Effects of temperature and photoperiod on larval size and survivorship of a burrowing mayfly (Ephemeroptera, Ephemeridae)

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There is great size variation in naturally occurring and laboratory-reared populations of larvae of *Hexagenia* species. We investigated differences in mean larval size and survivorship of *Hexagenia limbata* and *Hexagenia rigida* reared at different temperatures (12, 17, and 22°C) and photoperiods (24 h L, 24 h D, 12 h L : 12 h D) over two time intervals (60 and 120 d) in the laboratory. Owing to space limitations, two sets of replicates (3 replicates per set) were conducted in sequence for the 60-d trial. Larvae were hatched from eggs collected from imagoes at the Detroit River near Windsor, Ontario, and stored at 8°C. Of the factors examined, temperature alone influenced larval size after 60 d of growth. None of the factors had any significant effect on larval survival after 60 d. Larvae from the second 60-d replicate set (hatched from eggs incubated for 75 d longer than the first set) were larger and exhibited lower survivorship than larvae in the first set. This finding suggests that larval size may not be a good indicator of larval age, especially if eggs have been exposed to low temperatures for varying periods. Temperature and its interaction with photoperiod significantly affected both size and survivorship of larvae reared for 120 d. At 22°C, larvae reared under the 12 h L : 12 h D regime were larger than those reared under either constant light or dark conditions; no discernible trend in photoperiod was evident at the lower temperatures. Larval survivorship was lowest for treatments exhibiting conflicting environmental cues (12°C and 24 h L, and 22°C and 24 h D).

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Il existe une variation importante de la taille chez les larves d'*Hexagenia*, qu'elles proviennent de populations naturelles ou de populations élevées en laboratoire. Nous avons examiné les différences dans la taille moyenne et la survie chez des *Hexagenia limbata* et *Hexagenia rigida* élevées en laboratoire à différentes températures (12, 17, 22°C) et photopériodes (24 h L, 24 h D, 12 h L : 12 h D) durant deux intervalles différents (60 et 120 jours). À cause de problèmes d'espace, deux séries d'expériences parallèles (3 par série) ont été menées de front au cours de l'intervalle de 60 jours. Les larves provenaient d'éclosions d'oeufs recueillis chez des imagos issus de la rivière Détroit, près de Windsor, Ontario, et ont été gardées à 8°C. Parmi les facteurs examinés, seule la température influençait la taille des larves après la croissance de 60 jours. Aucun des facteurs n'avait un effect significatif sur la survie des larves au bout de 60 jours. Les larves de la seconde série d'expériences de 60 jours (écloses d'oeufs gardés pour 75 jours de plus que la première série) étaient plus grosses et avaient une survie moins grande que les larves de la première série. Il semble donc que la taille des larves puisse constituer un bon indicateur de leur âge, particulièrement si les oeufs ont été exposés à de basses températures pour des périodes de durées diverses. La température et son interaction avec la photopériode affectent significativement la taille et la survie des larves gardées en élevage pour 120 jours. À 22°C, les larves gardées à un régime de 12 h L : 12 h D étaient plus grosses que les larves gardées à lumière constante ou à obscurité constante; aucun effet de la photopériode n'a pu être décelé aux températures plus basses. La survie était plus faible chez les larves soumises à des traitements comportant des facteurs écologiques déclencheurs en conflit (12°C et 24 h L, et 22°C et 14 h D).

[Traduit par la rédaction]

Introduction

Interpretation of the life history of mayflies (Ephemeroptera), specifically burrowing mayflies, *Hexagenia* spp., is difficult, owing to the protracted flight period, the presence of multiple cohorts (Heise *et al.* 1987), delayed hatching of eggs, differential growth of males and females, and a wide variability in growth rates of individuals from the same egg mass (Hunt 1953). There is tremendous size variation in larval populations collected from a single lake or river, owing to overlapping cohorts and the presence of one or more species of *Hexagenia* (Fremling 1973; Flannagan 1979; Heise *et al.* 1987).

In mayflies, all growth occurs in the larval stage. The subimagoes (subadults) and sexually mature imagoes (adults) do not feed and seldom live for more than 3 days, during which time they may disperse and mate and the females oviposit (Edmunds *et al.* 1976; Corkum 1987). Thus, the size variation revealed in the adult populations of *Hexagenia* species is a reflection of size variation in the larvae. The evolutionary significance of variation in adult size lies in the positive association between size and fecundity (Clifford and Boerger 1974).

Sweeney (1984) considered temperature and photoperiod to have a major effect on several measures of fitness in mayflies, including embryonic development, larval growth, and adult emergence, as well as adult size and fecundity. Sweeney and Vannote (1978) argued that adult size and fecundity of hemimetabolous insects (including mayflies) depended on the thermal conditions during larval growth, suggesting that temperatures higher or lower than the optimum result in lower individual adult weight and fecundity.

The effect of temperature on larval growth of aquatic insects is well known. Although abnormally high temperatures result in a decrease in the growth rate and efficiency of organisms (Heiman and Knight 1975), several workers have shown a positive correlation between larval growth rate in mayflies and water temperature in both natural (Brittain 1976*a*; Corkum 1978*a*; Newell and Minshall 1978; Sweeney and Vannote 1978; Vannote and Sweeney 1980; Heise *et al.* 1987) and laboratory (Thomforde and Fremling 1968; Brittain 1976*b*; Sweeney 1978; Wright and Mattice 1981*a*; Wright *et al.* 1982; McCafferty and Pereira 1984) populations. However, in the

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studies cited, temperature alone did not account for all size variation observed in larval mayflies.

Although photoperiod has been shown to be important in determining the survivorship and reproductive behaviour of a variety of terrestrial insects (Danks 1987), few studies have examined the influence of photoperiod on aquatic insects. Of these, most have focused on Odonata (Corbet 1955; Lutz and Jenner 1964; Lutz 1968; Lutz 1974*a*, 1974*b*) and Diptera (Anderson 1968; Danks 1978, 1991), with the emphasis on diapausing rather than nondiapausing organisms (Mousseau and Dingle 1991). No studies have been conducted on the effect of photoperiod on the larval development of mayflies.

The potential ecological significance of both temperature and photoperiod on larval growth is great. Sweeney and Vannote (1981) have suggested that, as day length and temperature increases, there is a corresponding increase in the feeding period of visual predators. Accordingly, the feeding activity of prey should be enhanced during periods of darkness, when predation risk is lowered. We expected an increase in larval size and survivorship of burrowing mayflies, whose diet consists of algae, diatoms, detritus, and mud (Neave 1932; Zimmerman and Wissing 1980), at elevated temperatures and constant dark conditions.

Recent work (Hanes 1989)[1] has shown that some environmental factors do not elicit developmental responses by larvae of burrowing mayflies until after a 2-month growth period. Hanes (1989)[1] suggested that endogenous factors may be most important during early larval development, whereas responses to exogenous factors (such as temperature and photoperiod) may be exhibited in later stages of development. Other workers have shown that the response of insect larvae to temperature and photoperiod may vary among instars and according to the season examined (Lutz and Jenner 1964; Lutz 1968).

In this study, we present the results from a laboratory experiment designed to examine the effects of temperature and photoperiod on larval size and survivorship of burrowing mayflies (*Hexagenia*) reared for two time intervals (60 and 120 d).

Methods

Experimental organism

Larvae used in the experiment were hatched from eggs collected from female imagoes of two *Hexagenia* species. *Hexagenia limbata* (Serville) and *Hexagenia rigida* McDunnough are common in the Great Lakes region (Wood 1973; Schloesser and Hiltunen 1984; personal observation), and typically co-occur in large rivers and lakes throughout eastern and central North America (Neave 1932; Edmunds *et al.* 1976; Flannagan 1979). Imagoes were captured on warm, relatively calm evenings, near sunset, on the bank of the Detroit River in Windsor, Ontario (42°20′27″N, 82°56′56″W).

We collected imagoes on 6 July 1989, using a battery-powered ultraviolet lamp that was placed on a white sheet. Female imagoes that landed on the sheet were grasped by their wings and placed in bags (ca. 50 adults per 6-L bag) with ca. 1.5 L of aerated distilled water. Females immediately released eggs into the water. A small amount of clay mixed with water was added to the bags to minimize clumping of eggs (M. G. Henry, Minnesota Cooperative Fish and Wildlife Research Unit, St. Paul, personal communication). In the laboratory, eggs that were stored in the collection bags were cooled at 4°C steps to 8°C for incubation (Friesen 1981). Cold storage induced dormancy; diapause does not occur in eggs of *Hexagenia* species in this region (Hanes *et al.* 1990). Eggs were held at 8°C for 9 months, then removed from the incubator on 4 April 1990 and transferred directly to room temperature (20°C); larvae hatched 4 days later. Approximately 90% of all eggs hatched over the next 6 days; 65% of the eggs hatched within the first 2 days (i.e., days 5 and 6).

Rearing system

Larvae were reared in 1-L clear plastic tanks (70 cm^2 × 13 cm deep). In each tank, we placed 200 mL of standard sediment (4 cm deep) consisting of potting soil and potter's clay in a 2:1 ratio (dry weight) to which we added 750 mL of distilled water (M. G. Henry, Minnesota Cooperative Fish and Wildlife Research Unit, St. Paul, MN, personal communication; Hanes 1989, see footnote 1). Each rearing tank was aerated continuously by a system of capillary tubing and needles (Corkum and Hanes 1989).

Eight newly hatched (1 day old) larvae were pipetted into each rearing tank. This density is representative of field populations (1140 larvae/m^2) of *Hexagenia* species in the Great Lakes region (Schlosser and Hiltunen 1984) and has been demonstrated to permit high larval growth and survivorship in laboratory studies (Hanes 1989, see footnote 1). Rearing tanks were placed into one of nine treatments (3 temperatures × 3 light regimes).

Larvae in all tanks were fed a mixture of yeast, Tetramin® fish food flakes, and alfalfa powder (M. G. Henry, Minnesota Cooperative Fish and Wildlife Research Unit, St. Paul, personal communication) twice weekly (0.7 mg/tank per day). This feeding regime is known to provide excess food to larvae (Hanes 1989, see footnote 1). A layer of food was visible on the surface of the mud in all tanks throughout the experiment.

Three large (125 × 40 × 40 cm), light-tight, black Plexiglas chambers were each maintained at one of three temperatures (12, 17, 22°C). Rearing tanks were placed in the temperature-controlled water bath of each chamber. Cooling water in the chambers held at 12 and 17°C was refrigerated using Aquachiller® cooling units (one per chamber). The water in the chamber held at 22°C was controlled with an aquarium heater (Thermal Compact Pre-set®); water was circulated within this chamber using a network of aeration tubing.

Each temperature-controlled chamber was partitioned into three equal-sized compartments. Each compartment was randomly assigned a photoperiod (24 h L, 12 h L : 12 h D, 24 h D). A pair of removable black Plexiglas dividers (40 × 40 cm) that fit into grooves between adjacent compartments prevented light transfer between them but allowed water to circulate. A light-tight black Plexiglas lid covering each compartment was hinged to the back of the chamber. In those compartments requiring light, two fluorescent lamps (Mini Lampi®) (providing a light intensity of 12 lx at the water surface) were secured to the underside of each lid.

Experimental design

A 3 × 3 factorial design was used to examine differences in mean size and survivorship of larvae reared at the three different water temperatures and 3 different light regimes. The temperatures selected (12, 17, 22°C) were above the threshold temperature for growth (Heise *et al.* 1987), yet below the temperatures that inhibit metamorphosis (Wright and Mattice 1981*a*). Photoperiods were selected to represent a typical field situation (12 h L : 12 h D) in this region, conditions in deep or shaded water (24 h D), and a complementary extreme light regime (24 h L). Constant 24 h light conditions have been reported for populations of *Hexagenia* species near their northern limit of distribution at South Indian Lake, Manitoba (D. J. Giberson, University of Manitoba, personal communication).

We investigated the influence of temperature and photoperiod on larval size and survivorship after 60 and 120 d growth. Initially, larvae were added to 81 tanks (9 tanks × 9 treatments). Larvae in six of the nine tanks per compartment were maintained for 120 d. The three other tanks per compartment were harvested after 60 d (i.e., first replicate set). These tanks were replaced with 27 other tanks containing newly hatched larvae from eggs incubated for an additional 75 d at 8°C (i.e., second replicate set). Thus, larvae in six

[1]E. C. Hanes. 1989. Effects of density and food limitation on larval size and mortality of *Hexagenia* (Ephemeroptera, Ephemeridae). B.Sc. thesis, Department of Biological Sciences, University of Windsor, Windsor, Ont.

TABLE 1. Summary of two-way ANOVA of the influence of temperature, photoperiod, and their interaction on larval size and survivorship after 60 d (first and second replicate sets)

	First replicate set				Second replicate set			
	Size				Size			
Variable	df	F	P	Survivorship	df	F	P	Survivorship
Temperature	2,26	22.393	<0.001	ns	1,17	4.980	<0.05	ns
Photoperiod			ns	ns			ns	ns
Interaction			ns	ns			ns	ns

NOTE: The second replicate set included only two of the three temperatures (data for 12°C were deleted from the analysis because of low larval survivorship); ns, not significant.

replicate tanks were examined for larval growth after 60 d (with replacement) and 120 d.

Data were analysed using a two-way analysis of variance (ANOVA) test (Sokal and Rohlf 1981). Variables analysed were mean head width of larvae in each tank and percent survival in each tank (8 larvae/tank; 6 tanks/treatment). Separate analyses were performed on each of the 60-d trials and on the 120-d trial.

Recovery

Larvae were retrieved from the tanks by slowly pouring off the overlying water through a 250-μm sieve and replacing it with carbonated water. The larvae rose to the surface and were removed using a Pasteur pipette. Afterwards, the sediment was sieved through a 250-μm sieve to insure that all larvae were retrieved. Larvae were preserved in 70% ethanol. Head width (measured across the eyes) and body length (total length excluding cerci) of all larvae were measured (to the nearest 0.075 mm) using a dissecting microscope with an ocular micrometer. Survivorship was calculated for each tank.

In the laboratory, we used head width (HW) rather than body length (BL) as a measure of larval size, since the coefficient of variation was less for HW (22.6%) than for BL (25.1%) (n = 35). The relationship between HW and BL was linear: BL (mm) = −0.230 + 8.454 HW (mm); R^2 = 0.83, n = 35. The mean head width of larvae within a tank was used for statistical analysis of size.

Results

Since identification of larvae of *Hexagenia* species is often unreliable, and female imagoes cannot be distinguished (McCafferty 1975), we identified the mayflies using male imagoes that were collected at the same time as the eggs. Based on the collection of males that emerged on 4 and 6 July 1989, we estimated that the ratio of *H. limbata* to *H. rigida* in the population of burrowing mayflies present at the collection site was 2:1 (n = 251).

Since there were two species, *H. limbata* and *H. rigida*, present at the study site, some of the variation observed among individuals may be attributed to size differences between species. Based on 1991 male imagoes collected at the study site, the mean BL (±1 SE) of the two species differs (*H. limbata*: 18.8 ± 0.13 mm; *H. rigida*: 17.7 ± 0.12 mm); however, the size ranges of the two species overlap (*H. limbata*: 13.2–24.0 mm; *H. rigida*: 12.3–20.7 mm) (Hanes *et al.* 1990). Since larvae were randomly distributed among rearing tanks, this size variation between species should be similar among all nine treatments, thus the influence of temperature and photoperiod may still be quantified. We also assumed that males and females occurred randomly among rearing tanks and that mortality rates were equal between the species and between the sexes among treatments.

Sixty-day trial

Significant differences in larval size were observed among the nine treatments ($F_{[8,53]}$ = 29.047, P < 0.001), the first and second replicate sets ($F_{[1,53]}$ = 11.395, P < 0.001), and the interaction between treatment and replicate sets ($F_{[8,53]}$ = 8.446, P < 0.001). Because of differences in larval size between the first and second sets of replicate tanks, subsequent analyses were performed separately on each data set.

The results of the two-way ANOVA tests of temperature and photoperiod effects on larval size for the two sets of replicates were similar (Table 1). Although temperature had a significant effect (P < 0.001) on larval size, neither photoperiod nor its interaction with temperature had any significant effect on larval size (Table 1).

Larval size increased with temperature in both replicate sets (Fig. 1). Moreover, the larvae reared in the second replicate set were larger than larvae in the first replicate set (especially at 22°C) (Fig. 1). No trends in larval size were detected among light regimes.

Results of a two-way ANOVA indicated that there was a significant difference in larval survivorship between the first and second replicate sets ($F_{[1,53]}$ = 14.078, P < 0.001). However, there were no significant effects due to treatment (temperature and photoperiod) or their interaction on larval survivorship. Again, because of differences in survivorship between the first and second sets of replicate tanks, subsequent analyses were performed separately on each data set.

In both replicate sets, neither temperature, photoperiod, nor the interaction of the two factors had any effect on larval survivorship of larvae reared for 60 d (Table 1). Lack of a significant temperature effect on larval survival ameliorates possible temperature shock on survival when newly hatched larvae at room temperature (20°C) were added to tanks at 12, 17, or 22°C.

Larval survivorship was higher in the first replicate set (55.1 ± 5.4%) than in the second (26.4 ± 5.9) (Fig. 2). In the second replicate set, larval survivorship was much lower at 12°C (14.1 ± 4.7%) than at either 17°C (35.8 ± 11.9%) or 22°C (31.9 ± 10.2%) (Fig. 2).

One-hundred-and-twenty-day trial

Results of a nested ANOVA indicated that there was significant variation in larval size among treatments ($F_{[8,206]}$ = 19.833, P < 0.001) and among tanks ($F_{[35,206]}$ = 2.192, P < 0.005) within treatments. Since the magnitude of the variance among treatments is greater than the variance among tanks, one can still determine whether variation among treatments is greater than expected (Sokal and Rohlf 1981).

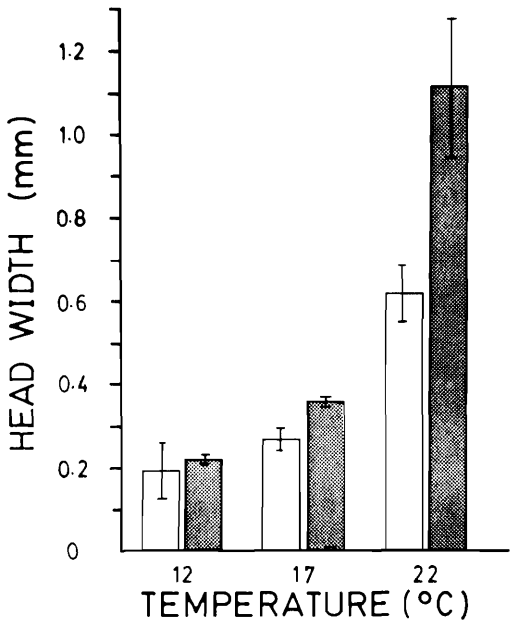

FIG. 1. Mean head width of larvae reared for 60 d at 12, 17, and 22°C for the first (open bars) and second (shaded bars) replicate sets. Data are combined for all photoperiods. Vertical lines show standard error.

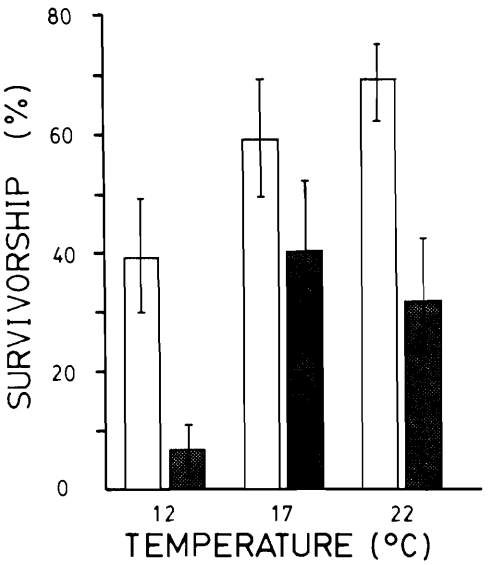

FIG. 2. Mean survivorship of larvae reared for 60 d at 12, 17, and 22°C for the first (open bars) and second (shaded bars) replicate sets. Data are combined for all photoperiods. Vertical lines show standard error.

Results of the two-way ANOVA indicated that after 120 d, temperature ($P < 0.001$) as well as the interaction of temperature and photoperiod ($P < 0.025$) significantly influenced larval size; photoperiod alone had no effect on larval size (Table 2). The F-statistic for temperature (102.43) was much larger than for the interaction term (3.19), indicating the overriding importance of temperature on larval size after 120 d (Table 2). Final larval size increased with rearing temperature (Fig. 3). After 120 d, larvae reared at 22°C were half-grown (BL = 10 mm, $n = 84$), whereas larvae reared at 12°C (BL = 2 mm, $n = 53$) were not much larger than newly hatched individuals (BL = 1 mm, $n = 30$). In addition, larvae that were reared under the 12 h L : 12 h D regime were larger than those reared under either constant light or constant dark conditions at 22°C (Fig. 3). No other effect due to the interaction of temperature and photoperiod was discerned.

Larval survivorship was significantly affected by temperature ($P < 0.05$) as well as by the interaction of temperature and photoperiod ($P < 0.05$) after 120 d; photoperiod alone had no significant effect on survival (Table 2). Larvae reared at higher temperatures had higher survivorship than larvae reared under lower temperatures (17 and 22°C vs. 12°C at 24 h L; 22°C vs. 12 and 17°C at 12 h L : 12 h D) (Fig. 4). Larval survivorship was lowest under 24 h of light at 12°C. Larvae reared at 22°C under either 12 or 24 h light had higher survivorship than those exposed to constant dark conditions (Fig. 4). There was no apparent difference in larval survivorship among the three light regimes at intermediate (17°C) temperatures. Although these interaction terms indicated significant effects on larval survivorship, the trends were not consistent among treatments.

Discussion

Temperature and photoperiod were selected for studying larval size and survivorship because of the potential of these factors to influence individual fitness (Sweeney 1984). Fit organisms are "those better represented in future generations than their relatively unfit competitors" (Stearns 1976). Since adult mayflies do not feed during their brief life-span of 1–3 days, their size depends on growth during the larval stage.

The positive correlation between larval development of aquatic invertebrates (including mayflies) and sublethal temperatures has been well documented (Ward and Stanford 1982; Sweeney 1984). In our study, we showed that water temperature significantly influenced larval size of *H. limbata* and *H. rigida* after 60 and 120 d of growth. Larval size increased with temperature (12, 17, 22°C), and this positive association has been shown in a related species, *Hexagenia bilineata* (Say), half-grown larvae of which were obtained from field collections and reared to emergence (Wright and Mattice 1981*a*).

None of the variables examined influenced larval survival after 60 d of growth, suggesting that exogenous factors such as temperature and photoperiod do not influence larval survival during the early stages of development. We did observe a significant effect of temperature on larval survival, but only after 120 d; larvae reared at higher (22°C) temperatures had higher survival than larvae reared at lower (12 or 17°C) temperatures. Wright *et al.* (1982) also showed that the temperature at which larvae of *H. bilineata* were reared affected survival, with highest survival at about 22°C.

In contrast to their response to temperature changes, the response of invertebrate development to changes in photoperiod is variable. Whereas some species of terrestrial insects grow more slowly when exposed to short photoperiods than when exposed to long photoperiods, other species show the opposite response (Danks 1991). In aquatic insects, development of the larvae of some visual predators (dragonflies) was accelerated when they were exposed to a longer photoperiod (14 vs. 11 h of light) within a prescribed temperature regime (Lutz and Jenner 1964; Lutz 1968). We had anticipated that larval size and survivorship of collector–filterers (sensu Merritt and Cummins 1984) such as *Hexagenia* species would increase during dark conditions, owing to the reduced risk from visual predators during foraging (cf. Sweeney and Vannote 1981).

Mayfly larvae appear to be photonegative in both lentic and

TABLE 2. Summary of two-way ANOVA of the influence of temperature, photoperiod, and their interaction on larval size and survivorship after 120 d

	Size			Survivorship		
Variable	df	*F*	*P*	df	*F*	*P*
Temperature	2,53	102.556	<0.001	2,53	3.378	<0.05
Photoperiod			ns			ns
Interaction	4,53	3.194	<0.025	4,53	3.303	<0.05

NOTE: ns, not significant.

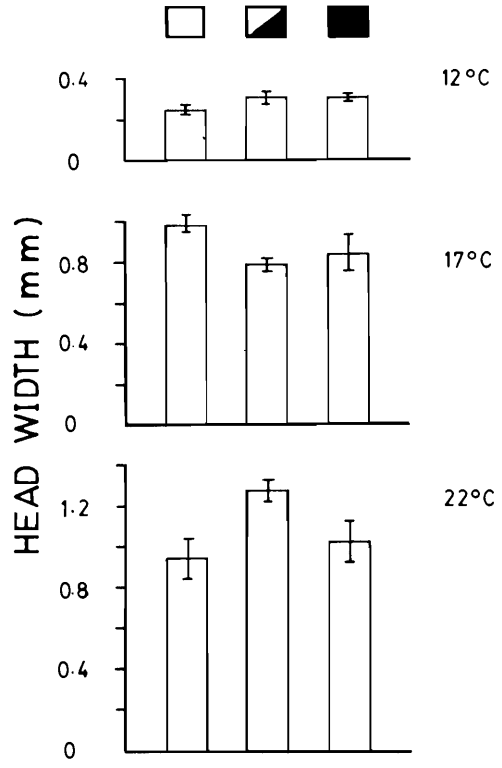

FIG. 3. Mean head width of larvae reared for 120 d at 12, 17, and 22°C for each photoperiod. The photoperiod regime is indicated at the top by open (24 h L), half-open (12 h L : 12 h D), and shaded (24 h D) bars.

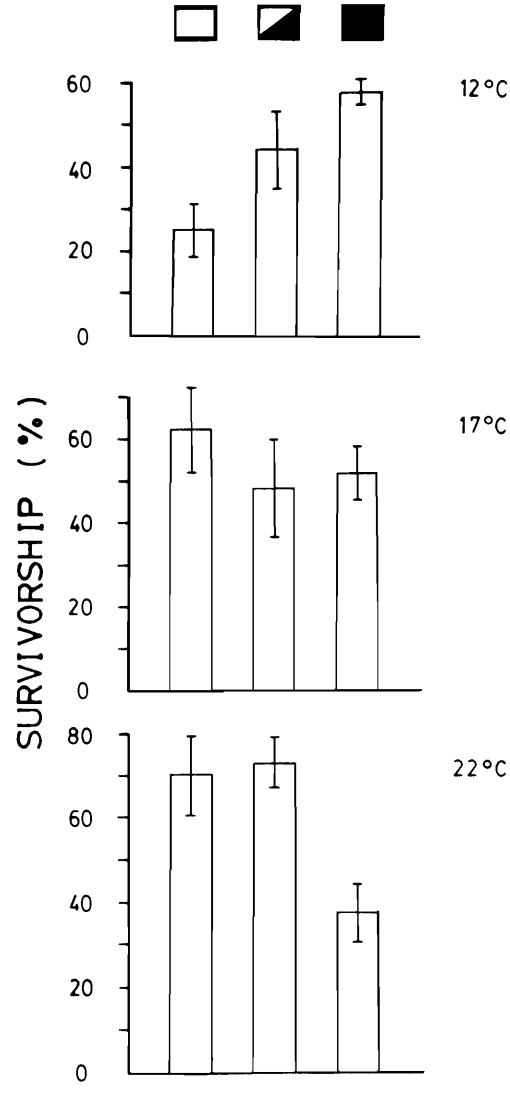

FIG. 4. Mean survivorship of larvae reared for 120 d at 12, 17, and 22°C for each photoperiod. The photoperiod is indicated at the top by open (24 h L), half-open (12 h L : 12 h D), and shaded (24 h D) bars.

lotic habitats. Larvae remain beneath the substrate during the day, but crawl to the upper surface or swim within the water column at night (Elliott 1968; Solem 1973; Corkum 1978*b*). People fishing in winter have observed larvae of *Hexagenia* species crawling on the lake bottom or swimming in the water column under the ice (during dark conditions) (Hunt 1953). In the laboratory, Hunt (1953) reported larvae with their heads near or at the mouth of burrows at night; they would retreat into their burrows with an increase in light intensity. However, Zimmerman *et al.* (1975) reported that larvae of *H. limbata* ingested and processed food under both constant light and constant dark conditions.

Our results indicated that there were no significant effects of photoperiod alone on larval size or survivorship after 60 or 120 d of growth (i.e., during the early stages of larval development). Since food was abundant, young larvae may have obtained sufficient food by feeding in their burrows, and additional foraging on the surface sediment (supposedly at night) would have been unnecessary. Alternatively, the actual presence of predators may be required to elicit a response by the larvae to leave their burrows at night to forage on the substrate surface. Perhaps the effects of photoperiod are not manifested until later in larval development (Corkum 1978*b*).

We demonstrated that although there was a significant interaction between temperature and photoperiod on both larval size and survivorship after 120 d of growth, no interaction effect was noted on larval size or survivorship after the shorter (60 d)

period of development. In the 120-d trial, survival appeared to decrease when the photoperiod differed dramatically from that expected on the basis of temperature. In the field, longer days are associated with high temperatures and shorter days with low temperatures. In our laboratory experiment, larval survivorship was lowest for treatments incorporating conflicting environmental cues (12°C and 24 h L, and 22°C and 24 h D).

Comparison of the replacement series of tanks for larval growth after 60 d showed that larval size increased (but with reduced survival) when the eggs had been subjected to a longer period of cold storage. Differential growth between replicate sets may have resulted from an increase in the quantity of food available to larvae in tanks in which survivorship was reduced. However, since in this study an unlimited supply of food was provided to larvae in all tanks, any effect of food quantity on larval growth in this experiment seems unlikely.

The only difference between the larvae used in the two 60-d replicate sets was the length of time the eggs remained in cold storage: those in the second set of replicates remained in cold storage for 75 days longer than those in the first set. These results suggest that, in field-collected samples, larger larvae might actually be younger than smaller larvae. Therefore, larval size does not necessarily reflect age.

If eggs are oviposited in microhabitats that are below the threshold temperature, hatching will be delayed. The temperature threshold for larvae of *H. limbata* to hatch is about 10°C (Hunt 1953; Heise *et al.* 1987); however, this threshold may vary with latitude and (or) altitude. In many aquatic insects the oviposition locations do not correspond to areas of larval abundance (Wright and Mattice 1981*b*). Typically, female imagoes of *Hexagenia* species oviposit on the water surface near shore. The oviposited eggs fall slowly through the water column and may be carried by currents or wave action. Eggs may be physically transported from warm nearshore areas to colder profundal regions shortly after oviposition, resulting in delayed hatching of eggs. Thus, the direct and delayed hatching of eggs may contribute to the asynchronous development of *Hexagenia* species.

Although egg diapause has been demonstrated for several species of mayflies (Bohle 1972) and for populations of *H. limbata* plus *H. rigida* in northern regions (D. J. Giberson, University of Manitoba, personal communication), the arrest in egg development of *Hexagenia* species in our study region merely represents a period of dormancy initiated by environmental cues (low temperatures, darkness) (see Butler 1984). In our region, most (90–95%) eggs of *H. limbata* and *H. rigida* hatch at room temperature with or without cold storage. Furthermore, subsequent recooling and warming of unhatched eggs does not result in any substantial hatch of larvae (<0.01%), suggesting that egg diapause does not occur in *Hexagenia* species in this region (Hanes *et al.* 1990).

The enhanced larval growth resulting from delayed hatching of eggs that we observed in this study may help to explain the complicated life cycle of *H. limbata* (and perhaps other aquatic insects) reported in the literature. Recently, Sweeney *et al.* (1991) have shown that eggs from the mayfly *Ephemerella septentrionalis* McDunnough obtained from the northern limit of its geographic range hatched later, yet emerged at the same time as populations from more southerly locations when the populations were reared under the same laboratory conditions.

Heise *et al.* (1987) detected seven overlapping cohorts of *H. limbata* larvae in Dauphin Lake, Manitoba, during a 2-year period. The life-history pattern of cohorts in which eggs apparently overwintered exhibited a shorter larval growth stage (15 months) than other cohorts (2 years) whose eggs apparently did not overwinter. Thus, the reduced time spent in larval development under field conditions may be explained by the faster growth of larvae hatched from eggs that are held at low temperatures for a period of time.

If mayflies have only a brief "window" of time in which to emerge, and since temperature and photoperiod are reliable indicators of season (Mousseau and Dingle 1991), these factors can be used to anticipate the time period over which emergence can be expected to occur. When Nebeker (1971) exposed field-collected larvae of *H. limbata* to elevated (16°C) water temperatures in the laboratory, adults emerged 5 months earlier than under natural conditions. In another laboratory study, McCafferty and Pereira (1984) reared half-grown larvae under four escalating thermal regimes (6–26, 12–26, 18–26, 24–26°C) and showed that mean maturation time decreased with increasing mean temperature. Nevertheless, emergence occurred over a range of temperatures. McCafferty and Pereira (1984) noted that although no critical temperature was required for emergence, a minimum number of degree-days was necessary.

Because of delayed hatching, variability in response of larvae to a suite of environmental cues, and variation among individuals in the development of adult tissues (Sweeney and Vannote 1981), it is not surprising that one finds large size variation among adult female mayflies (especially *Hexagenia* species). In mayflies with a long emergence period or a bivoltine life cycle, early-emerging females are larger and are more fecund (Brittain 1982). Ide (1940) showed that early-emerging imagoes of the mayfly *Paraleptophlebia mollis* (Eaton) tend to be larger and have higher fecundity (900 eggs per female) than those emerging 4–5 weeks later (300 eggs per female). The average fecundity of female imagoes of *H. limbata* ranges from 3500 to 4700 (Neave 1932; Hunt 1953; Clifford and Boerger 1974), larger females being more fecund (Hunt 1953). Eggs obtained from two imagoes of *H. rigida* numbered 1800 and 2400 (Neave 1932). Since the size of female imagoes of *H. limbata* and *H. rigida* is reduced over the length of the emergence span of about 4 weeks (E. C. Hanes, unpublished data), we anticipate that fecundity would be correspondingly reduced.

Owing to the relationship between adult size and larval size and survivorship, our study showed that the interaction of temperature and photoperiod (seasonal factors) driving larval growth has the potential to influence individual fitness. Within a lake, we expect larval size and survivorship to be more uniform in aerated profundal regions than in nearshore areas, where shading and basin shape contribute to patchy environmental conditions. The advantage of variation in size of female imagoes is unknown. However, we can speculate that a female imago has a greater chance of reproductive success if her offspring exhibit size variation, so that during the extended period of emergence, at least some larvae will emerge during optimal weather conditions. Thus, temperature and photoperiod may either stimulate or inhibit development, so that reproductive effort is not wasted as a result of mortality (Danks 1991).

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