Life-history responses of a mayfly to seasonal constraints and predation risk

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Abstract. 1. An experiment was conducted in the laboratory to examine the effects of photoperiod and predation risk on life-history variation in the mayfly *Ephemerella subvaria*.

2. Both photoperiod and predation risk affected age at maturity significantly but neither factor affected size at maturity. Mayflies perceiving themselves to be late in the growing season matured in fewer days than those perceiving themselves to be early in the growing season. The presence of predators delayed mayfly maturity significantly.

3. These results suggest that the large variation in life-history traits observed in aquatic insects may be attributed partially to seasonality but that other biotic and abiotic factors may also underlie variation in these traits.

Key words. Age at maturity, *Ephemerella subvaria*, predation risk, seasonal constraints, size at maturity.

Introduction

Several taxa of aquatic insect exhibit a characteristic emergence pattern. Within the same cohort, individuals that metamorphose early in the season do so at a larger size than individuals that metamorphose later in the season (Sweeney & Vannote, 1981; Forrest, 1987; Rowe & Berrill, 1989; Comiskey *et al.*, 1999; Peckarsky *et al.*, 2001). This large degree of phenotypic variation in body size is striking because the fecundity benefits of large body size are pronounced and have been demonstrated across many taxa (Vannote & Sweeney, 1980; Sokolovska *et al.*, 2000). The purpose of the work reported here was to examine the role of biotic and abiotic factors on the timing of metamorphosis and size at metamorphosis in the mayfly *Ephemerella subvaria* McDunnough (Ephemeroptera: Ephemerellidae).

Several hypotheses have been proposed to help understand this taxonomically broad variation in size at metamorphosis. Because size at emergence decreases gradually as the season progresses, this pattern does not suggest that individuals emerging later in the season are following an alternative life-history strategy to those emerging earlier in the season. Rather, the *mayfly hypothesis* (Rowe & Ludwig, 1991) and other similar hypotheses (Rowe *et al.*, 1994; Abrams *et al.*,

1996; Lytle, 2001) posit that the timing of metamorphosis is influenced by time constraints. For example, if the next generation of eggs or larvae needs to reach a certain developmental stage before the onset of an upcoming unfavourable period (e.g. winter or dry season), a delay in metamorphosis by larvae of the current generation decreases the survival probability of the next generation of offspring. Selection should favour a larva-to-adult transition that balances the potential increase in individual fecundity associated with delayed emergence with the potential costs of delayed emergence (Rowe & Ludwig, 1991).

Other hypotheses propose that the timing of metamorphosis is influenced by the risk of mortality (Werner & Gilliam, 1984; Abrams & Rowe, 1996; Abrams *et al.*, 1996). For example, as spring progresses the number of potential mayfly predators increases (Peckarsky *et al.*, 2001). Aquatic insects are very vulnerable to predation during the process of metamorphosis. Thus, a mayfly that delays the transition to adulthood may increase its fecundity after metamorphosis but may also increase its likelihood of being eaten during metamorphosis. Thus, selection should favour mayflies to forgo further growth for increased survival probabilities if the risk of predation is high.

Here, both the time constraint hypothesis and the risk of mortality hypothesis were tested by exposing larval mayflies simultaneously to one of two day-length treatments (following Johansson & Rowe, 1999; Johansson *et al.*, 2001) and one of two predation risk treatments. Larvae

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reared in a photoperiod mimicking late in the growing season were expected to accelerate their development and metamorphose earlier, and at a smaller size than larvae reared in a photoperiod mimicking earlier in the season. Larvae reared in a high predation risk treatment were expected to metamorphose earlier and at a smaller size than larvae reared in a low predation risk treatment.

Methods

Study organism and rearing conditions

Ephemerella subvaria inhabits riffles in temperate to sub-arctic streams in eastern North America (Allen & Edmunds, 1965). Larvae hatch in mid-August and grow during the autumn, winter, and early spring. Adults emerge from mid-May to June, mate, and lay eggs within 2–3 days of emergence (Ciborowski, 1976).

Mayfly larvae were collected from Reesor Creek, Ontario, Canada (43°N, 79°W). Individual larval rearing containers consisted of transparent 200 ml plastic cups with two $4 \times 5\,cm^2$ pieces cut out of the sides and replaced with 0.25 mm Nitex screen. Rearing containers were held partially submerged with styrofoam collars in larger bins of water. Each large bin (62 cm long × 40.5 cm wide × 14.5 cm deep) held 20 individual rearing cups and was filled 10 cm deep with dechlorinated tap water. Five litres of the water in these holding bins were replaced every 5 or 6 days.

Larvae were provided with conditioned (following Bird & Kaushik, 1984) $4 \times 4\,cm$ squares of maple leaf for food as well as for substrate. *Ephemerella subvaria* reared on maple leaves have good growth and survival (Bird & Kaushik, 1984). Larvae received new leaves every 3–4 days and were never food limited throughout the experiment.

Predation risk on *E. subvaria* was imposed by exposing larvae to the fish *Luxilus cornutus* (Cypriniformes: Cyprinidae, the common shiner). The distribution of *L. cornutus* overlaps extensively with that of *E. subvaria* and it is a predator of larval and adult aquatic insects (Scott & Crossman, 1998). In the predator risk treatments, two *L. cornutus* were placed in the large holding bins and allowed to swim freely among the partially submerged rearing containers. The fish could not prey directly on the mayfly larvae in the individual cups. Predation risk was thus imposed indirectly on mayfly larvae via chemical and visual cues. Many species of insect have been shown to respond to such cues (e.g. chironomids: Ball & Baker, 1996; mayflies: McIntosh *et al.*, 1999). Fish were fed Tetra Tropical Fish Staple Flakes (Pfizer, Morris Plains, U.S.A.) daily along with live *E. subvaria* larvae every 3 days.

Experimental design

Two photoperiod treatments (early, late) and two predator risk treatments (present, absent) were used in the experiment. The start of the *early* treatment mimicked that of early March (L:D 11.5:12.5 h), while the start of the *late* treatment mimicked that of mid-April (L:D 13.5:10.5 h). Given that *E. subvaria* have an emergence period of ≈1 month in nature (Sweeney & Vannote, 1981), this 6-week difference in starting photoperiods extrapolates to the day lengths that early- and late-emerging *E. subvaria* would experience in nature.

Lights were controlled using digital timers and adjusted weekly to follow the natural day length progression of the appropriate treatment. The two photoperiod and two predator treatments were crossed to form four treatments in total (early/predator risk, early/no predator risk, late/predator risk, and late/no predator risk). Each treatment was assigned randomly to two bins. Thus, there were eight bins each containing 20 individual rearing containers. Half-way through the experiment, all bins were rotated and repositioned to minimise effects due to bin positioning. Temperature was kept constant at 15 °C for the duration of the experiment.

Response variables

Larval head widths were measured as the maximum distance between the eyes, using a dissecting microscope fitted with an ocular micrometer. One ocular unit was equal to 0.25 mm. Head widths were measured at the start of the experiment, on days 9, 19, and when larvae reached the last larval (black wing pad) stage (Brittain, 1982). Larval mouthparts have degenerated completely by this time; larvae cease growing and emerge 1–2 days after reaching this stage. Size and age at the black wing pad stage are commonly used as measures of age and size at maturity in studies of mayfly life history (e.g. Peckarsky *et al.*, 1993; Scrimgeour & Culp, 1994). Thus, size at maturity is defined here as the larval head width at the black wing pad stage.

Age at maturity was defined as the number of days needed to reach the black wing pad stage after the start of the experiment. This duration is not the actual age of the larvae, because larvae were not reared from eggs, but because each treatment received a random sample of wild-collected young larvae, any variation in age or size at maturity attributable to variation in starting size can be accounted for by including initial size as a covariate in the statistical analyses.

Statistical analyses

To ensure that the variation in the response variables was not due to uncontrolled effects unique to specific bins, a nested ANOVA with bins nested within treatment was conducted.

An ANOVA was used to examine whether photoperiod or predation risk affected age at maturity significantly in *E. subvaria*. A scatter plot of the standardised predicted residuals by the standardised observed residuals revealed a wedge-shaped pattern. Age at maturity data were therefore

ln transformed to normalise the distribution of the data. To account for the fact that larvae were not all the same size at the start of the experiment, mayfly size at day 1 was included in the model as a covariate. Because male mayflies tend to emerge earlier than female mayflies, sex was included in the model as a random effect factor. Sex was treated as a random effect because mayfly larvae were too small to sex at the start of the experiment and, consequently, the sexes were distributed randomly to each treatment.

The above ANOVA model was also conducted to examine the effect of photoperiod and predation risk on size at maturity in *E. subvaria*. In this case, sex was included as a factor because males develop compound eyes whereas females do not. Males were thus expected to have larger head widths than females. Size at maturity data did not need transforming.

A repeated-measures (sampling date) ANOVA was conducted to examine whether mayflies in the different photoperiod and predation treatments grew at different rates. The three sampling dates included in the model were larval size at day 9, size at day 19, and final size. All statistical analyses were conducted using SPSS version 10.0.7 (Norusis, 2000).

Results and discussion

There was no significant effect of bins on age at maturity ($F_{4,87} = 0.79$, $P = \mathrm{NS}$) or on size at maturity ($F_{4,87} = 0.78$, $P = \mathrm{NS}$). Thus, in all subsequent analyses, each mayfly was considered to be a single replicate. Larval mortality was observed in all treatments but did not differ statistically among treatments ($F_{3,7} = 0.111$, $P = \mathrm{NS}$).

Both photoperiod and predation affected age at maturity in *E. subvaria* (Fig. 1a). Mayflies in the late photoperiod treatment matured in fewer days than those in the early treatment ($F_{1,86} = 151.82$, $P = 0.001$) and larvae exposed to predation risk delayed maturity and metamorphosed later than larvae in the predator-free treatment ($F_{1,86} = 41.64$, $P < 0.01$). There was a significant effect of sex ($F_{1,86} = 73.29$, $P < 0.001$) as predicted, but no significant effect of the interaction between photoperiod and predation ($F_{1,86} = 1.661$, $P = \mathrm{NS}$) or the three–way interaction among photoperiod, predation, and sex ($F_{3,86} = 0.02$, $P = \mathrm{NS}$) was found.

Neither photoperiod ($F_{1,86} = 0.17$, $P = \mathrm{NS}$) nor predation ($F_{1,86} = 3.02$, $P = \mathrm{NS}$) affected size at maturity significantly (Fig. 1b). An effect of sex was found ($F_{1,86} = 46.67$, $P = 0.005$) but no effect of the interaction between photoperiod and predation ($F_{1,86} = 0.27$, $P = \mathrm{NS}$) or the three–way interaction among photoperiod, predation, and sex ($F_{3,86} = 0.32$, $P = \mathrm{NS}$).

The repeated-measures ANOVA revealed a significant effect of sampling date on mayfly head width ($F_{2,180} = 15.30$, $P < 0.001$) but the interactions between sampling date and photoperiod ($F_{2,180} = 0.47$, $P = \mathrm{NS}$), between sampling date and predation ($F_{2,180} = 0.80$, $P = \mathrm{NS}$), and among sampling date, photoperiod, and predation
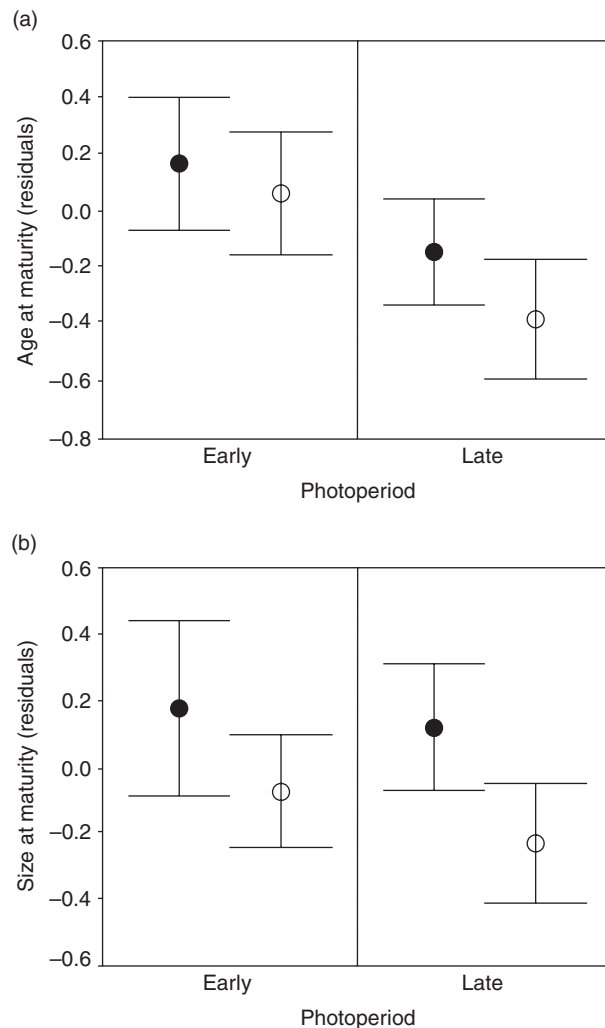

Fig. 1. The effect of photoperiod and predation on (a) age at maturity and (b) size at maturity of *Ephemerella subvaria*. For (a) the residuals from the regression of ln(age at maturity) on size at day 1 are plotted on the *y* axis. For (b) the residuals from the regression of size at maturity on size at day 1 are plotted on the *y* axis. Error bars are ±1 SEM. ● = predation risk present, O = no predation risk. Sample sizes: early, predation = 24; early, no predation = 24; late, predation = 24; late, no predation = 23.

($F_{2,180} = 0.43$, $P = \mathrm{NS}$) were not significant. These results revealed that larval head widths changed over time but that they did not change at different rates across photoperiod and predation treatments.

This study was designed to help to explain the interesting pattern of variation in size at emergence observed in many species of aquatic insect. The *mayfly hypothesis* and other related hypotheses hinge on organisms being able to assess their relative position in the season. These models predict that larvae that emerge later in the season are smaller than their earlier-emerging counterparts because the former mature faster and forfeit further growth in favour of increased mating opportunities and increased offspring

survival probabilities. Here, larvae perceiving themselves to be late in the season were shown indeed to accelerate their development rate but, in this study, accelerated development did not result in a statistically significantly smaller size at maturity. The lack of a size effect would be expected if larvae in the late treatment exhibited higher growth rates than those in the early treatment. This effect was not seen. These results contrast partially with those of Johansson and Rowe (1999) and Johansson *et al.* (2001). In their studies, damselflies exposed to late photoperiods accelerated development and emerged at smaller sizes than damselflies in the early photoperiod treatment. The lack of size difference in the current experiment may have been due to low statistical power or to larvae being fed *ad libitum*. An unlimited food source may diminish the size trade-off usually associated with accelerated development. Alternatively, although *E. subvaria* larvae have been shown to have good growth on maple leaves (Bird & Kaushik, 1984), larvae in all treatments metamorphosed at smaller than average sizes compared with wild-caught larvae of the same developmental stage. A nutrient deficiency in the laboratory may prevent larvae in the early photoperiod treatment from attaining their maximum size.

Several models have also proposed that variation in age at maturity and size at maturity may be influenced by seasonal variation in predation pressure or other forms of mortality. Selection should favour mayflies in a high-risk environment to accelerate development in order to minimise the time spent in this environment. Although this effect has been shown in other species of aquatic insect (e.g. Ball & Baker, 1996; Johansson *et al.*, 2001; Peckarsky *et al.*, 2001), *E. subvaria* exhibited a slower development rate in the predation risk treatment than in the predation-free treatment. These results suggest that predator presence impedes rather than accelerates the development of these larvae. Development rate may decrease if predators induce behavioural, immunological, or physiological anti-predator responses. Behavioural responses to predators are not common in *E. subvaria* (Soluk & Collins, 1988; Peckarsky, 1996) but the existence of the other types of response has not been investigated. If resources were diverted to these types of anti-predator response, rather than to development, a decrease in development rate would not be unexpected. Overall, although photoperiod and predation risk explained much of the variation in age at maturity, these factors explained much less of the variation in size at maturity. These results suggest that although many species exhibit seasonal variation in life-history traits, an array of biotic and abiotic influences probably underlies this variation.

Acknowledgements

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