EFFECT OF A LOW TEMPERATURE PERIOD ON THE EGG HATCHING OF THE JAPANESE BURROWING MAYFLY, *Ephoron shigae*

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ABSTRACT

The eggs of *Ephoron shigae* require a period of cold temperature to promote hatching. The effects of temperature and length of the cold period on egg hatching were examined in the laboratory. Eggs were obtained from females collected in the Asahi River in western Japan. After 50 days at 20°C, the eggs were placed at different cold temperatures for different periods of time. The longer the period at the colder temperature, the more effectively and the more synchronously the eggs hatched after the cold period. More than 80% of eggs hatched successfully after a period of 75 days at 8°C or colder temperatures. In the Asahi River, egg hatching must be promoted effectively because the water temperature remains between 4°C and 8°C for about 100 days in winter.

INTRODUCTION

It has been known that the eggs of the genus *Ephoron* develop effectively by going through a period of cold temperature (Edmunds *et al.* 1956; Britt, 1962; Giberson and Galloway, 1985; Nakamura, 1985). Studies have shown that the eggs of *Ephoron shigae* (Takahashi) required three stages of development for successful hatching: Firstly, the eggs have to be kept at relatively high temperature after oviposition for pre-diapause development. Then they almost stop developing at an advanced embryonic stage characterized by the appearance of dark eyespots, and require a period of low temperature (PLT) for diapause development. After the diapause period, the eggs further require relatively high temperatures for post-diapause development to hatching (Nakamura *et. al*, 1987; Matsumura, 1989).

Thus, although the outline of the effect of temperature on egg hatching are known, the required conditions of the PLT for diapause development had not been exactly determined. In the present paper, we examine the effect of the temperature and length of PLT on the egg hatching of *E. shigae*, paying particular attention to hatching success and synchronization.

MATERIALS AND METHODS

Eggs of *E. shigae* were obtained from female subimagos collected with a light trap in the lower reach of the Asahi River near the centre of Okayama City (long. 133°56'E, lat. 34°40'N) on 10 September 1988 (Females of *Ephoron* lay eggs and die as subimagos). The light trap was set at around 20.00 hours when almost all females seemed to be mated (Watanabe *et al.* 1989). As soon as the abdomen of a female subimago was dipped into water, two egg masses were expelled. Many females were forced in this way to lay eggs in a beaker filled with water. After the eggs had been mixed in the beaker, they were distributed as evenly as possible with a syringe into 26 petri dishes (80 mm in diameter) half filled with water. The eggs became attached to the bottom of the dishes after a while. The water was changed every three days throughout the experiment and there was no forced aeration in the dishes. The photoperiod in the chamber was adjusted bi-weekly to natural sunrise and sunset using artificial light.

All dishes were kept at 20°C for 50 days which provided a period long enough for the pre-diapause development (Nakamura *et al.* 1987; Matsumura, 1989). After that, they were divided into five groups of five dishes except for a single dish which was kept at 20(19.7-20.5)°C as a control. The groups were placed in 0(-0.8-1.2)°C, 4(3.1-5.3)°C, 8(7.3-9.0)°C, 14(13.2-14.2)°C and 18(17.7-18.2)°C, respectively, using a temperature gradient chamber (Type TG-100-AD; NIPPON MEDICAL & CHEMICAL INSTRUMENTS CO., LTD). The five dishes in each group were kept there for 15, 30, 45, 60 and 75 days, respectively. After PLT, the dishes were brought back to 20°C. When egg hatching commenced, the newly-hatched larvae were removed and counted under a binocular microscope almost every day. On 19 February 1989 (159th day after ovipositon), the experiments were terminated and the number of eggs in each dish, including hatched and unhatched ones, was counted.

RESULTS AND DISCUSSION

Fig. 1 shows the daily cumulative percentage of eggs hatching to the total number of eggs used in each experiment. The longer the PLT means the shorter the period after PLT because all the experiments were finished on the 159th day after oviposition. As shown in the figure, the colder and the longer the PLT eggs were exposed to, the more effectively egg hatching was promoted, except that the cold treatment at 4°C was more effective than 0°C. More than 95% of the eggs hatched successfully after treatment at 0°C or 4 °C for 75 days. On the other hand, when the temperature of cold treatment was higher than 14°C, or when PLT was shorter than 30 days, egg hatching lasted for a long time and was not complete by the end of the experiments.

Giberson and Galloway(1985) also demonstrated that hatching success of *Ephoron album* eggs treated at -2°C was positively correlated to the duration of the exposed period, although eggs treated at 4 and 10°C did not hatch. Bohle(1972) reported that longer and colder treatments led to higher hatching success for *Ephemerella ignita* having embryonic diapause stage.

The eggs of *E. shigae* not subjected to PLT were not completely dormant because they began to hatch more than 100 days after oviposition. However, even the PLT at 18°C clearly stimulated hatching compared with the control. Schaller(1968) and Sawchyn and Church(1973) reported also for some species of Odonata having the embryonic diapause stage that the development of eggs continuously exposed to higher temperatures proceeded very slowly.

Table 1 shows the number of days required for 1%, 50% and 99% of the eggs to hatch (percentage in relation to the total number of eggs hatched at the end of the experiment). In general, the start of hatching (days after PLT for 1% of the eggs to hatch) became earlier as the PLT was longer. However, at any temperature lower than 14°C the eggs started hatching within 10 days after a PLT that was longer than 45 days. Besides, the length of the post-diapause period(days after PLT for 50% to hatch) did not much differ when the PLT had been longer than 45 days: this means that the eggs exposed to proper cold temperature almost completed the diapause development in 45 days.

The length of the hatching period (days for 1-99% to hatch) became shorter with a longer PLT. Thus a longer PLT enhances the synchronization of egg hatching as shown by Schaller(1968) for a dragonfly *Aeschna mixta*.

Nakamura *et al.*(1987) reported that eggs which had been kept at 16-29°C for more than 36 days hatched normally after a PLT. The water temperatures during mid-September when *E. shigae* oviposited in the Asahi River was 22-24°C and it decreased gradually after that (Fig. 2). Temperature remained above 16°C for 40-50 days after the emergence period: this seems to provide a period long enough for the pre-diapause development. In winter, water temperatures of 4-8°C continued for about 100 days. This cold period, according to the results of the present study, probably leads the majority of eggs to hatch synchronously although we cannot determine from our study the temperature required to break the diapause.

The synchronous emergence of *Ephoron shigae* during mid-September has been known (Watanabe *et al.* 1989). The adaptive value of the adult synchronization of aquatic insects has been suggested to reside in increasing mating success(Corbet 1964) or predator satiation(Sweeney and Vannote 1982). The synchronous emergence of *E. shigae* may be ensured, at least partially, by synchronizing mechanisms at hatching through embryonic diapause.

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Table 1. The number of days after PLT required for 1%, 50%, and 99% eggs to hatch. The values are enclosed with squaresin the cases where hatching was almost finished by the end of the experiment.

Low temperature period (PLT)		The days after PLT requied for 1%, 50%, 90% eggs to hatch			The length of hatching period (days for 1-99% eggs to hatch)
Temp. (℃)	Period (days)	1%	50%	99%	
0	15	47	80	93	46
	30	20	61	79	59
	45	8	9	48	40
	60	9	14	21	12
	75	9	11	19	10
4	15	44	67	92	48
	30	20	53	78	58
	45	9	15	48	39
	60	9	14	25	16
	75	10	16	22	12
8	15	37	76	94	57
	30	20	52	77	57
	45	6	12	64	58
	60	6	28	46	40
	75	6	16	31	25
14	15	35	83	94	59
	30	20	61	79	59
	45	10	26	64	54
	60	6	15	49	43
	75	6	10	33	27
18	15	39	82	94	55
	30	22	67	79	57
	45	16	46	64	48
	60	5	23	49	44
	75	5	22	34	29
20	0	52	93	109	57

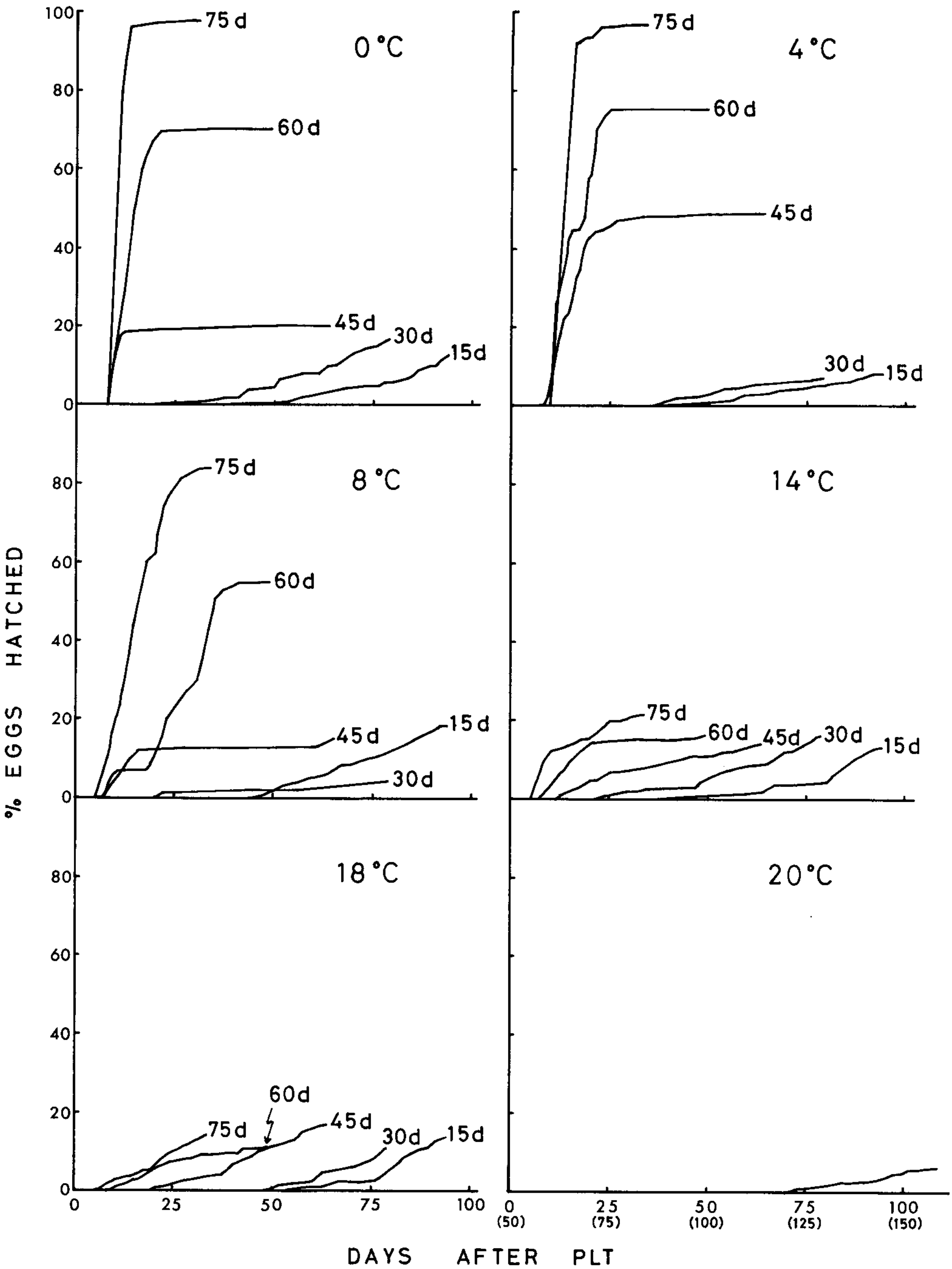

Fig. 1. Daily cumulative percentage of eggs hatching to the total eggs after different lengths of PLT at the different temperatures and the control without PLT(20°C). The numbers in the parentheses on the right bottom are the days after oviposition for the control experiment at 20°C.

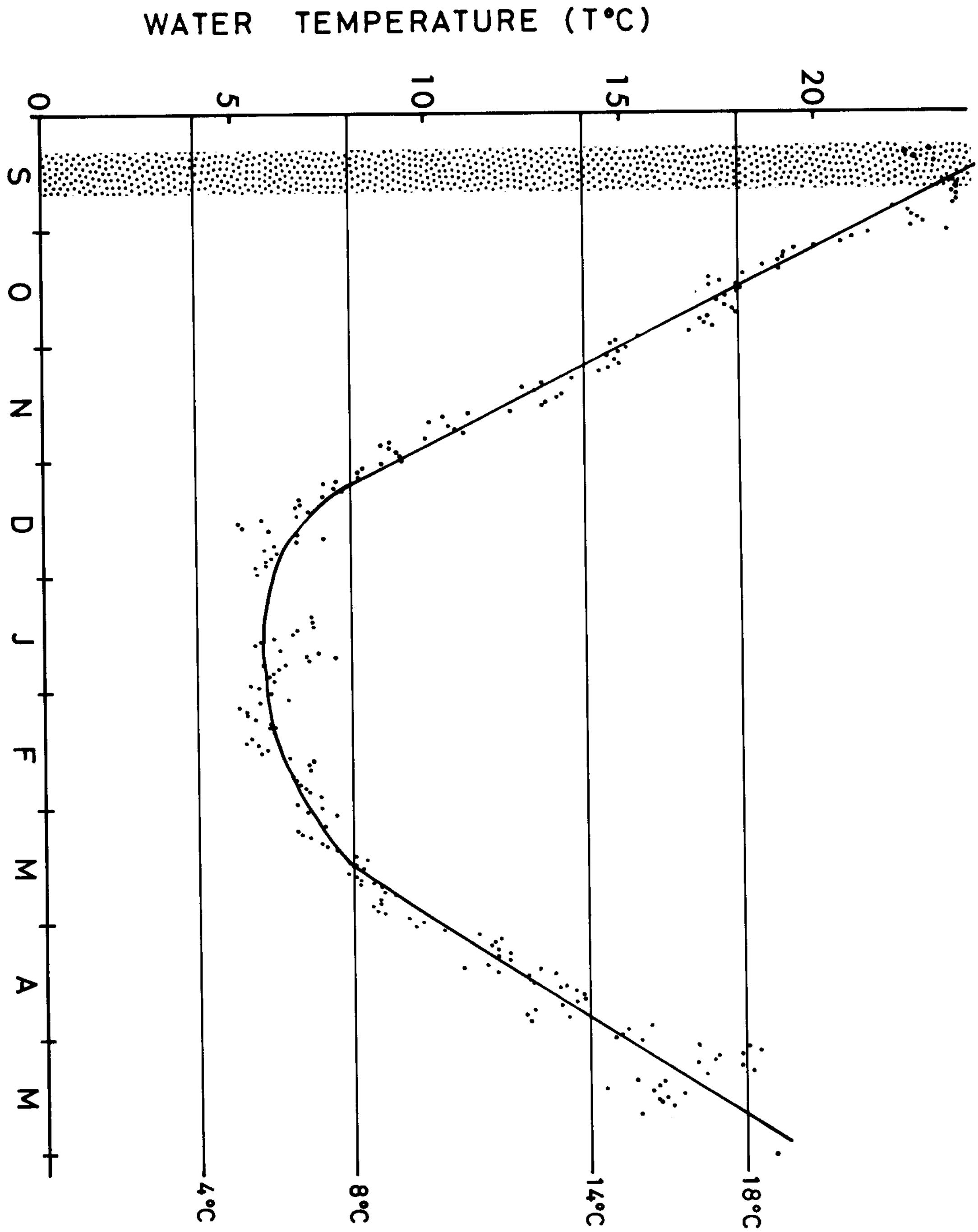

Fig. 2. Water temperatures from September 1988 to May 1989 in the lower reach of the Asahi River. Points are daily mean temperatures measured at Heidan Automatic Control Observatory of the Okayama River Management Office. The shaded area indicates the emergence period.