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Effect of temperature and sex on growth patterns in nymphs of the mayfly *Hexagenia bilineata* in the laboratory

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SUMMARY. Eggs collected from *Hexagenia bilineata* females were successfully reared in the laboratory at temperatures of 15, 20, 25 and 30°C. Eggs did not hatch at 10°C and although hatching was successful at 35°C, all nymphs at this temperature died while in early instars.

Survival of nymphs between the approximate size interval of 4–14 mm showed a significant decrease with increased temperatures. Nymphs at 15°C, however, generally did not survive transformation to the subadult stage.

The growth pattern of individual nymphs was well described by a logistic curve at most temperatures. Furthermore, growth pattern was significantly affected by both temperature and sex.

Rate of development from oviposition to first emergence increased with increasing temperatures in a linear fashion between 15 and 30°C. The relationship was equally well described by a hyperbolic equation and a power-law equation. By extrapolation from the hyperbolic equation, the lower threshold temperature for development was estimated to be 10.1°C ± 3.1°C. The degree (°C)-days required for development from oviposition to first emergence was calculated to be 2337 days with 95% confidence limits of 2045–2727 days under laboratory conditions.

Introduction

With increased siting of energy-producing facilities on lakes, reservoirs and large rivers, thermally altered habitats may become more widespread and therefore of ecological concern. Burrowing mayflies of the genus *Hexagenia* are frequently abundant in these habitats and are an important food resource for many fishes (Hoopes, 1960; Wade, 1968). Before population responses of *Hexagenia* to thermal alterations of their habitat can be predicted, basic information on the developmental response of *Hexagenia* to temperature is required.

Although *Hexagenia* is a frequently investigated mayfly genus, quantitative analysis of effects of temperature on the larval growth pattern and on development time from oviposition to the adult stage has not been published for any species of *Hexagenia*. The relationship between embryonic development time and temperature has been quantified by Friesen, Flannagan & Lawrence (1979) for *Hexagenia rigida* (McDunnough) and by Tennessen & Miller (1978) for *Hexagenia bilineata* (Say). Other investigators have observed that higher than normal temperatures shorten nymphal development time of *Hexagenia* sp. (Fremling,

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1967; Hunt, 1953; Nebeker, 1971) but the observations were limited to one or two temperatures and the pattern of growth was not quantitatively described.

In fact, with few exceptions (Sweeney, 1978; Clifford, Hamilton & Killins, 1979), most quantitative studies of factors affecting growth of any mayfly species have been limited either to one stage (egg or nymph) or to a limited number of temperature regimes. Embryonic development rates for various mayfly species have been quantified as a function of temperature by several investigators (summarized by Humpesch & Elliott, 1980). Brittain (1976) reared nymphs from eggs hatched in the laboratory, but only quantified the relationship between body length increase and time after hatching at one temperature regime. Humpesch (1979) quantified both the growth pattern (length increase over time) and specific growth rate versus temperature for *Baetis* spp. but started with stage II and III nymphs collected from the field. Although both Sweeney (1978) and Clifford *et al.* (1979) reported on egg development and nymphal development in their respective papers, only Clifford *et al.* (1979) began nymphal growth studies with newly hatched nymphs.

Our study reports laboratory investigations of the effect of a range of constant temperatures on growth rate throughout the whole life cycle (from oviposition to emergence) and on nymphal growth pattern from time of hatch to emergence for *Hexagenia bilineata*. The relationship of these parameters to temperature are quantified in order to provide useful models for evaluating growth responses to either natural or modified temperature regimes. Additionally, we describe other observed responses to temperature such as egg development and hatching success, nymphal survival, emergence success and size at emergence.

Methods

Large numbers of *Hexagenia bilineata* eggs were collected on 28 July 1978, near Watts Bar Lake, Tennessee (35°52′N latitude, 84°30′W longitude), from females swarming to lights at *c.* 22.00 h. Females were dropped on to the surface of a tray of water where they extruded their eggs. Glass slides placed on the bottom of the trays provided a convenient substrate for attachment of the adhesive eggs. Since we were unable to return to the laboratory until the next day, eggs were kept at ambient temperatures (22°C) overnight (*c.* 14 h). They were then transferred to flow-through aquaria at controlled water temperatures of 10, 15, 20, 25, 30 and 35°C. Acclimation of the eggs was considered unnecessary, based on the results of Friesen *et al.* (1979). Temperatures were accurate to ± 0.5°C except during three short-term (< 12 h) laboratory waterflow disruptions, which primarily affected the 10 and 15°C regimes. During one disruption, the eggs at 10 and 15°C were moved to an environmental chamber which varied between 15 and 18°C for a 5-day period. Photoperiod for all temperature regimes was controlled by a photocell providing ambient daylight regimes for the latitude.

Egg development

Observations of egg development were conducted at each of the test temperatures, primarily to determine the approximate date of 50% hatch. One slide with eggs distributed in one layer was placed in a clear container for close observation at each temperature. A clump of 100 eggs was marked off and observed daily (except weekends) at 20, 25, 30 and 35°C and twice weekly at 15 and 10°C, under a dissecting microscope, to determine rate of development to hatching. Data recorded on each observation date included number of eggs hatched, unhatched and dead. Observations of eggs held at 15°C were terminated after 60 days, when more than 50% of the eggs had hatched and the remaining eggs were covered with diatoms which made it difficult to determine egg stage. For the same reason, observations of eggs held at 10°C were terminated on the 84th day after fertilization.

Nymphal growth pattern

Studies of development were conducted on nymphs hatching from the eggs collected at the same time as those used for egg development observations, but reared separately at test temperatures of 15, 20, 25 and 30°C. Several slides with hundreds of adherent eggs on each slide were placed on the surface of a mud substrate in 200-l aquaria at each test temperature. The mud was collected from sites in Watts Bar Lake which were known to have the type of substrate preferred by *H. bilineata*

(Wright & Mattice, 1981b); it was autoclaved wet at 128°C and 9.1-kg pressure for 1 min to kill any macroinvertebrates present. The starting date of the nymphal growth experiment was designated as the day on which 50% egg hatch occurred on slides used for the egg development observations. As nymphs grew to the size where burrows could be recognized, sections of mud were removed and all nymphs measured. Measurements for analysis of the growth pattern were not started until nymphs were larger than 4 mm because handling of smaller nymphs caused mortality.

Replicate containers for nymphal growth pattern observations were inoculated with nymphs when their mean size was 4–6 mm at each temperature. The size variability present was retained by removing small numbers at one time from the source population and distributing all nymphs removed among six replicates at each temperature to obtain a similar mean body length and variance in each container. Five nymphs held at 15°C were discarded because they were 6–8 mm larger than all of the others. Each replicate consisted of thirty nymphs in a small, round bucket filled to a depth of at least 8 cm with autoclaved and conditioned mud. The mud surface area was about 227 cm^2.

Nutrient enrichment of the substrates was accomplished by adding a high protein fish food [Staple Flake Food for Tropical Fish (Hartz Mountain Corporation, Harrison, NJ 07029) blended with water] to the mud surface, providing a layer of organic matter generally lasting for 2–7 days, depending on temperature. By visually inspecting the replicates about three times per week, the amount and frequency of food addition was gauged to maintain a visible layer of organic matter without producing fungal growth. These food additions together with substrate changes at the time of each measurement presumably provided excess food.

Data collected on each nymph included body length to the nearest 1 mm (from tip of head to tip of abdomen), sex (after 10-mm body length was reached), and stage of wing-pad development. All nymphs were removed from a container, anaesthetized with carbonated water, measured under a dissecting microscope, then returned to the container of fresh autoclaved substrate. Very few nymphs were killed directly during the measurement process, but subsequent mortality resulting from handling stress could not be determined. Frequency of measurement was variable, 2–3 weeks at 30°C, 3–4 weeks at 25 and 20°C, 3 months at 15°C. A total of five or six measurements was taken between the 4–6-mm stage and emergence at each temperature.

Emergence was determined by daily observation of the experimental replicates. A screened hole near the rim of the buckets allowed water flow-through while retaining nymphal exuviae. Observations were continued until all nymphs had emerged, except at 15°C when observations were terminated about 2 months after an initial emergence attempt had occurred in each replicate (475 days). Size at emergence was evaluated by measuring the body lengths of nymphs with large wing-pads. Although adult body length measurements would have been desirable, the adults frequently escaped before measurement could be made.

Development rate from oviposition to adult

The date of first emergence was noted for each replicate within each test temperature. All eggs used in these tests were collected on 28 July. Using the time interval between oviposition and first emergence in all replicates at each temperature, the relationship of development time to temperature was evaluated. Both a power-law equation and a hyperbolic equation were fitted to the data.

Results

Egg development

Eggs hatched at all test temperatures between 15 and 35°C within 60 days. At 60 days only very slight development could be seen in eggs at 10°C and observations were complicated by growth of diatoms on the eggs. However, 84 days after oviposition some eggs at 10°C appeared empty as though hatching had occurred, even though late embryonic stages were never observed. Due to uncertainty in interpretation of this observation and the fact that 10°C eggs were held at 15–18°C for a period of 5 days (see Methods), no conclusions about development at 10°C could be made.

Days after oviposition at which first hatch, *c.* 50% hatch and last hatch were recorded are given in Table 1. Some of the days were

estimated by extrapolation because we did not make weekend observations at 20, 25 and 30°C or frequent observations at 15°C. The extrapolated days (enclosed within parentheses in Table 1) are probably correct within 1 day at test temperatures of 20, 25 and 30°C and correct within 2 days at 15°C.

By the date of last observed hatch, nearly 100% of the eggs had hatched at temperatures of 20, 25 and 30°C. At 35°C only 85% of the eggs hatched, the rest appeared to be dead. Total percentage hatch at 15°C was undetermined, due to discontinuance of observations after *c.* 60 days; however, very few dead eggs (brown or deteriorating) were noted at that time.

Nymphal survival and size at emergence

Nymphs successfully emerged at 20, 25 and 30°C. Emergence was attempted at 15°C; however, the sub-imagos were rarely able to successfully escape the nymphal skin. Those which did escape never left the water surface and died as sub-imagos.

Nymph survival decreased with increasing temperature (Table 2). Nymphs at 35°C apparently did not survive past the first few instars as burrow formation was never observed. Comparison of survival among temperatures was based on the survival of nymphs between the approximate mean lengths of 4 and 14 mm. This growth interval, rather than a time interval, was chosen for comparison of survival to reduce the effect of differential mortality resulting from increased moult mortality at higher temperatures (Clifford *et al.*, 1979). During this growth interval (4–14 mm), one length measurement was made of nymphs at 30°C and two measurements of nymphs at 25, 20 and 15°C. Thus, mortality due to handling should not have biased the results.

TABLE 1. Days to first hatch, 50% hatch, and last hatch for about 100 *Hexagenia bilineata* eggs reared at five test temperatures

	Temperature (°C)				
Egg hatch	15	20	25	30	35
First hatch	(46)*	(21)	10	(7)	(7)
50% hatch	(60)	24	10	(7)	9
Last hatch	?	30	12	9	11

*Values in parentheses were estimated by extrapolation between two observation dates.

TABLE 2. Percentage survival ± 95% CL of nymphs at different temperatures

Temperature (°C)	Size interval (mm)	Initial number	Survival (%)
15	5.9–14.8	180	80 ± 8
20	4.0–12.4	180	65 ± 22
25	4.0–13.7	180	43 ± 27
30	5.5–12.1	180	21 ± 13

Survival was assumed to be equivalent for both sexes at all temperatures, and sex ratio was assumed to be initially close to 50:50 at all temperatures. In fact, at the point when the sex of all surviving individuals could first be unequivocally identified, the mean proportion of males at 15, 20, 25 and 30°C was 31, 48, 44 and 44%, respectively. Although at all temperatures the proportion of males was less than 50%, only the low percentage at 15°C differed significantly from a 50:50 ratio ($P < 0.05$). This suggests a possible interaction between sex and temperature on survival, but no further sex-dependent mortality was noted after sex was identified. Other explanations for the skewed sex ratio are possible, such as parthenogenetic development (Humpesch, 1980; Friesen & Flannagan, 1976). However, eggs were collected from many females and randomly distributed among test temperatures, reducing the possibility that any single rearing temperature would have received unfertilized eggs.

Nymph size at the large wing-pad (LWP) stage was differentially affected by temperature for males and females (see Table 3 and the 'measured data' in Fig. 2). Among females, body length at the LWP stage showed a significant linear increase with temperature from 15 to 25°C ($P < 0.05, r^2 = 0.30$). With addition of the 30°C data the relationship between size and temperature in females becomes non-linear (see Fig. 2).

TABLE 3. Mean body length ± 95% CL (mm) of nymphs at the large wing-pad stage of development

	Males		Females	
Temperature (°C)	No.	Mean	No.	Mean
15	14	21.8 ± 0.9	21	24.0 ± 0.9
20	12	20.0 ± 0.7	5	25.0 ± 1.8
25	10	20.8 ± 1.3	7	26.7 ± 1.3
30	2*	18.7	2*	19.5

*Measurements are of cast skins.

Linear regression of body length on temperature for the males was not significant ($P > 0.05$) between 15 and 25°C. However, addition of the 30°C data does result in a significant linear decrease in body length with temperature ($P < 0.05$, $r^2 = 0.09$). The very low r^2 value indicates there is considerable variation in the male data which is not explained by the linear relationship. The 30°C data are important for interpretation of trends based on the measured lengths, because without this information one might infer that length is not affected by temperature for males and that length increases with increasing temperatures for females. Unfortunately, the mean lengths at 30°C were based on only four measurements of last instar nymphal exuviae (two male and two female). However, the small body lengths of the four 30°C exuviae are consistent with other observations we have made on *Hexagenia* nymphs reared at high temperatures (Wright & Mattice, 1981a).

Nymph growth pattern

Growth rate, measured by increase in body length over time, appears to follow a similar pattern for both males and females at all test temperatures (Fig. 1). A logistic model was found to provide a better fit than linear or exponential models at all test temperatures, based on comparison of the residual sums of squares. The logistic model used here was of the general form:

$$L(t) = \frac{L_0 L_m e^{Bt}}{(L_m - L_0) + L_0 e^{Bt}} \qquad (1)$$

where $L(t)$ is the mean body length at time t since 50% hatch (i.e. the observed data). The parameters L_0, L_m and B are associated with initial length, maximum length and rate (day^{-1}) of length increase. To simplify calculations, L_0 was fixed at 0.9 mm based on our own measurements of nymphs at hatch and on data reported by Hunt (1953). The remaining parameters, L_m and B, were estimated using non-linear least squares methods.

The regression of mean body length against time using the logistic model, was performed separately for each surviving replicate thereby generating five or six estimates of L_m (maximum length) and B (rate of increase) within each temperature–sex combination (Table 4). Some of the predicted values at 30°C were unreasonable (e.g. 224.4, 46.9 and 34.5 mm) and thus were excluded from further analysis. Estimates of L_m were accepted as 'reasonable' if they were between the lengths of 14 and 33 mm, since this was the maximum range of lengths found for *H. bilineata* nymphs in Watts Bar Lake and in the Mississippi (Jergens, 1965). The unreasonable estimates at 30°C were probably due to inadequate data; accidental mortality (resulting from waterflow disruption) and natural mortality eliminated most nymphs in the 30°C replicates well before maturity.

The arithmetic mean value of maximum body length (L_m) and rate of body length increase (B) for each temperature–sex combination (Table 4) was used to generate the curves superimposed on the data in Fig. 1. The dashed portions of the curves represent the extrapolation beyond the observations to the day when 99% of the maximum length (L_m) would be attained. Comparison of the predicted maximum body length (L_m) values with the measured lengths of nymphs in the large wing-pad stage (assumed to be at maximum nymphal length) provides support for the logistic model (Fig. 2). The predicted and measured values of maximum length do not differ significantly ($P > 0.05$), except for the 30°C female group. The comparison at 30°C is very tenuous as exuviae—not large wing-pad nymphs—were measured and only two were available.

Further statistical analysis of the effects of temperature and sex on nymphal growth was performed by using the L_m and B parameters estimated from each replicate (Table 4). Due to the inherent correlation between L_m and B values, some type of multivariate technique was considered to be most appropriate. Canonical analysis provides a good visual representation of the mutual position and orientations of the populations specified by the different temperature–sex combinations or treatment groups. If

$$\boldsymbol{x} = (\hat{B}, \hat{L}_m) \qquad (2)$$

represents the 2×1 observation vector from a replicate for a particular temperature–sex combination, where $\hat{B}$ and $\hat{L}_m$ refer to the estimates of B and L_m, respectively, a transformation:

$$\boldsymbol{y} = \boldsymbol{C}\boldsymbol{x} \qquad (3)$$

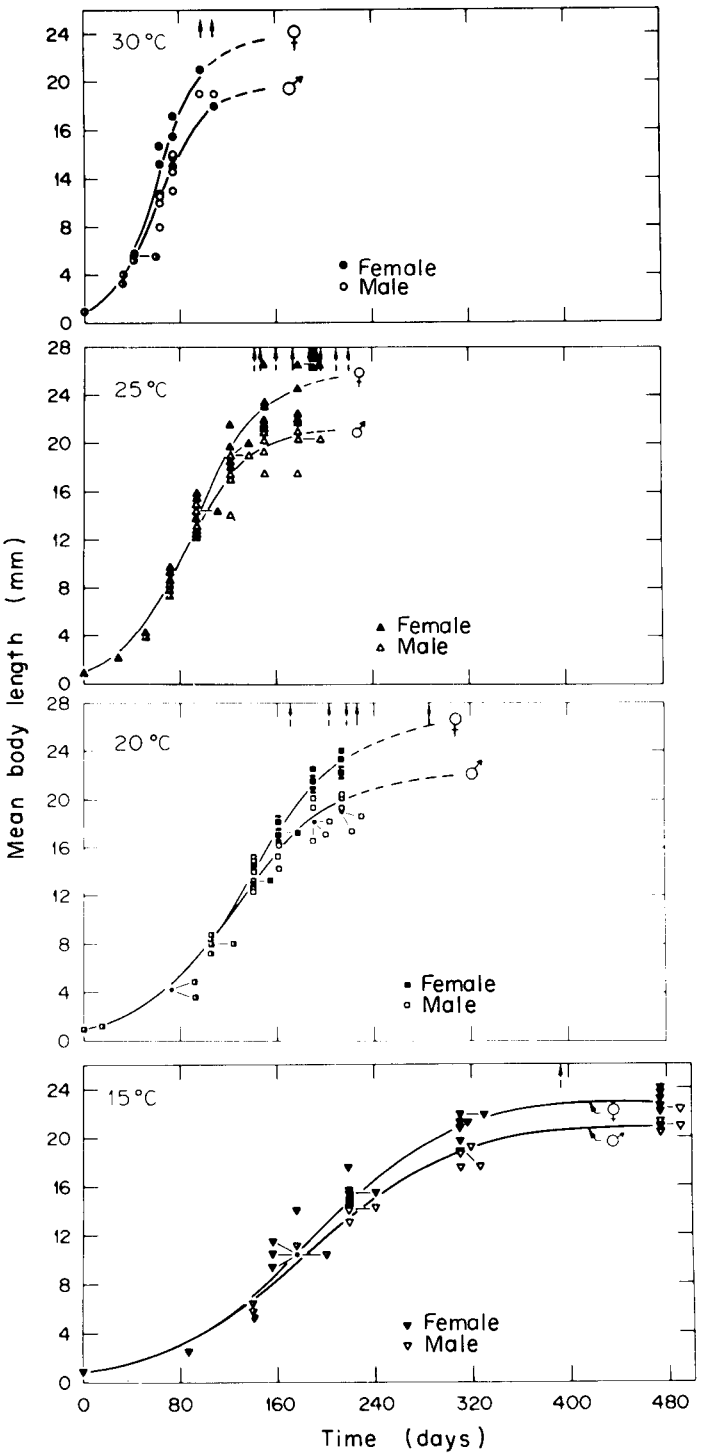

FIG. 1. Change in body length as a function of time. Points indicate mean body length (mm) of each replicate. Curves superimposed on the data for each temperature–sex combination were predictions (eqn (1)) based on the mean L_m and B values shown in Table 4 for each group. Broken arrows along the top of each graph indicate a single emerging sub-adult; solid arrows indicate that two or more sub-adults emerged on that day.

TABLE 4. Values for maximum body length (L_m, mm) and rate of increase (B, day^{-1}) estimated for each replicate and mean L_m and B values estimated for each combination of temperature and sex

	15°C		20°C		25°C		30°C*	
Replicate	B	L_m	B	L_m	B	L_m	B	L_m
1	0.0173	21.1	—	—	0.0369	21.1	0.0482	20.3
2	0.0167	20.9	0.0261	22.8	0.0372	18.1	0.0497	22.0
3	0.0166	21.1	0.0272	21.3	0.0390	22.5	—	—
4	0.0163	22.3	0.0233	23.6	—	—	0.0490	15.1
5	—	—†	0.0252	21.7	0.0405	22.5	0.0376	224.4‡
6	0.0171	21.1	0.0246	21.8	0.0365	21.7	0.0504	21.4
Mean	0.0168	21.2	0.0253	22.1	0.0384	21.1	0.0493	20.8
1	0.0171	23.7	—	—	0.0374	23.0	0.0471	22.5
2	0.0168	22.5	0.0259	25.4	0.0351	27.5	0.0457	46.9‡
3	0.0168	24.6	0.0233	29.9	0.0378	28.2	0.0529	28.6
4	0.0166	23.9	0.0243	27.2	—	—	0.0532	34.5‡
5	0.0191	23.0	0.0254	25.5	0.0381	25.2	—	—
6	0.0172	23.5	0.0238	27.0	0.0354	23.8	0.0565	20.8
Mean	0.0172	23.6	0.0246	26.8	0.0367	25.5	0.0522	24.0

* Only one replicate at 30°C contained nymphs surviving past the mean length of 13 mm. Mortality was due to an accidental water-flow disruption.

† All nymphs died in replicate early in the experiment.

‡ These values were excluded from calculation of the mean and other statistics because they were not within 'reasonable' limits as explained in the text.

can be derived to emphasize the differences between the means of the different treatment groups, where C represents a 2 × 2 transformation matrix determined from the observed data. The two new variables contained within the transformation vector, y, are the canonical variables. The first component of the transformed vector, y, is derived to show maximum separation between the treatment means (i.e. the greatest variability among the treatment means). The second component of y is derived to be orthogonal to the first and to show the next greatest separation among the treatment groups. Because we have only two canonical axes, a plot of the variables makes it possible to visually determine the mutual positions or orientations of the treatment groups.

Figure 3 shows a distance separation of the temperature levels, especially along the first canonical axis. Circles around each point represent the approximate 95% confidence regions to give a rough indication of the relative uncertainty of each mean. Sex differences represented by the second canonical axis appear to be significant at 20, 25 and 30°C, but the differences are not as evident as those for temperature. Even though the canonical variables can be used for statistical comparisons between the groups, the physical interpretation of these variates is difficult because they are linear combinations of the parameters L_m and B in the logistic model [eqn (1)]. For complete explanations of this analysis, see Seal (1964).

The more commonly used multivariate technique, multivariate analysis of variance, also demonstrated significant temperature ($P = 0.0001$) and sex effects ($P = 0.0001$), but no significant temperature and sex interaction ($P = 0.18$).

Development rate

Time to first emergence (D) and rate of development ($1/D$) varied as functions of temperature (T) (Figs 4 and 5). Both the linear form of the power-law

$$\log_e D = \log_e a - b \log T$$

and the hyperbolic equation ($1/D = a + bT$) describe the data equally well; both are highly significant ($P < 0.0001$). Time to first emergence versus temperature was described by the power-law equation as

$$\log_e D = 11.5928 - 2.0247 \log_e T$$

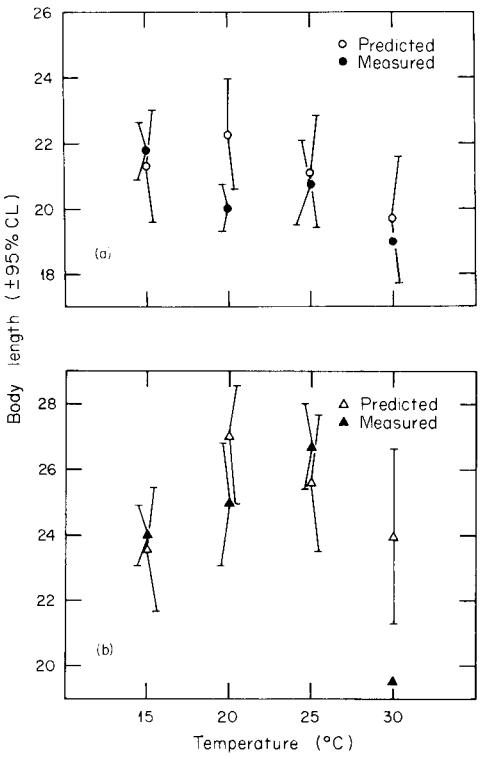

FIG. 2. Comparison of predicted L_m values (O, △) and measured values (●, ▲) of length (and their 95% confidence limits), in mm, at the large wing-pad stage of development for both male (a) and female (b) *Hexagenia bilineata*. At 30°C, only two values were obtained.

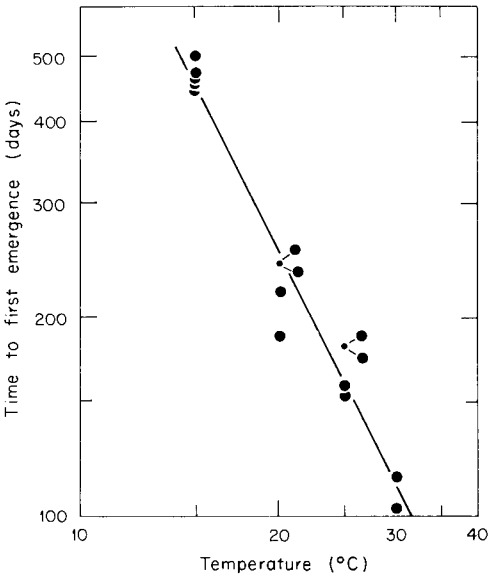

FIG. 4. Regression of days to first emergence (D) on temperature (T) using the linear form of the power law ($\log_e D = \log_e a - b \log_e T$). Points (●) indicate the mean value for each group.

(Fig. 4). The proportion (r^2) of the variance of D explained by regression of D on T was 0.96. Development rate versus temperature was described by the hyperbolic equation as $1/D = -0.004324 + 0.000428T$ and the corresponding r^2 was 0.94 (Fig. 5).

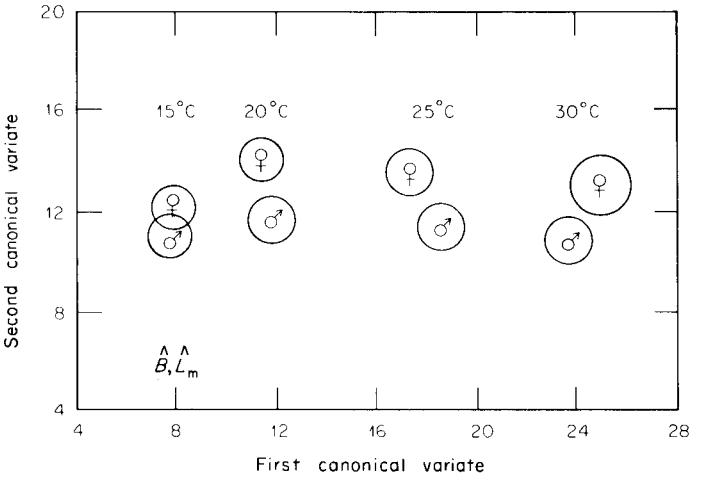

FIG. 3. Plot of the canonical variables derived from the observation vectors, ($\hat{B}$, $\hat{L}_m$) for each temperature–sex combination. Each circle centre represents the mean of the observation vectors and the circles around each point represent the approximate 95% confidence regions. Further explanation in the text.

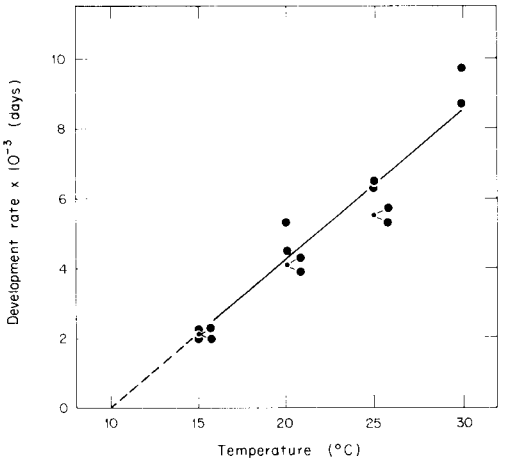

FIG. 5. Regression of development rate ($1/D$) on temperature (T) using the linear form of the hyperbolic equation ($1/D = a + bT$). Points (●) indicate the mean value for each group.

Lack-of-fit tests (Draper & Smith, 1966) also did not differentiate between the two models, indicating for both a significant lack of fit at the 0.05 level but a non-significant lack of fit at the 0.01 level. Thus, in both cases there is a significant amount of variation that is not explained by the regression of D and $1/D$ on temperature.

Based on the statistical comparisons, it is not possible to choose one model as a better fit to the data, but the hyperbolic model has advantages for predictive purposes. The total number of degree days required for development can be estimated by taking the reciprocal of the regression coefficient (b), and the theoretical threshold temperature (i.e. the temperature at which development rate equals zero) can be estimated by extrapolation of the regression line. Based on our data, the degree days (with 95% CL) required for development of *Hexagenia bilineata* from oviposition to first emergence is 2337 (2045–2727)°C days above a threshold temperature (with 95% CL) of 10.1 (7.0–13.2)°C.

Although the variability in our data produced wide 95% confidence limits on our estimates of degree days required for development, the mean value appears to agree with field data reasonably well. Observations from the intake channel of the Kingston steam plant on Watts Bar Lake, TN (35°52′N latitude, 84°30′W longitude) indicated that first emergences of *H. bilineata* in the intake channel occurred on 9 June 1976 (Mattice & Dye, 1978), 23 June 1977 and 19 June 1978. Using weekly mean temperatures from records supplied by the Tennessee Valley Authority, we estimated that the degree-days (°C) above 10°C between first emergences in 1976, 1977 and 1978 were 2312 days for 1976–77 and 2293 days for 1977–78. Since water temperatures are normally between 22 and 25°C at the time of the first major emergence (late June, early July), the laboratory estimate of 2337 mean degree-days represents a difference from the field observations of only 2–5 calendar days.

Discussion

The number of days to first egg hatch for *Hexagenia bilineata* showed a similar pattern with respect to temperature as that found for *Hexagenia rigida* by Friesen *et al.* (1979) and for *H. bilineata* by Tennessen & Miller (1978). Our unreplicated egg development results are not entirely within the 95% confidence limits of the curve predicted by Tennessen & Miller (1978) from their observation of five replicates of *H. bilineata* eggs. The range of variation was not reported by Friesen *et al.* (1979), but it appears that our data agree more closely with the results of Tennessen & Miller (1978).

The logistic model predicted that a temperature of 20°C would result in the largest nymphs. This conformed to our expectation since the results of a previous experiment suggested that the optimal constant temperature for growth would be between 15 and 22.5°C (Wright & Mattice, 1981a). According to Vannote & Sweeney (1980), nymphal body length should be maximized under an optimal temperature regime. It is therefore somewhat unsatisfactory that the mean body lengths of the measured nymphs at the large-wing-pad stage did not closely follow the same trend as the predicted mean lengths. The 95% confidence intervals of the predicted and measured lengths overlap in all cases, however, except at 30°C for females (see Fig. 2). Thus, the observed data, at least, do not statistically contradict the predictions.

At 30°C the size of emerging nymphs was considerably smaller than at lower temperatures. Although our observed estimate of size at

emergence is based on only four nymphal exuviae of emerging sub-adults, we think they were representative, because previous growth experiments also produced small adults at 30°C (Wright & Mattice, 1981a).

A linear relationship between temperature and development rate of both eggs and nymphs is valid only over a limited range of temperatures (Davidson, 1944; Andrewartha & Birch, 1954). However, the linear temperature-summation or degree-day concept has proven useful for predicting the first emergences of many terrestrial insects (Gaylor & Sterling, 1975; Reissig *et al.*, 1979; Aliniazee, 1975; Hammond, Posten & Pedigo, 1979; Howe, 1967). Howe (1967) notes that predictions based on a linear relationship are fortuitously accurate for many insects if the predicted lower temperature threshold is interpreted as the minimum for completion of a stage. Humpesch & Elliott (1980) reported that linear equations (either the power-law or hyperbola) were successful in relating egg hatching to temperature in the laboratory for twelve species of mayflies and for six species the adequacy of the equations had also been proven in the field.

The logistic growth pattern displayed by *Hexagenia* at laboratory temperatures is the expected growth pattern for most animals when the whole growth phase is considered (Sutcliffe *et al.*, 1981; Weatherley, 1972). However, laboratory investigations of other mayfly species have described body length increase with time using an exponential model (Clifford *et al.*, 1979; Humpesch, 1979; Brittain, 1976) or a linear model (Hunt, 1953). The differences in growth models for mayflies are most likely a result of differences in the methods of data collection. Since the growth pattern of *Hexagenia* was exponential over a major portion of the growth phase (see Fig. 1) it was only by obtaining measurements near the date of emergence that the logistic pattern of growth became evident, especially at higher temperatures. Hunt (1953) obtained too few measurements to demonstrate the logistic pattern and it is probable that the final measurements of Humpesch (1979) and Brittain (1976) were not sufficiently close to the emergence date. Clifford *et al.* (1979) obtained measurements at each instar prior to emergence; however, the exponential pattern displayed by their data may be a result of performing the growth experiment at temperatures above those characteristic of the normal environment for *Leptophlebia cupida*. Vannote & Sweeney (1980) hypothesized that at temperatures above optimum, adult tissue maturation begins early and proceeds rapidly, thus reducing the time available for nymphal growth. Consequently, the logistic character of growth at high temperatures may be obscured by the compressed period of adult maturation. This is supported by our data which show a much stronger logistic pattern at the lower temperatures. Another aspect which emphasized the logistic growth pattern is that our data show population growth rather than individual growth. Measurements on each group were continued through the emergence period when the nymphs emerging were presumably the largest while the smaller nymphs were still growing, thus the mean size tended to remain the same.

Our laboratory studies on the response of *Hexagenia bilineata* to a range of constant temperatures should be useful for predicting the effects of temperature modification of the natural environment. The accuracy of the degree (°C)-day prediction for determining data of first emergence can best be determined by additional field observation. Research into the possible interaction effects of light regime, food and temperature would probably help to explain some of the variance observed in the data.

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