosus* were collected with a light trap on 12 June, 10 July, 21 August, 12 September and 2 October 1993. All specimens collected were subimagos. The eggs from subimagos were inseminated artificially by the modified method of HUMPESCH (1980). An egg mass extracted with forceps from a female subimago was divided into 4-5 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 abdomen of a male subimago was crushed, and the sperm was transferred to and mixed with the eggs in the dish with a preparation needle. About 5 min after the insemination, the dish was filled with dechlorinated water. The eggs shortly became attached to the bottom of the dishes which were then placed under seven different temperatures av. 7.3, 10.5, 12.9, 15.0, 20.0, 25.1 and 30.1°C, using a temperature gradient chamber (Type TG-100-AD, Nippon Medical & Chemical Instruments). Four replicates in August and October, and seven replicates in June, July and September, respectively, were started at each experimental temperature. Egg batches from the same female were placed under different temperatures. The water was changed every three days, and some eggs detached from the bottom were removed with waste water. Natural daylight and darkness were used although the chambers were generally dark (<50 lux) even in the daytime because of a small window. There was no forced aeration in the dishes. The temperatures were measured randomly day and night over the whole experimental period.

When the hatching began, the newly hatched larvae were removed and counted daily under a binocular microscope. On the 150th day (1 March, 1994) after the commencement of the last experiment, all the experiments were terminated and the number of hatched and unhatched eggs in each dish was counted with a pen-type colony counter.

RESULTS

A summary of the experiments is shown in Table 1. Water temperature in each experimental chamber was much the same throughout the five experiments. Hatching failed to occur in any dishes at temperatures at and below av. 10.5 °C, and the eggs in the late emergence period

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Table 1. Summary of information on the experiments on the duration of embryonic development of *Potamanthus formosus* started at five different dates during the emergence period.*: A, No. of successful dishes/No. of dishes; B, range of hatching rates (%).

Temperature (°C) mean(range)	*	Dates of insemination 12 Jun	10 Jul	21 Aug	12 Sep	2 Oct
30.1(29.6-30.6)	A	5/7	7/7	4/4	4/7	2/4
	B	0.4-2.6	0.4-73.2	7.5-21.9	0.3-5.0	5.7-7.3
25.1(24.7-25.4)	A	4/7	7/7	4/4	4/7	2/4
	B	0.6-9.7	0.8-43.6	15.9-44.1	0.3-8.0	4.5-8.2
20.0(19.4-20.7)	A	2/7	7/7	4/4	1/7	4/4
	B	0.3-0.6	9.8-34.4	20.4-36.4	0.5	0.4-10.3
15.0(13.7-16.5)	A	4/7	6/6	4/4	0/7	2/4
	B	0.1-6.1	0.2-80.2	0.6-3.3	-	1.1-5.8
12.9(11.5-14.5)	A	3/7	5/7	0/4	0/7	0/4
	B	0.1-0.9	0.2-38.1	-	-	-
10.5(9.9-11.6)	A	0/7	0/7	0/4	0/7	
	B	-	-	-	-	
7.3(6.7- 7.9)	A	0/7	0/7	0/4	0/7	
	B	-	-	-	-	

did not hatch at av. 12.9 °C. Therefore, the lowest temperature for hatching success tended to become higher in the later experiments. Hatching occurred in all dishes at av. 15 °C or higher temperatures at the experiments in July and August, but the number of successful dishes was variable in the other experiments. Besides, the percentage of eggs hatching per dish varied considerably from dish to dish, and it was very low in many dishes. It may be due to the technique of artificial insemination and only a low percentage of eggs might be fertilized.

Table 2 shows the developmental time (the number of days required for hatching) for 50% of the total number of hatched eggs in each dish. The developmental time was inversely related to water temperature in every experiment. At the temperatures of av. 30.1 and 25.1°C, the developmental time did not differ among different experiments, i.e. different seasons. However, it was significantly different among different experiments at av. 20 °C and lower temperatures. It generally extended in the later experiments at av. 15 °C and 12.9 °C except for an inversion between August and October at av. 15 °C.

Fig. 1 shows the relationship between rate of development for 50% of the eggs to hatch (1/Y) and water temperature (T) for the experiment started on 12 June. As seen from this example, the relationship was linear for every experiment. This means that the relationship between the developmental time (Y) and water temperature (T) is given by a hyperbolic equation (1) as reported by WATANABE (1992): $Y = D/(T - t)$, where D is the total number of effective degree-days required for 50% of the eggs to hatch, and t is the threshold temperature for egg development. The regression coefficients and intercepts of the regression lines of 1/Y and T for the five experiments are shown in Table 3. There was no difference in the slopes of the regressions among the five experiments (ANCOVA, $p>0.25$). The inter-

Table 2. Developmental time (days) for 50% of eggs to hatch at different constant temperatures. Values are indicated by the mean ±95% confidence limits. Difference in the developmental time at a given temperature among the experiments is statistically analyzed on the extreme right.

Average Temp.	12 June n		10 July n		21 Aug. n		12 Sep. n		2 Oct. n		ANOVA *p*
30.1 °C	5	13.4±0.9	7	12.7±1.1	4	14.9±1.9	4	14.6±0.6	2	12.3±1.0	> 0.1
25.1 °C	4	18.5±0.8	7	17.9±0.7	4	18.9±2.1	4	20.0±1.3	2	19.4±4.6	> 0.25
20.0 °C	2	38.7±1.7	7	44.2±3.1	4	38.0±0.6	1	53.0	4	41.9±5.5	< 0.05
15.0°C	4	46.0±1.2	6	47.4±3.4	4	143.1±26.9	0	-	2	115.0±0	< 0.005
12.9 °C	3	136.3±58.9	5	218.1±2.4	0	-	0	-	0	-	< 0.01

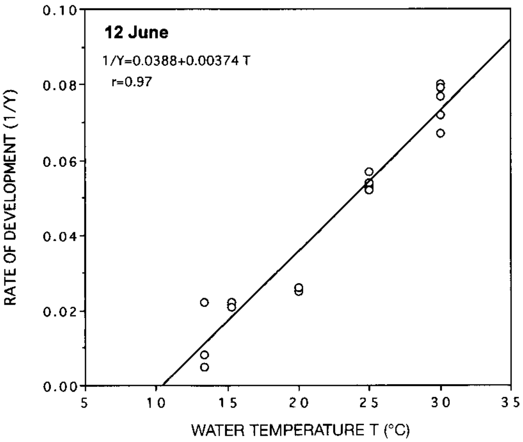

Fig. 1. Relationships between rate of development for 50% of eggs hatched (1/Y days) and temperature (T °C) for the experiments started on 12 June.

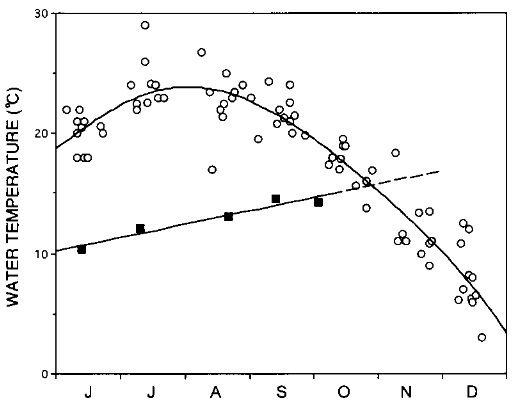

Fig. 2. Seasonal change in water temperature in Koto River (open circles) and in the threshold temperatures of egg development (solid squares). The linear regression is expressed by the equation, t = 3.64 + 1.10 D, where t is the threshold temperature and D is water temperature. Data of stream temperature were obtained from Annual Report of Water Quality published by Kagawa Prefectural Government. The temperature were measured monthly at 10:00-11:00 a.m. (nearly daily mean temperature) in the past eleven years at a point about 200 m upstream from the sampling site of subimagos. The curve of the stream temperature was fitted by a polynominal equation.

cepts, however, significantly differed among the experiments (ANCOVA, p<0.01). The calculated t (the temperature at which 1/Y was zero) becomes generally higher in the later experiments.

DISCUSSION

The effect of water temperature on the egg development of *P. formosus* changed seasonally in a population. The eggs in later emergence period stopped hatching or required a longer developmental time at the temperatures where the eggs in early emergence period hatch in relatively short period. The other examples of intra-population variation of egg development were reported for a stonefly, *Diura bicaudata* (KHOO, 1968) and for a mayfly, *Hexagenia limbata* (GIBERSON & ROSENBERG, 1992b), both of which have diapause and non-diapause eggs. In those cases, the diapause character of the eggs was considered to be an adaptation to avoid a rapid development resulting in early hatching before winter and in a few survivors in the following spring. The eggs of *P. formosus* in later emergence period seem to have the similar adaptive significance.

The time limit in which eggs can hatch before winter can be estimated from the present results. Fig. 2 shows the seasonal changes in water temperatures in the Koto River and in the threshold temperatures of egg development obtained by the present experiment. As shown in the figure, the calculated threshold temperature increased linearly during the emergence period. The stream temperature becomes lower than the threshold temperature of egg development after late October. It means that the egg

Table 3. The regression coefficient (a) and intercept (b) of the regression line of water temperature (T) and the rate of development for 50% of the eggs to hatch (1/Y) for every experiment. The column T indicates the threshold temperatures for egg development. The coefficients of correlations are always ≥ 0.96.

Dates	n	a	b	T
12 Jun.	18	0.00374	-0.0388	10.37
10 Jul.	32	0.00426	-0.0514	12.07
21 Aug.	16	0.00412	-0.0540	13.11
12 Sep.	9	0.00449	-0.0653	14.54
2 Oct.	10	0.00494	-0.0705	14.27

development should be completed until late October to hatch within the year. Even if the actual threshold be slightly lower than the calculated threshold as has been reported (GIBERSON & ROSENBERG, 1992), the eggs may develop very slowly, at most, after late October because of the low temperature regimes. At a rough estimate by degree-day calculation, eggs need to be deposited before early September to hatch by late October. Therefore, the eggs deposited after early September are unlikely to hatch before winter. We did not check whether or not the unhatched eggs in the low temperatures would hatch when they were transferred into higher temperatures. WATANABE (1988), however, reported that tiny nymphs of *P. formosus* increased rather abruptly in May. This fact suggests that many eggs in later emergence period overwintered and hatched in the following spring.

We do not know whether the unhatched eggs of *P. formosus* entered a true diapause or a low temperature quiescence in the present experiments. The eggs in later emergence period, however, required a very long developmental time at a low temperature (av. 15°C), even when they hatched. These hatched eggs also are unlikely to hatch before spring in the stream where the water temperature falls into nearly 5°C in winter (WATANABE, 1992). In addition, the length of hatching period (5-95% of eggs hatched) of *P. formosus* extended at lower temperatures (WATANABE, 1992), although it was not clear in the present experiment owing to the variable and generally low rates of hatching. Therefore, there seems to be no qualitative difference between the hatched and unhatched eggs at low temperatures, and the suppressed development at low temperatures probably resulted in the unhatched eggs by the end of the experiment.

WATANABE (1988) reported that the emerging adults of *P. formosus* consist of three different cohorts in the Hatsukagawa Creek about 130 km distant from the Koto River: a cohort emerged in June to early July (cohort W1). Its offspring rapidly developed under high temperature regimes and emerged in late August to early October (S). Another cohort emerged between the above two. This cohort (W2) being derived from tiny nymphs which were abruptly increased in May was considered to be offspring of cohort S in the last year and to be parents of the next W1. Accordingly, the fundamental life cycle seems to be a repetition of W1→S→W2→W1.

The life cycle of *P. formosus* in the Koto River is probably similar to that in the Hatsukagawa Creek because the temperature regimes and the emergence periods of *P. formosus* are much the same in the both streams (WATANABE, 1989, 1992). If that is true, the eggs obtained in the different seasons for the present experiments came from the different cohorts. Therefore, the different responses to temperatures by the eggs in different seasons are not fixed genetically, since the cohorts are not different strains.

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