

Life Cycle and Synchronization of Nymphal Development of the Mayfly *Ephoron shigae* in Japan (Ephemeroptera: Polymitarcyidae)

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ABSTRACT

The life cycle of *E. shigae*, including eclosion, nymphal growth, last instar recruitment and adult emergence, was studied in a Japanese river. The synchronization of nymphal development is discussed. Eggs hatched sporadically from mid-March to early April as determined from continuous sampling of eggs from the river substratum. Nymphs attained the last instar in mid- to late August and emerged in mid-September. Size of nymphs at the time of last instar recruitment decreased gradually as did the size of emerging mayflies. Smaller individuals entering the last instar later developed more quickly and required a smaller number of degree-days to complete the last instar than did larger individuals. Consequently, larval development synchronized during the period between last instar recruitment and adult emergence.

KEYWORDS: Polymitarcyidae, *Ephoron shigae*, life cycle, eclosion, synchronization of development.

INTRODUCTION

A burrowing mayfly *Ephoron shigae* (Takahashi) has been known for mass emergence in many Japanese rivers. Because of its conspicuous appearance, the biology of this species has been studied from many aspects.

E. shigae is univoltine over its entire range in Japan, a conclusion based on studies on seasonal development (Nozaki, 1983; Ban et al., 1994) and seasonal emergence patterns from four rivers including rivers from the northern and southern periphery of its range (Watanabe et al., 1998). The univoltine life cycle seems to be maintained under a wide variation of climates through embryonic diapause. Experimental studies have shown that the eggs of *E. shigae* enter an obligatory diapause at an advanced embryonic stages (characterized by the appearance of dark eyespots) and require a period of chilling before development can continue (Nakamura et al., 1987; Watanabe & Takao, 1991), as do other species of *Epho-*

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ron (Edmunds et al., 1956; Britt, 1962; Giberson & Galloway, 1985; Kureck & Fontes, 1996). Watanabe (1998) demonstrated a geographic variation in diapause and post-diapause embryonic development depending on winter water temperatures. However, overwintering and spring hatching of *Ephoron* mayfly eggs have never been proven by field studies.

The biology of nymphs was studied by Ban et al. (1994) who reported that the presence of half-buried cobbles was the most important factor for the occurrence of *E. shigae* nymphs on the muddy sand bottom. Information about food and habitat preferences of nymphs has also been given by Nozaki (1983) and Hasuda (1989).

Watanabe and Ishiwata (1997) surveyed the geographic distribution in Japan and reported geographic parthenogenesis: some populations have few or no males (unisexual populations) whereas other populations have many males and females (bisexual populations). In bisexual populations, male subimagines emerged synchronously about an hour after sunset and moulted to imagines in a 25 min period immediately prior to an abrupt increase in female emergence (Watanabe et al., 1989). Females in unisexual populations, however, began to emerge as early after sunset as males in bisexual populations (Watanabe et al., 1998).

In the present study using field data, we focus on the seasonal timing of life cycle events including egg hatching, nymphal growth, and last instar recruitment and growth, in order to understand the processes leading to synchronization of adult emergence.

MATERIALS AND METHODS

Study site

This study was conducted in the lower reach of the Asahi-gawa River near the center of Okayama City, Honshu (long. 133°56' E., lat. 34°40' N.) There the river was ca. 130 m wide and water flowed in the center of a weir. The study site was a stagnant zone near the left bank just downstream from the weir, with a cobble substratum at the surface and cobbles and sandy mud below the surface. Seasonal variations in mean daily water temperatures from March to September, 1991 and 1992, taken about 100 m downstream are given in Figure 1 (data from Okayama River Management Office, Ministry of Construction).

Eggs

To study eclosion, a small amount of sand (ca. 120 cc) was sampled with a trowel from the river bottom at various points in the study site. Samples were taken six times at irregular intervals from 11 February to 2 April 1991. Fifteen to 20 eggs per day were examined to determine whether egg capsules were cracked and empty (Fig. 2). More frequent and larger samples (ca. 50 eggs/day) were made in 1992 for precise estimation of the eclosion period, i.e., at intervals of one to three days from 12 to 16 March, every day from 18 March to 6 April, and every three days from 6 to 15 April. Hatching rate between two successive sampling occasions was calculated from the increasing rate of empty eggs.

Nymphs

Nymphs of *E. shigae* were sampled every two to three weeks from 21 May to 9 July, and weekly from 17 July to 16 September 1991. More than 30 nymphs were randomly collected at each sample. However, it became difficult to collect sufficient numbers of nymphs in mid-September when adult emergence started. In 1992, nymphal samples were taken only in summer, i.e., weekly from 10 August to 4 September, to examine the timing of last instar recruitment. The substratum was sampled with a

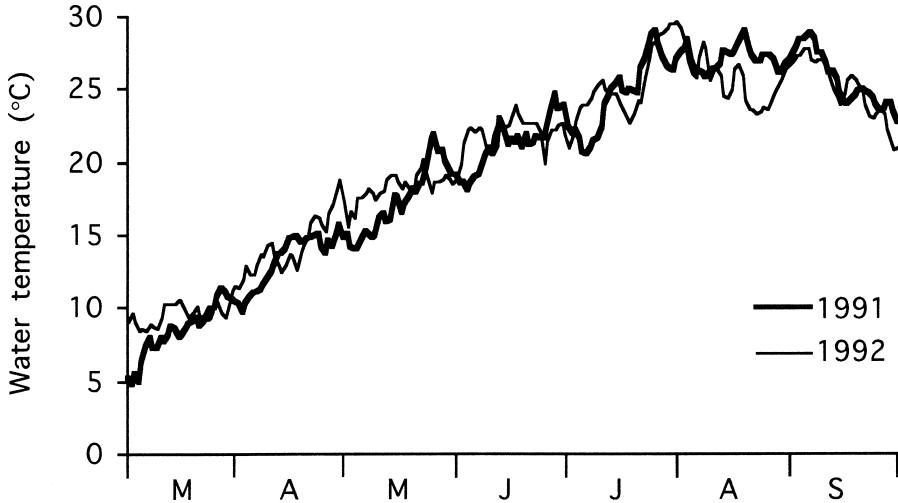


Fig. 1. Seasonal variations in mean daily water temperature in the Asahi-gawa.

wire-gauze shovel (0.471 mm pore opening; see Watanabe & Harada, 1976) which was pushed 7–10 cm into the substratum. Nymphs sorted from the substratum were fixed immediately in 5% formalin solution in the field and transferred to 70% ethanol in the laboratory. Head width and length of the right wing pad were measured to the nearest 0.05 mm with an objective micrometer. Nymphs larger than ca. 1.0 mm in head width were sexed by the appearance of developing genitalia, and last instars were distinguished from earlier instars by the length of the wing pads (Fig. 3).

Adults

Imagines and subimagines swarming over the water surface were collected by sweep net (40 cm diameter) every day during the emergence period in 1991 and 1992. A 20-stroke sweep was made every 5 min during the swarm, and the daily sum of the number caught was regarded as the relative abundance of adults on the day. Winged mayflies were preserved in 5% formalin and were then sexed and counted in the laboratory.

RESULTS

Figure 4 shows the cumulative percentage of hatched eggs and the daily rate of eclosion, assuming that hatching occurred evenly between successive sampling occasions. Empty eggs first appeared on 25 March 1991, and all eggs examined had hatched on 30 March and 2 April. In 1992, eclosion began on 13 March and continued sporadically through 12 April. The highly synchronized eclosion shown for 1991 may be imprecise because it is based on low frequency of sampling and a small number of examined eggs. The average date of hatching, however, was calculated as 24 March for both years (Table 1). Mean daily water temperatures during the eclosion period ranged from 9.2–11.4 °C in 1991 and 9.3–14.5 °C in

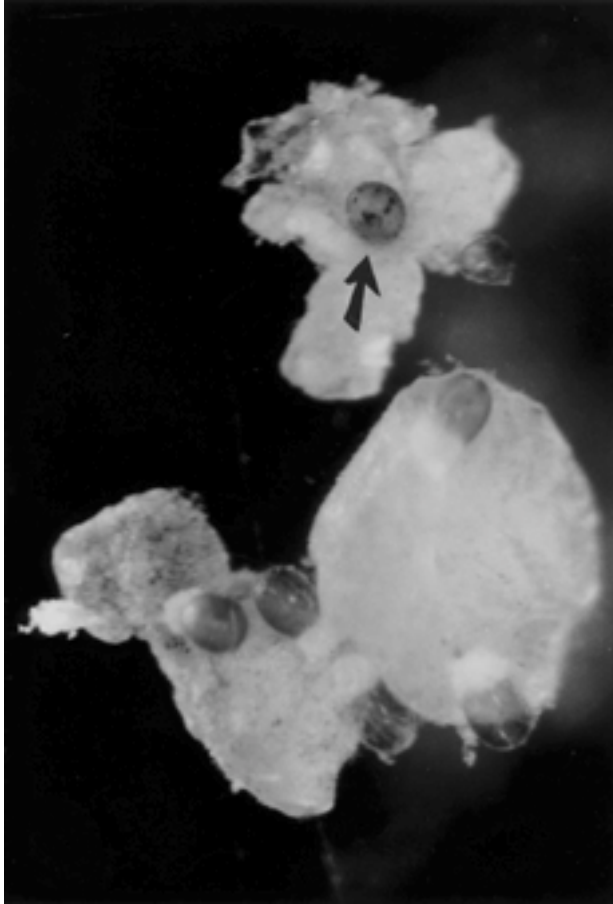


Fig. 2. Egg capsules sticking to sand grains. An arrow indicates unhatched egg the eyespots of which can be seen. The other egg capsules are hatched.

1992. Average water temperatures at eclosion calculated from mean daily temperatures and daily rates of eclosion were 10.2° C for 1991 and 10.3° C for 1992.

The seasonal development of nymphs in 1991 is shown in Figure 5 based on head width. Nymphs were first collected on 21 May. Nymphs larger than 1.0 mm head width were generally sexed after 11 June, but a few could not be sexed until late July. Females were generally larger than males, and head widths of both sexes gradually increased until late August. Last instars first appeared on 13 August (females) and 20 August (males) and dominated the population by the end of August.

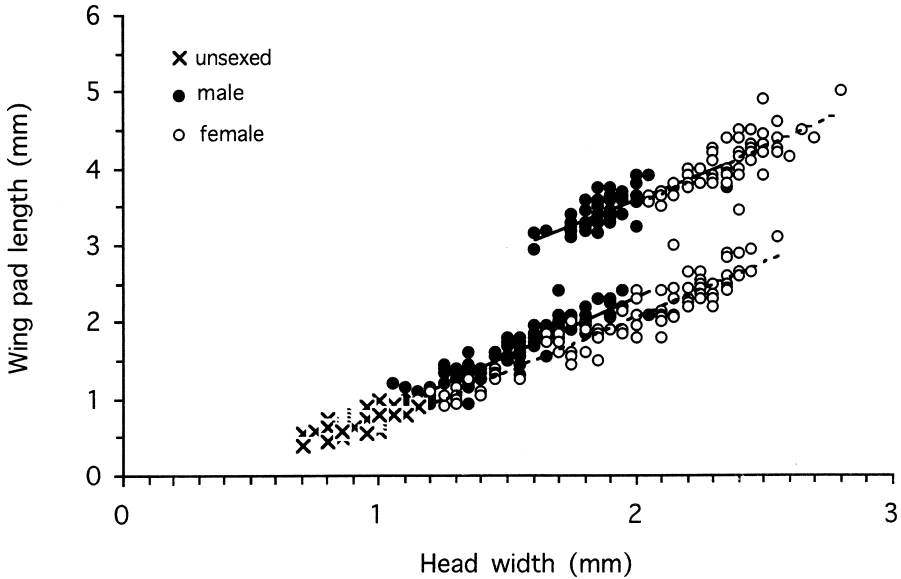


Fig. 3. The relationship between head width and the length of right wing pad for all the *E. shigae* nymphs with measurable wing pads in 1991. Regression equations are as follows: male: the last instars ($n = 57$), $y = 1.31x + 1.00$, $r^2 = 0.53$; earlier instars ($n = 105$), $y = 1.50x - 0.68$, $r^2 = 0.87$; female: the last instars ($n = 55$), $y = 1.51x + 0.51$, $r^2 = 0.60$; earlier instars ($n = 102$), $y = 1.41x - 0.74$, $r^2 = 0.90$.

Figure 6 gives the seasonal change in the coefficient of variation (C.V.) of nymphal head width. All small nymphs were combined for the C.V. for 21 May. Data from 11 June to 17 July were omitted because many unsexed nymphs were included, and all unsexed nymphs from 24 July were considered to be males based on size-frequency distribution (Fig. 5). Figure 6 shows a great variation in size on 21 May, which abruptly decreased to mid-August and then gradually decreased from mid-August to early September as nymph moulted to the last instar.

Seasonal changes in the relationship between head width and wing pad length for late instars in 1991 and 1992 are shown in Figure 7. (Last instars are distinguishable from earlier instars by their long pads as shown in Figure 3.) The recruitment of last instars began with larger nymphs in mid-August in both years and proceeded gradually to smaller ones, although there seems to be a lower size limit for recruitment at about 2 mm head width (female) or 1.5 mm (male). All nymphs had moulted to the last instar by the beginning of September. Thus, the size of last instar nymphs gradually decreased, resulting in a decrease in adult size during the emergence period as reported by Watanabe et al. (1998). Last instar recruitment of small nymphs curtailed additional growth and nymphal development synchronized during this period.

TABLE 1. Seasonal timing of eclosion, last instar recruitment and emergence, with the degree-days ($>0^{\circ}\text{C}$) between two stages. The date of each stage is represented by its mean, and mean minus and plus a standard deviation (in parentheses), and degree-days between the respective dates of each stage were calculated.

	Seasonal timing of three stages $M (\pm SD_{0.95})$			Degree-days between mean dates (between $M \pm SD_{0.95}$, respectively)		
	Eclosion	Last instar recruitment	Emergence	Eclosion -Last instar	Last instar -Emergence	Eclosion -Emergence
1991 male	3/24 (3/22-3/27)	8/26 (8/21-8/30)	9/13 (9/12-9/14)	3131.9 (3015.8-3205.8)	491.7 (601.9-410.4)	3623.6 (3617.7-3616.2)
1991 female		8/21 (8/15-8/26)	9/13 (9/11-9/15)	2996.0 (2847.0-3099.7)	627.6 (744.4-540.7)	3623.6 (3591.4-3640.4)
1992 male	3/24 (3/18-3/31)	8/24 (8/20-8/29)	9/13 (9/11-9/14)	3149.6 (3113.0-3199.9)	523.8 (568.7-424.5)	3673.4 (3681.7-3624.4)
1992 female		8/23 (8/17-8/28)	9/14 (9/12-9/16)	3125.8 (3039.0-3174.2)	571.6 (667.7-500.6)	3697.4 (3706.7-3674.8)

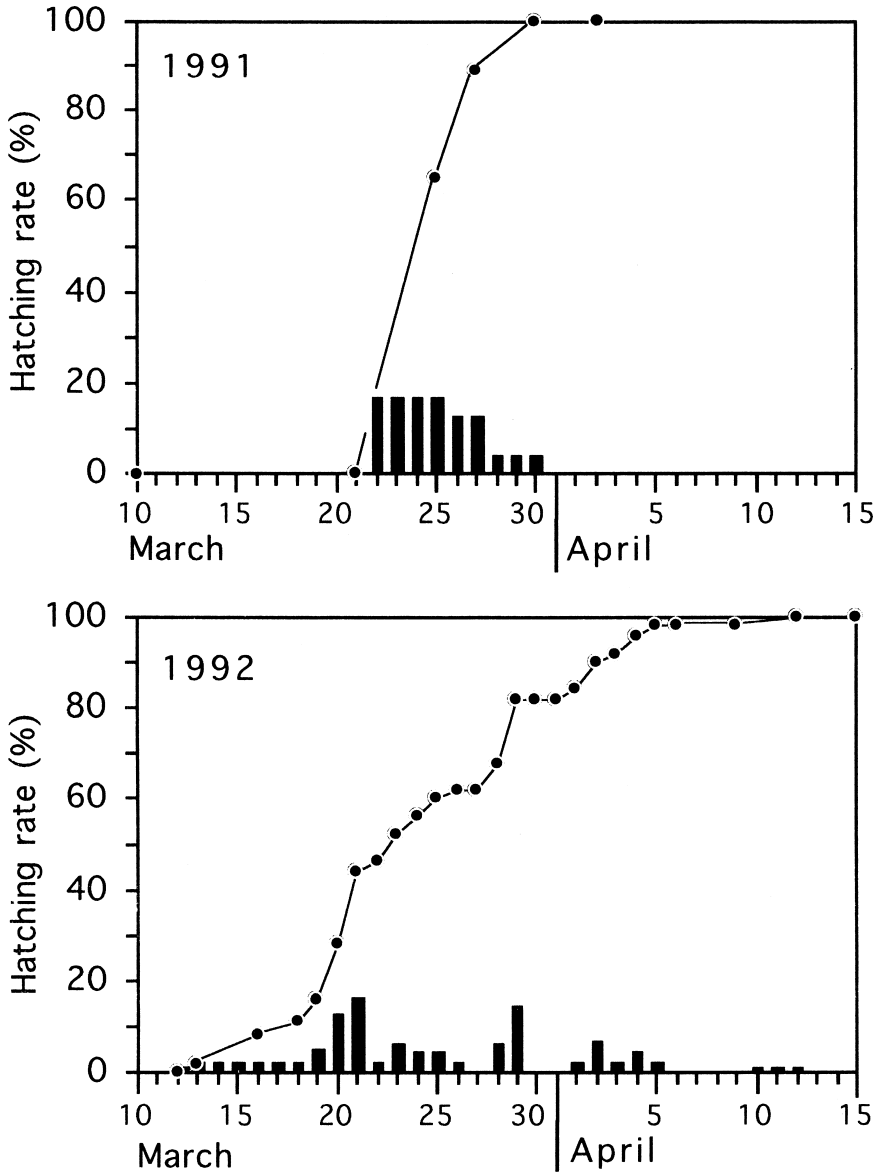


Fig. 4. The cumulative percentage of hatched eggs of *E. shigae* (dots and lines) and estimated daily rate of eclosion (bars) in the Asahi-gawa River.

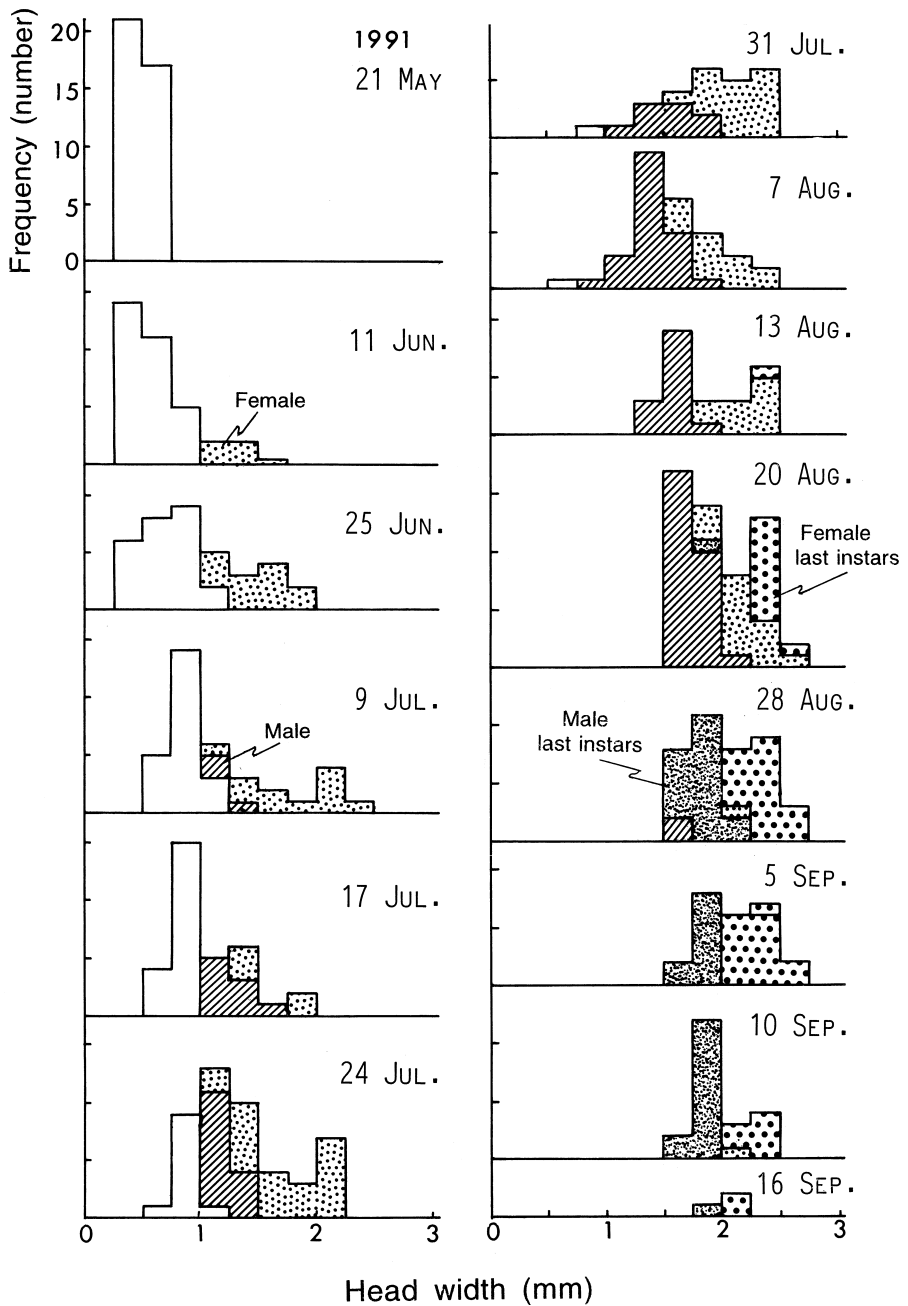


Fig. 5. Seasonal change in frequency distribution of head widths of *E. shigae* nymphs.

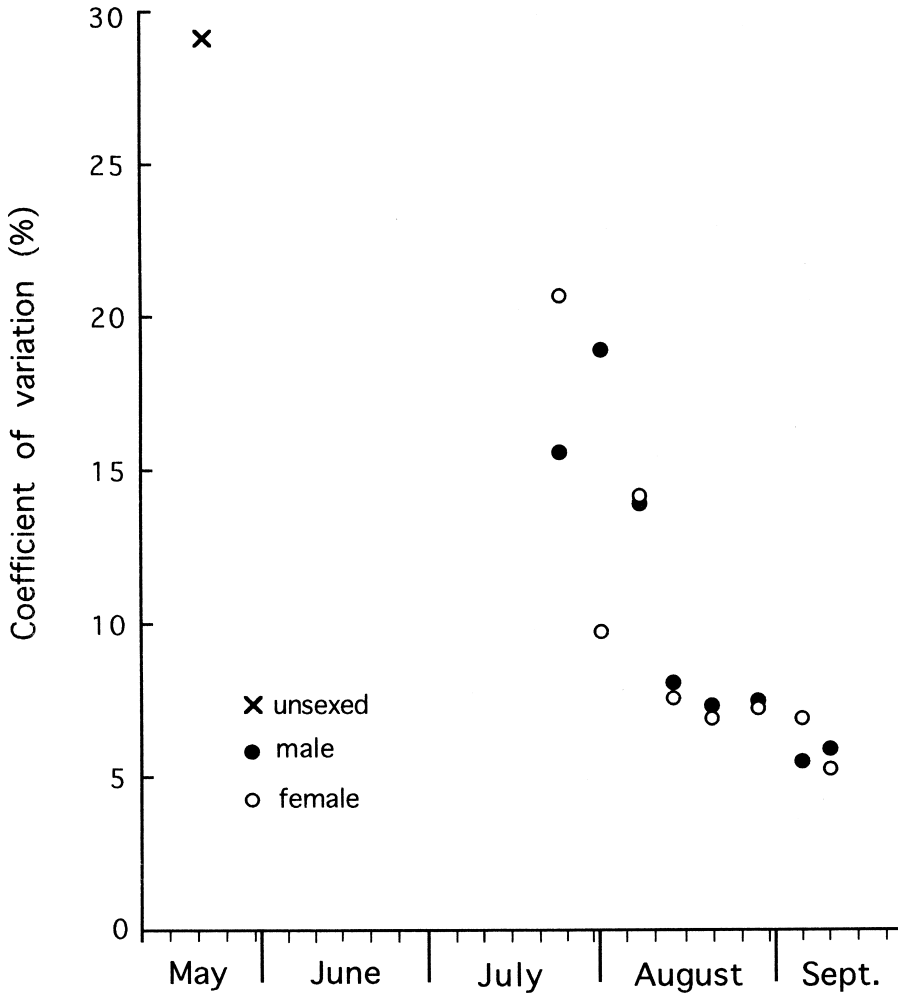


Fig. 6. Seasonal change in the coefficient of variation of head width of *E. shigae* nymphs.

The average dates and standard deviations of eclosion, last instar recruitment and emergence for *E. shigae* in the Asahi-gawa in 1991 and 1992 are given in Table 1 (see Watanabe et al., 1998 for seasonal patterns of emergence) with the average number of degree-days between these dates. The periods of emergence, expressed as one standard deviation prior to and after the mean date of emergence, were much smaller than the periods of last instar recruitment for both sexes in both years. This indicates that emergence synchronized during this period. Take-mon (1990) reported a similar synchronization of development during the last instar of *Ephemera strigata*.

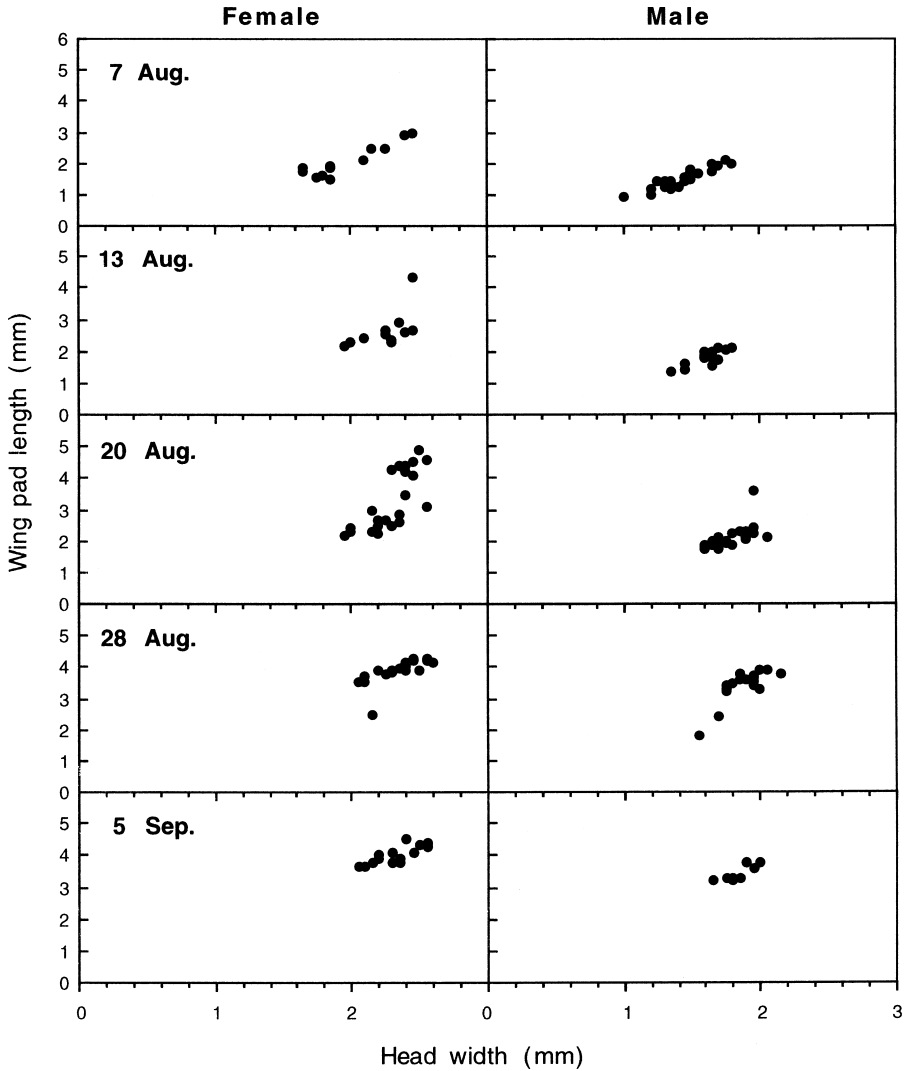


Fig. 7a. Seasonal change in the relationship between head width and right wing pad length for last instar nymphs of *E. shigae* in 1991.

Degree-days were calculated from one stage to the next, assuming that mean, and mean minus and plus one standard deviation represented mean, and early and late recruits, for each stage respectively. Degree-days from hatching to the last instar seem to be slightly greater in late recruits than in early recruits. Degree-days from last instar recruitment to adult emergence were much greater for early recruits than for late recruits.

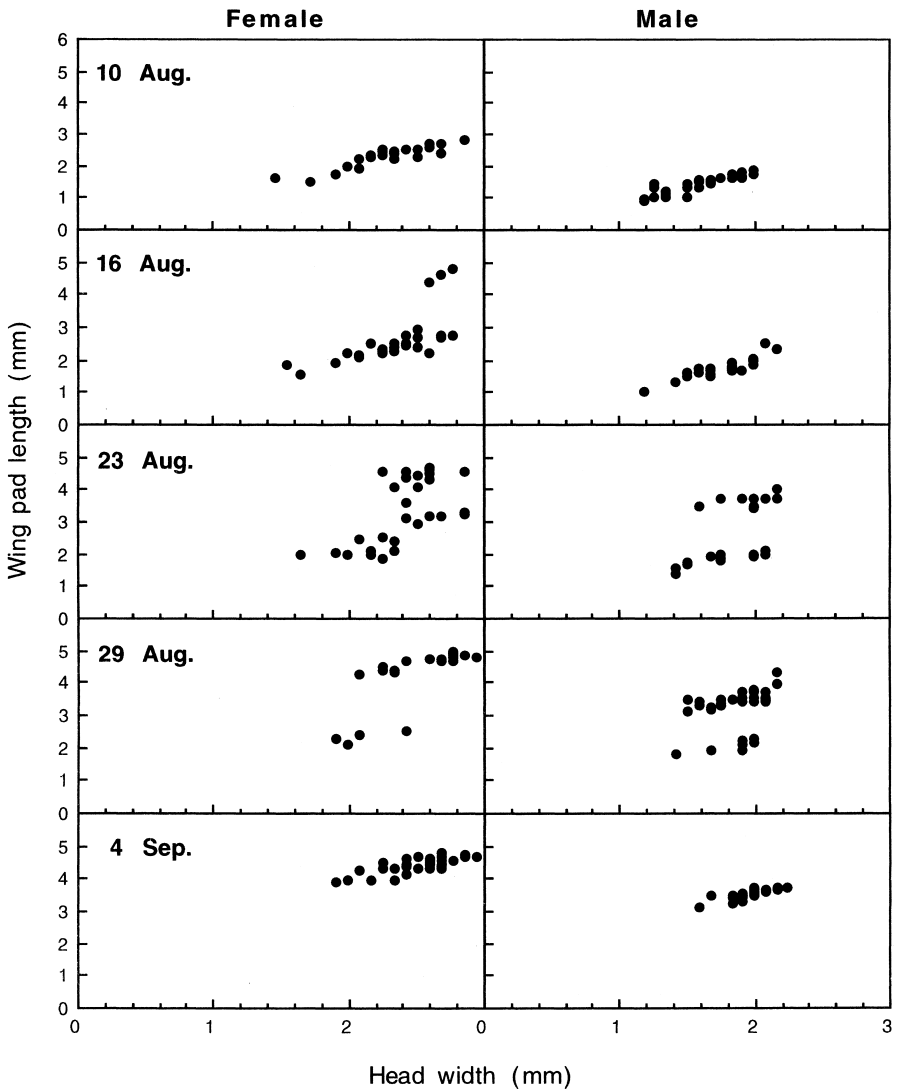


Fig. 7b. Seasonal change in the relationship between head width and right wing pad length for late instar nymphs of *E. shigae* in 1992.

DISCUSSION

Life cycle of Ephoron shigae and comparison with other Ephoron mayflies

From present results and previous studies, the outline of the life cycle of *E. shigae* in the Asahi-gawa is summarized as follows:

Eggs hatch from mid-March to early April when water temperature reaches about 10° C. The size of newly hatched nymphs is 0.14 mm average head width (Watanabe, unpublished). Nymphs grow under increasing temperature regimes from spring to early summer and develop to the last instar in mid- to late August when water temperatures fluctuate widely. Emergence occurs in mid-September when water temperatures begin to decrease. Eggs develop in autumn to an advanced embryonic stage characterized by the appearance of dark eye spots and undergo diapause in winter (Watanabe & Takao, 1991). Thus, *E. shigae* has a univoltine life cycle with an egg stage lasting over half a year.

Other species of *Ephoron* also have univoltine life cycles with embryonic diapause and spend much of their lives as eggs. The lengths of embryonic development exceeded eight months in *E. album* from western North America (Edmunds et al., 1956; Britt, 1962; Giberson & Galloway, 1985; Phillips et al., 1994), *E. leukon* from north-central Virginia (Snyder et al., 1991) and *E. virgo* from the Rhine in Köln, Germany (Kureck & Fontes, 1996). In *E. album* the life cycle of which has been studied at several localities, the egg period was the longest reaching almost ten months in the northernmost locality, Manitoba, Canada; small adult size and low fecundity due to the short growth period were reported there (Giberson & Galloway, 1985).

The life cycle of *E. shigae* in the Asahi-gawa with a relatively short egg period and a resultant long nymphal period seems adapted for warm condition. Geographical variations in life cycle and in adult size of *E. shigae* remain to be studied.

Period of egg hatching in the Asahi-gawa

Eggs of *E. shigae* hatched sporadically for almost a month in the Asahi-gawa in 1992. Synchronized egg hatching has been reported for many mayflies under constant temperatures in the laboratory (reviewed by Elliott & Humpesch, 1980). Eggs of *E. shigae* also hatched synchronously at 20° C after completion of low temperature diapause (Watanabe & Takao, 1991). However, when eggs of *E. shigae* were incubated at different constant temperatures in the range of 8-20° C after chilling at 4° C, the hatching period (5-95% hatching) became longer at the lower temperatures (Watanabe, 1998). This fact explains the extended hatching period under low temperatures in early spring. In addition, eggs covered with substratum may possibly have delayed hatching in the field as reported by Hunt (1951). In any case, this extended hatching period seemed to result in the wide variation in nymphal head width shown on 21 May 1991.

Factors affecting seasonal decrease in adult size and synchronization of development

Decrease in adult size during the emergence period has been reported in many aquatic insects (e.g., Sweeney, 1978; Sweeney & Vannote, 1981; Takemon, 1990). Sweeney and Vannote (1981) hypothesized a threshold temperature beyond which a greater part of assimilated energy was assigned to adult tissue synthesis over

larval growth, therefore synchronizing nymphal development as water temperature exceeds the threshold, with adult size largely dependent on the size of larva at the onset of adult tissue synthesis. However, Sweeney et al. (1995) withdrew the above hypothesis because adult size decrease was recognized even in a tropical mayfly, *Euthyplocia hecuba*, which inhabited a river with nearly constant temperatures throughout the year. Besides, Hagiwara (1977) reported adult size decrease of the winter stonefly *Eocapnia nivalis* the larvae of which grew under decreasing water temperatures.

Sizes of imagines and subimagines of *E. shigae* in the Asahi-gawa also decreased gradually during the September emergence period (Watanabe et al., 1998). This pattern results from last instar recruitment of progressively smaller nymphs. In addition, the emergence period of *E. shigae* was much shorter than the period of last instar recruitment, indicating that nymphal development synchronized during the last instar. Synchronization occurred during fluctuating, not increasing or decreasing, water temperatures (Fig. 1).

Smaller individuals of *E. shigae* entering the last instar later and emerging later experienced fewer day-degrees. This indicates that the rate of development during the last instar was negatively related to nymphal size regardless of temperature or other environmental factors, which explain the synchronization process from last instar recruitment to emergence. In *Daphnia*, smaller individuals completed each instar more quickly than large individuals (Anderson et al., 1937; Munro & White, 1975). If the shorter instar duration of smaller nymphs demonstrated here for the last instar holds also for penultimate and some later instars, the synchronized last instar recruitment and the decreasing variation in head width during later nymphal stages could be explained.

ACKNOWLEDGMENTS

We are grateful to the Okayama River Management Office, Japanese Ministry of Construction, which provided us with data on water temperatures in the Asahi-gawa River. This study is partly supported by the Foundation of River Management and the Nissan Science Foundation.

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