

Comparisons of Temperature-Related Life Histories in Two Ephemerid Mayflies (*Ephemera separigata* and *E. strigata*: Ephemeridae, Ephemeroptera, Insecta) from a Mountain Stream in Korea

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강원도 치악산 계류에서 하루살이 두 종 (가는무늬하루살이, 무늬하루살이: 하루살이과, 하루살이목, 곤충강)의 온도와 연관된 생활사. 이성진 · 배연재*¹ · 윤일병 · Naoshi C. Watanabe² (고려대학교 생물학과, ¹서울여자대학교 생물학과, ²Faculty of Agriculture, Kagawa University)

강원도 치악산 계류에서 하루살이과에 속하는 가는무늬하루살이와 무늬하루살이의 온도와 연관된 생활사 적응에 대하여 연구하였다. 하루살이 개체군의 고도분포에 따라 3개의 조사지점(상류와 중류지점: 가는무늬하루살이; 하류지점: 무늬하루살이)을 선정하였다. 채집은 1997. 2~1998. 1 동안 월 1회 실시하였고, 우화기에는 월 2회 실시하였다. 수온은 1997. 5~1998. 1 동안 시간대로 모니터링 하였다. 결과로서, 3개의 조사지점은 서로 다른 온도역(평균온도 상류: 9.39°C; 중류: 10.38°C; 하류: 12.70°C)을 나타냈다. 체장과 유충단계의 분석 결과 두 종 모두 명백히 연 1세대였고, 각 개체군은 2개의 무리(cohort)를 포함하였다. 우화는 늦은 5월부터 늦은 6월까지 일어났지만 하류로 갈수록 일렀다. 유충의 충원은 우화기(여름형)와 그 5~6개월 이후(겨울형)에 시작되었다. 이처럼 2회에 걸친 유충의 충원에 대하여 알의 휴면 또는 무성생식이 원인으로 작용하는 것으로 가정되었다. 상류와 중류에서 가는무늬하루살이의 총 적산온도는 각각 2380DD와 2668DD였고, 하류에서 무늬하루살이는 3220DD였다. 가는무늬하루살이의 경우 연평균수온이 1°C 내려감에 따라 알의 부화는 약 22일, 평균 유충성장은 약 15일 지연되는 것으로 추정되었다. 가는무늬하루살이는 냉온에 적응된 생활사 양상을 나타냈고, 다른 한국산 및 일본산 근연종과 비교하여 종내 및 종간 생활사 적응에 대하여 추가로 논하였다.

Key words: *Ephemera separigata*, *Ephemera strigata*, Ephemeroptera, Life history, Temperature, Korea

INTRODUCTION

Temperature has been known one of the major factors affecting temporal and spatial distributions of aquatic insects (Ward, 1992). Larvae of

the mayfly genus *Ephemera*, known as burrowing mayflies, are common in northern temperate streams, including Korea and Japan. Among the species of the genus, *Ephemera separigata* Bae, *E. strigata* Eaton, and *E. orientalis* McLachlan are widespread in Korea (Bae, 1995; Lee *et al.*,

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1995, 1996), whereas *E. japonica* McLachlan occurs in Japan instead of *E. separigata* (Kuroda *et al.*, 1984; Watanabe, 1985; Bae, 1995; Lee *et al.*, 1995, 1996). When they inhabit in the same watercourse, they show a stratified pattern of altitudinal distribution occupied by *E. separigata* or *E. japonica* in the uppermost section of the stream, and followed by *E. strigata* and *E. orientalis* (Kuroda *et al.*, 1984; Watanabe, 1985; Bae, 1995; Lee *et al.*, 1995). It has been a keen interest to know the factors affecting the altitudinal distributions, which may be involved in the adaptation, speciation, and evolution of the species.

The purpose of this study is to elucidate the intraspecific and interspecific life history adaptations of *E. separigata* and *E. strigata* from a mountain stream in Korea with relation to temperature as the major determinant.

MATERIALS AND METHODS

Study area

Field study was conducted from a mountain stream in the Chiaksan National Park, located in the central area of Korea, from February 1997 to January 1998. The stream originates from the Namdaebong peak (37° 18' 29" N and 128° 3' 55" E; alt. 1181.5 m) and its upper section runs through the Sangwonsa valley where the forest has been well preserved.

Three sampling sites were chosen in the upper section of the stream based on altitude as well as the stratified distributions of the species of *Ephemera*. The upstream site (alt. 800 m) is a small headwater stream (width 2 m) completely canopied by the riparian forest. Only *E. separigata* in the genus inhabited in the site. The midstream site (alt. 470 m) is a mid-sized mountain stream (stream order 3; width 10 m) partially canopied by the forest. *E. separigata* was predominant at the site throughout the study period, but minor population of *E. strigata* also became to occur. Below the midstream site, *E. separigata* was gradually disappeared and *E. strigata* was predominant to the downstream site. The downstream site (alt. 370 m) is a mid-sized plain stream (stream order 4; width 20 m) flowing through a small village. *E. strigata* was predominant at the site, but minor population of *E. orientalis* also occurred which is more and more abundant below the site. Detailed habitat information and distributions of general aquatic insects were re-

ported in Park *et al.* (1997) and Bae *et al.* (1998).

Sampling and field experiment

Qualitative samplings were monthly taken during the study period, but semimonthly taken from May to August in 1997 when the populations of *Ephemera* were expected to emerge (Kuroda *et al.*, 1984; Lee *et al.*, 1996). Larvae were collected by a hemispheric-shaped wire net with handle (diameter: 200 mm; mesh size: 0.5 mm) because they were found within 1cm depth in the sand dunes sometimes mixed with gravel and pebbles. Approximately thirty or more individuals were collected at each sampling trial except for the upstream site where the population was limited. Larvae were killed with 80% ethanol in the field, fixed with Kahle's fluid in the lab for about two days, and then preserved in 80% ethanol. Larval body length and wingpad length were measured by a micrometer on the dissect microscope (10~80x).

Water temperature was monitored at intervals of two hours at each study site from May 1997 to January 1998 by an automatic temperature recorder made by Onset Computer Corporation (Model: Optic StowAway). Thermometers were installed on the bottom of the running water area (20~50cm deep) avoiding the direct sun light. Thermometers at the upstream and midstream sites, however, were missing during the rainy season (Fig. 1-a). To calibrate this data loss, a tertiary polynomial regression was used in which indicated relatively high correlation (upstream site: $r^2 = 0.92$; midstream site: $r^2 = 0.94$; downstream site: $r^2 = 0.89$) (Fig. 1-b). ANOVAs also showed no significant differences ($p = 0.520$) between the real temperature data and the model data at the downstream site. Because the seasonal fluctuation pattern of the water temperature would be similar in a stream, we assumed that each regression values could represent the characteristics of water temperature at each site.

Developmental stages and degree day calculation

Since mayfly instars have not been able to be discriminated, arbitrary larval developmental stages mainly based on wingpad development as well as genital development have been used in the analysis of mayfly populations (Clifford, 1970; McCafferty and Huff, 1978; Heise *et al.*, 1987). In this study, we slightly modified previous devel-

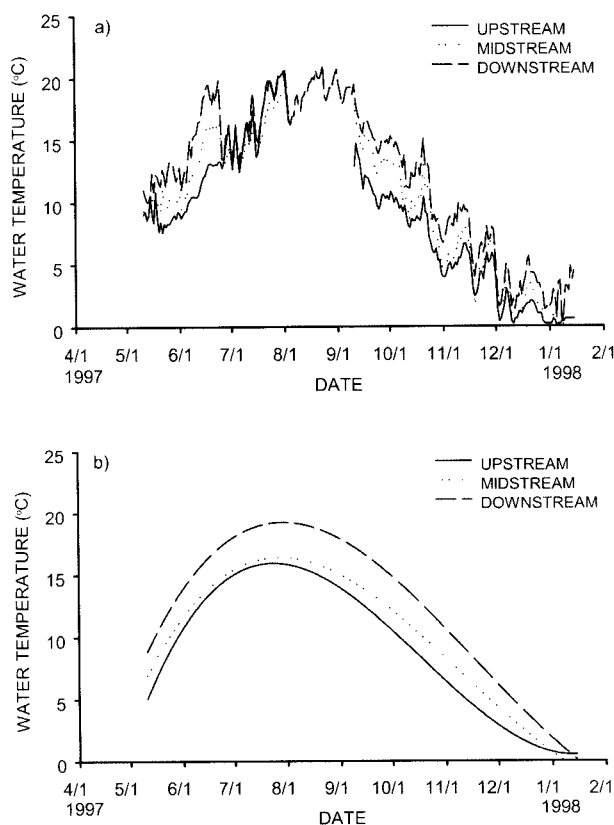


Fig. 1. Yearly water temperature change and its regression at the upstream ($Y = 6E - 6X^3 - 0.0029X^2 + 0.3332X + 4.7244$; $R^2 = 0.92$), midstream ($Y = 4E - 6X^3 - 0.002X^2 + 0.275X + 6.5256$; $R^2 = 0.93$), and downstream ($Y = 5E - 6X^3 - 0.0025X^2 + 0.3031X + 8.0995$; $R^2 = 0.90$) site in the study stream.

opmental stage analysis methods as follows. Stage I: wingpad absent, stage II: wingpad length shorter than head width; external sex organ absent, stage III: wingpad length shorter than head width; external sex organ visible, stage IV: wingpad length longer than head width; wingpad color white to gray, and stage V: wingpad color black. In the analysis of the life cycle, larval body length in a detailed scale (1 mm) was also used because it could be more informative. When two cohorts were existing in a same sampling date, they were separated from each other by Cassie's probability paper method (Cassie, 1954). Date of egg hatching in each species was estimated from extrapolating of the mean larval size and developmental stage information in each sampling date.

Water temperature was converted to degree day (DD) by the rectangle method shown in the

following equation. Rectangle $DD = (T_{max} + T_{min})/2 - T_b$ (where T_{max} and T_{min} are daily maximum and minimum water temperature, respectively, and T_b is the thermal threshold for development of a given population). In this study, T_b for each population was assumed to 0°C , because it has not been studied in detail for the populations of *E. separigata* and *E. strigata*. The Julian day was also assumed from the regression of the larval development data.

RESULTS

Daily water temperature

The patterns of the yearly water temperature change between the study sites were relatively similar, with the highest temperature in late July and the lowest in early January which are typical in northern temperate region (Fig. 1). Daily water temperatures calculated from the model ranged $0 \sim 15.93^\circ\text{C}$ (mean 9.39°C), $0 \sim 16.40^\circ\text{C}$ (mean 10.38°C), and $0.12 \sim 19.24^\circ\text{C}$ (mean 12.70°C) at the upstream, midstream, and downstream site, respectively. ANOVAs also indicated that the temperature differences between the sites were significant ($p < 0.05$). In the summer time, the temperature difference between the midstream and downstream sites was larger than that between the upstream and midstream sites.

Life cycle

All the populations of *E. separigata* and *E. strigata* in the study sites represented a univoltine life cycle with two distinct size groups (cohorts) (Figs. 2, 3), but differed in the timing of emergence and larval recruitment between the sites.

In the population of *E. separigata* in the upstream site, last instar larvae occurred from early June to late June, although the adults were actually found for some more extended period. Early instar larvae (stage I) were recruited twice for one year period. One of the early instar groups (summer cohort) occurred during the emergence period, from early June to early August, while the other group (winter cohort) occurred from late March to late April. In the midstream site, the last instar larvae occurred only in early June, and the summer and winter cohorts were recruited from late May to mid-July and from mid-January to late April, respectively.

In the population of *E. strigata* at the down-

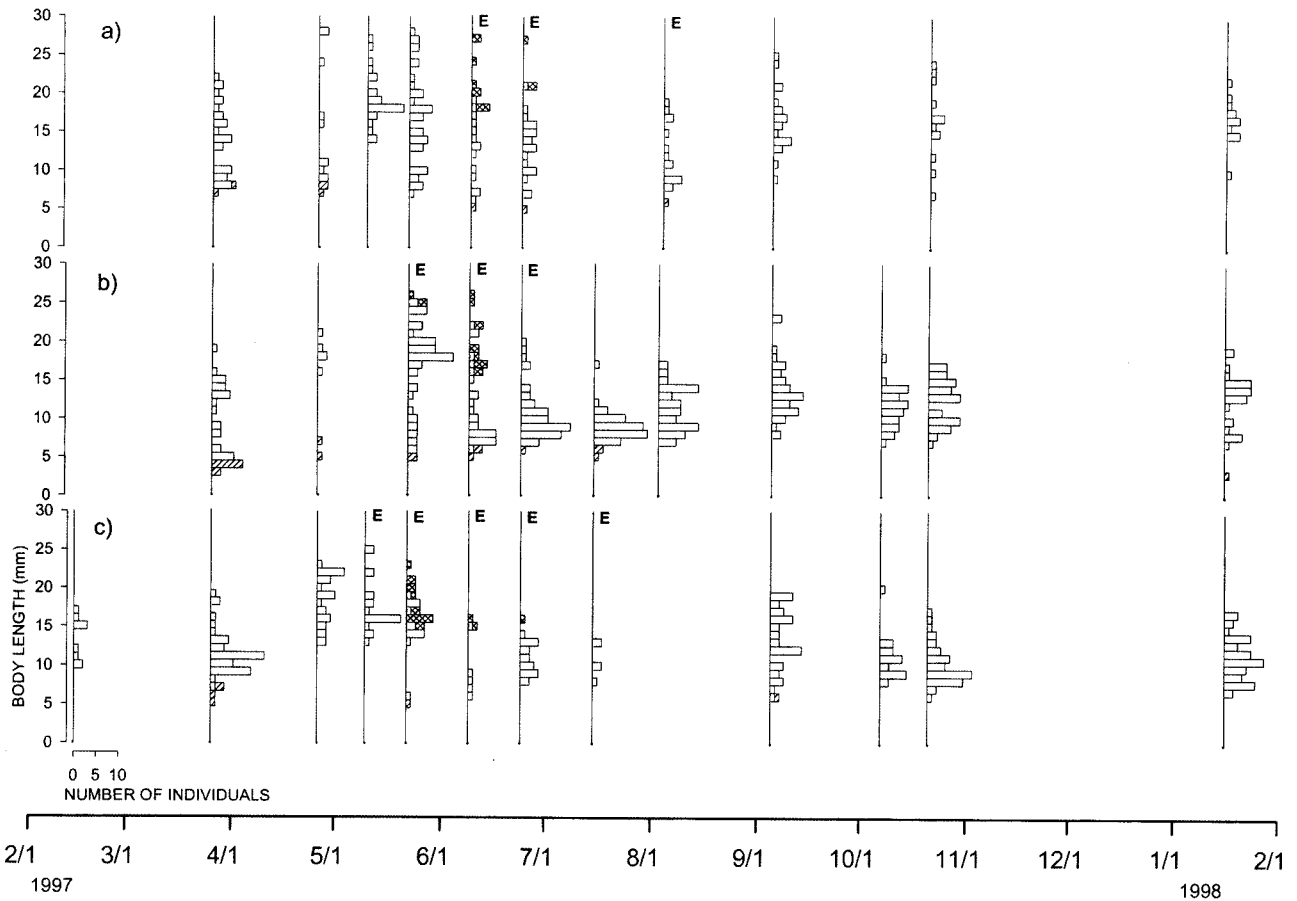


Fig. 2. Larval body size frequency distributions of the populations of *E. separigata* (a: upstream site; b: midstream site) and *E. strigata* (c: downstream site). (▨): stage I; (▩): stage V.

stream site, last instar larvae occurred from late May to late June, but majority of them was found in late May. Summer and winter cohorts were recruited in late May and late March, respectively.

Larval growth

As shown in the body length and larval developmental stage analyses (Figs. 2, 3), two distinct larval growth types were indicated. Larval growth was more or less gradual in the summer cohorts, but it was relatively abrupt in the winter cohorts. Earlier instar larvae grew more rapidly, and stage III larvae were most abundant throughout the year at all sites. The body length was significantly different between the summer and winter cohorts, but the difference became less significant as closing to the emergence time (Table 1).

Based on the regression of the degree day accu-

mulation associated with the average body length at each larval stage in the summer cohorts (Fig. 4), the first egg hatching day was estimated around April 19, March 27, and May 5 in the population of the upstream, midstream, and downstream site, respectively. The degree day accumulation also indicated that it took 2380 DD, 2668 DD, and 3220 DD for the full growth of the larvae in the order of the sites above (Fig. 4).

In the populations of *E. separigata*, the amount of cumulative DD was almost same to grow to the stage I and stage II larvae between the upstream and midstream sites. To grow to the stage III larvae, however, the amount was somewhat larger at the upstream site comparing with that at the midstream site. The population of *E. strigata* at the downstream site needed more cumulative DD to grow to the stage III larvae comparing with those of *E. separigata*. Estimated days to grow to the stage I, stage II, and stage III lar-

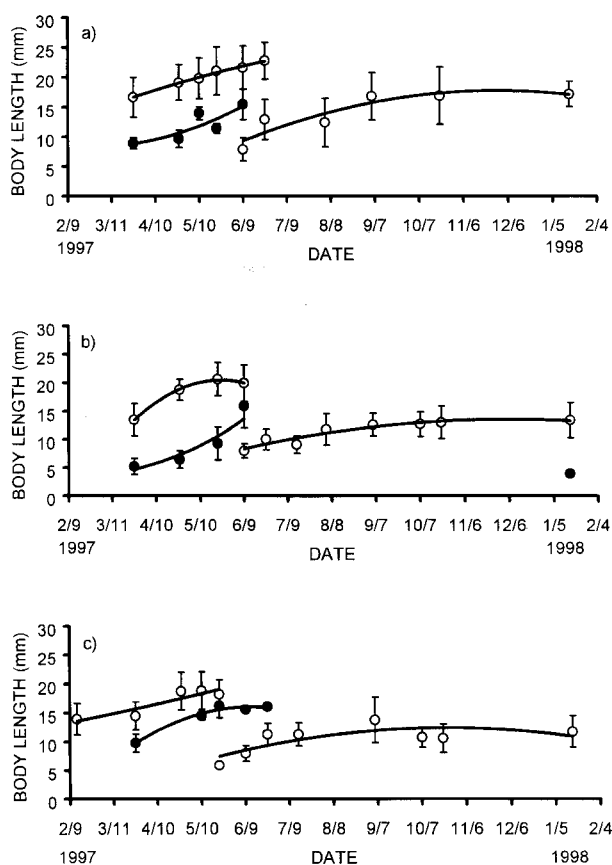


Fig. 3. Life cycle patterns of the populations of *E. separigata* (a: upstream site; b: midstream site) and *E. strigata* (c: downstream site). (○: summer cohort; ●: winter cohort).

vae at the sites in above order were 30, 59, and 185 days, 49, 77, and 194 days, and 23, 34, and 135 days, respectively (Fig. 4).

DISCUSSION

Intraspecific life history adaptation

Our results showed that all the populations of *Ephemera* in the study stream were univoltine with two distinct cohorts. Previous studies on Japanese *E. strigata* also showed similar results (Ban *et al.*, 1988). Lee *et al.* (1996), however, was not aware of the two different cohorts in *E. separigata* because winter and early spring samplings were lacking in that study.

Possible hypotheses have been presented to explain the cause of two periodically different early instar recruitment in a mayfly population. One of them is the effect of egg diapause, which

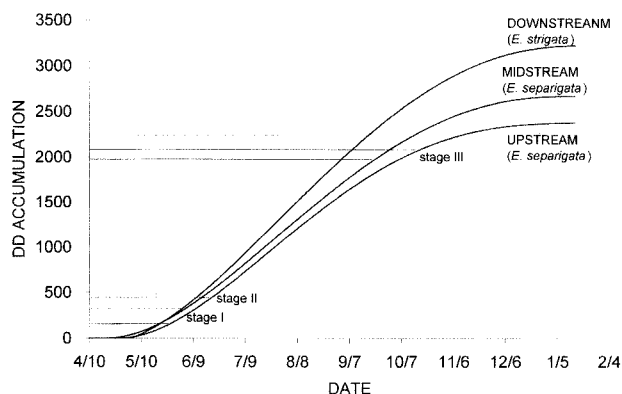


Fig. 4. Comparison of degree day accumulation required for the larval growth of the populations of *E. separigata* (upstream and midstream sites) and *E. strigata* (downstream site).

may cause the delay of egg hatching in part of the egg population. Clifford (1982) argued that approximately 10% of the univoltine mayfly species, which he examined, took summer diapause, although summer diapause is not common in aquatic hemimetabolous insects. Giverson and Rosenberg (1992) also reported that an ephemeropterid mayfly, *Hexagenia limbata*, produced an egg mass about a half of which underwent diapause under cold temperature ($< 4^{\circ}\text{C}$) condition.

Parthenogenesis could be alternative hypothesis, which may cause the delayed egg hatching in the *Ephemera* populations, because non-fertilized eggs hatched less successfully and developed more slowly in most cases of parthenogenesis (Humpesch, 1980). Both Hunt (1953) and Friesen and Flannagan (1976) reported the cases of non-obligatory parthenogenesis in ephemeropterid mayflies, *Hexagenia limbata* and *H. rigida*, respectively. Brittain (1982) reviewed many cases of non-obligatory parthenogenesis in mayflies. Watanabe and Ishiwata (1997), in a comprehensive study on the parthenogenesis in a Japanese ephemeropterid mayfly, *Ephoron shigae*, found that the sex ratio was the major clue in determining the bisexual and parthenogenetic populations, and that the parthenogenetic mayfly eggs would be produced in the end of the emergence period. In the populations of *E. separigata* and *E. strigata* in this study also produced more females in the end of the emergence period (unpublished data), which may indicate a possible parthenogenesis.

Our study sites represented different thermal

Table 1. Comparisons of body size between the summer and winter cohorts of *E. separigata* and *E. strigata* when both of the cohorts occur.

	Summer cohort			Winter cohort			p*
	μ	sd	n	μ	sd	n	
Upstream							
March 26	16.64	3.38	20	8.89	0.93	11	p<0.05
April 26	19.12	6.79	6	9.36	1.36	7	p<0.05
May 10	19.80	3.54	23	13.95	0.99	2	p<0.05
May 22	21.10	4.03	23	11.41	2.63	18	p<0.05
June 9	21.63	3.56	9	18.47	1.37	3	p>0.05
Midstream							
January 16	3.37	3.11	30	3.9	—	1	
March 26	13.44	2.88	19	5.18	1.42	17	p<0.05
April 26	18.74	1.87	5	6.4	1.56	23	p<0.05
May 22	20.58	2.90	40	9.28	2.90	16	p<0.05
June 9	19.89	3.15	14	15.88	3.86	9	p<0.05**
Downstream							
March 26	14.49	2.42	25	8.98	1.40	20	p<0.05
May 10	18.87	3.18	17	14.60	0.75	4	p<0.05
May 22	18.23	2.53	16	16.38	2.09	11	p>0.05

* t-test, ** p>0.01

regimes each other (Fig. 1). The average temperature difference between the upstream and midstream sites was about 1°C and that between midstream and downstream sites was about 2.3°C. In the populations of *E. separigata*, the life history features such as egg hatching time, larval growth, and emergence time were similar, but those were delayed to a certain period at the upstream site. Based on our temperature and life history data (Figs. 1, 3 and 4), it can be estimated that the egg hatching and average larval growth from the stage I to the stage III may be delayed to ca. 22 days and ca. 15 days, respectively, as the annual average water temperature drops to 1°C.

In comparison with the population of *E. strigata* in the downstream site, Japanese populations of *E. strigata* showed somewhat widespread larval recruitment and emergence time although their general life history features are similar (Kuroda *et al.*, 1984; Ban *et al.*, 1988). This could be caused by the geographic or altitudinal differences that may effect on the different temperature regimes.

Interspecific life history adaptation

In comparison with the larval growth between *E. separigata* at the upstream site and *E. strigata* at the downstream site, *E. strigata* required more degree days to grow to the stage I, stage II,

and stage III larvae (Fig. 4). This means, if the temperature regime is same, the larvae of *E. separigata* may grow more rapidly than the larvae of *E. strigata*. Early instar larvae of *E. separigata* in the winter cohort were recruited earlier (January), even in the lower temperature condition (near 0°C), than those of *E. strigata* (late March) (Fig. 3). This also may indicate that *E. separigata* is a cold-adapted species comparing with *E. strigata*.

Based on some previous studies on the life history of *E. japonica*, the uppermost stream representative among Japanese *Ephemera*, its general life history features are similar to those of *E. separigata*, but it has wider range of altitudinal distribution and longer period of emergence time (Kuroda *et al.*, 1984; Watanabe, 1985). This may indicate that *E. japonica* has adapted to a wider range of thermal regime.

ABSTRACT

Temperature-related life history adaptations in two ephemeropterid mayflies, *Ephemera separigata* and *E. strigata*, were studied from a mountain stream in Korea. Three study sites were chosen based on the altitudinal distributions of the *Ephemera* populations (upstream and midstream sites: *E. separigata*; downstream site: *E. strigata*). Samplings were taken monthly from Febru-

ary 1997 to January 1998, but semimonthly during the emergence period. Water temperature was monitored hourly from May 1997 to January 1998. As results, three sites represented their own temperature regimes (mean temperature upstream: 9.39°C; midstream: 10.38°C; downstream: 12.70°C). Both *E. separigata* and *E. strigata* showed evident univoltine life history pattern and two cohorts were recognized in each population based on the analyses of body length and larval developmental stages. The emergences took place from late May to late June, but earlier at the lower sites. Larval recruitments occurred during the emergence period (summer cohort) and 5~6 months later from the emergence period (winter cohort). It was hypothesized that either egg diapause or parthenogenesis could be the cause of the two periodically different early instar recruitments. Total accumulated degree day of *E. separigata* was 2380 and 2668 at the upstream and midstream site, respectively, and that of *E. strigata* was 3220 at the downstream site. It was estimated that the egg hatching and average larval growth of *E. separigata* could be delayed to ca. 22 days and ca. 15 days, respectively, as the annual average water temperature drops to 1°C. *Ephemera separigata* showed a cold adapted life history pattern, and more discussions on the intraspecific and interspecific life history adaptations were provided with other Korean and Japanese congeners.

ACKNOWLEDGEMENTS

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