Effects of Temperature on Adult Size and Emergence Success of *Hexagenia bilineata* Under Laboratory Conditions

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ABSTRACT

The simultaneous effect of sex, initial size, and temperature on growth rates, adult size, and size at large wing-pad (LWP) stage of *Hexagenia bilineata* (Say) mayflies was investigated. We found that female nymphs grew faster than males; that growth at the constant temperature of 15°C was significantly less than at 22.5 and 30.0°C; and that smaller nymphs tended to grow faster than larger nymphs over a period of 49 days. Size of nymphs at the LWP stage was significantly related to sex, with females being considerably larger. Within the same initial size groups, all nymphs reared at 30°C were significantly smaller at the LWP stage than nymphs reared at 22.5°C and 15°C. Metamorphosis was not successful at 15°C. Adults reared at 30°C were significantly smaller than those reared at 22.5°C. The nearly synchronous emergence times of nymphs initially in the 13.5-mm and 17-mm size classes at both 22.5 and 30°C, suggested that adult tissue maturation was initiated almost simultaneously in nymphs of those two sizes. The lag in emergence of nymphs at the initial size of 9 mm suggested that a size-dependent threshold for adult tissue maturation exists between 9 and 13.5 mm body length. Finally, while 15°C was too low to permit successful emergence, it did not inhibit adult tissue maturation.

INTRODUCTION

Hexagenia bilineata (Say) is a large burrowing mayfly commonly found in large rivers, lakes, and reservoirs. The periodic emergences of *H. bilineata* adults throughout the summer provide excellent fishing conditions as many "pan" fish take advantage of the plentiful food resource. Nymphs are also consumed throughout the year by bottom feeding fishes and are relatively important as a fish food organism. Knowledge of the response of *H. bilineata* to thermal alterations of aquatic habitats, such as may be caused by steam-electric or hydroelectric power generation, may be important in predicting impacts of these technologies on reservoir systems.

The thermal equilibrium hypothesis recently published by Vannote and Sweeney (1980) proposes that the stability of subpopulations of aquatic insects reflects a dynamic equilibrium among temperature and individual growth, metabolism, reproductive potential, and generation time. Thus they hypothesize that

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under an "optimum" thermal regime or "equilibrium" location, individual weight and fecundity is maximized and coincides with the location of greatest sub-population biomass. The data used for supporting the hypothesis showed differences in adult size resulting from temperature differences less than 5°C. If their hypothesis is generally applicable, we should expect to observe a difference in adult size in H. bilineata mayflies emerging from two coves differing in average temperature by ~5°C year round. Our studies show that these coves do have significant differences in population densities (Auerbach et al. 1977) and slight differences in emergence timing (Mattice and Dye 1978); however, no difference in adult size has been observed (Auerbach et al. 1977). Since field observations are frequently affected by many unknown factors, laboratory experiments were conducted to ascertain the responses of H. bilineata to temperature differences.

The information reported in this paper results from laboratory studies initiated with 9-mm or larger nymphs, with particular emphasis being placed on observing the effects of constant temperature, sex, and initial size on adult size, size at large wing-pad stage, and emergence success. Data on growth rates and survival and some observations on fecundity were also obtained. This information was not collected with the specific intent of testing the thermal equilibrium hypothesis of Vannote and Sweeney (1980). However, the information obtained can provide an indication as to whether the temperature responses of Hexagenia bilineata are consistent with that hypothesis.

METHODS AND MATERIALS

Mayfly nymphs and their preferred substrate type (Wright and Mattice, in press) were collected from Watts Bar Lake, Roane County, TN (35° 52' N latitude, 84° 30'W longitude) in mid-April 1978. The substrate was autoclaved for approximately 1 min at 126°C which we found was sufficient to kill all macro- benthos. Equal amounts of substrate were added to 18 small (~19 liters) aquaria and allowed to settle for one week. The mayflies were held in a large mud-filled tank in the laboratory at approximately 15°C for one week prior to distribution to the experimental aquaria. The experimental design was a replicated 3 x 3 block design with variables of temperature and initial size. Sex of mayflies was also considered. Similar temperature for each of the three initial sizes was assured by placing three of the experimental (19 liter) aquaria inside a large tank which served as a water bath. Two water baths were established at each temperature (15, 22.5 and 30°C).

Mayflies were measured, sexed, and separated into three size classes with a mean and range of 9 ± 1 mm, 13.5 ± 0.5 mm, and 17 ± 1 mm. Nymphs in each size class were separated into six groups (20 mayflies each) of equal size distribution. Two groups from each size class were then randomly distributed among the temperature classes. Sex ratios were equalized among the six groups within the size classes of 13.5 mm and 17 mm; this division was not possible for the 9-mm size classes because sexual characteristics had not developed. Initial composition of mayflies in each experimental group is summarized in Table 1. The orginial numbers of each sex were estimated for most of the 9-mm size class by assuming that mortality was the same for both sexes. The actual numbers present when sex was first determined for mayflies of this group are also shown in Table 1.

All nymphs were measured every 2 to 3 weeks until the majority of nymphs had emerged. At 22.5 and 30°C the observation period lasted for 49 to 62 days, but at 15°C measurements were continued for as long as 151 days. Measurements of nymphs were made after anesthetization with carbonated water. This procedure resulted in very few mortalities and quick recovery. Nymphs were then returned to the aquaria in which fresh autoclaved mud had been placed. At the time of measurement, nymphs reaching the large wing-pad stage were noted. This stage was easily identified because during a late instar molt, the wing-pad at least doubled in length. After the large-wing-pad (LWP) stage was reached, little or no increase in body length occurred; however, the last measurement before death or emergence was used for analysis.

Food was generally added twice per week, with amounts being higher at 30°C and lower at 15°C. Staple Flakefood for Tropical Fish[a] was mixed with water in a blender and poured into the

Table 1. Initial number of nymphs within each temperature-size-sex group.

		Temperature					
		15°C		22.5°C		30°C	
Initial Size[a]	Sex	A	B	A	B	A	B
S	♂	13(13)[b]	11(9)	14(10)	8(7)	10(6)	15(15)
	♀	7(7)	9(7)	6(3)	12(11)	10(5)	5(5)
M	♂	10	10	10	10	10	10
	♀	10	10	10	10	10	10
L	♂	7	7	7	7	7	7
	♀	13	13	13	13	13	13

[a] S = mean size of 9.1 mm; range = 8-10 mm nymphs
M = mean size of 13.5 mm; range = 13-14 mm nymphs
L = mean size of 17.0 mm; range = 16-18 mm nymphs

[b] Numbers in parenthesis are actual numbers sexed after start of experiment. Mortality was assumed to be the same for males and females in order to estimate initial numbers of each sex.

tanks, providing a layer of organic matter on the surface which generally was visible for 2 to 3 days. Information recently published by Zimmerman and Wissing (1980) suggests that Hexagenia normally feed on freshly deposited detritus and that the microbal biomass in the detritus may be important as food. Since the Flakefood was composed of approximately 53% crude protein, it probably promoted rapid growth of bacteria. Our previous experience indicated that addition of too much food stimulated growth of a fungus layer over the substrate surface. Thus, amount and frequency of food addition was varied to provide the maximum amount possible without stimulating fungal growth. Although our procedures were subjective, we believe that food additions plus substrate changes every two to three weeks were sufficient to provide an excess of available food.

Temperature increases of 2 to 5°C occurred twice in all tanks as a result of water flow interruptions in the laboratory. These fluctuations were less than 24 hr in duration. Normally, temperature was controlled within ± 1°C. Nymphs were exposed to the natural photoperiod occurring from mid-April through August since laboratory lights were controlled by a photocell.

Emerging adults were captured by covering each large water bath, containing the three size classes of nymphs in separate aquaria, with cheesecloth. Numbers of mayflies emerging from each size class could be determined because the cast exuviae remained floating on the water within each aquarium. Adults, however, were usually attached to the cheesecloth and could not be identified with an individual size class unless mayflies emerged from only one aquarium. Both exuviae and adults (subimagos and imagos) were preserved in 70% propanol and measured at a later date.

Growth rate for each replicate was determined by weighted regression of mean nymph length against time. The time period for comparison of growth rates was arbitrarily set at 49 days, because the first emergence occurred shortly before that time and because nymphs in the 9-mm and 13.5-mm initial size classes had grown to or beyond the initial size of the next larger group. Three-way analysis-of-variance (ANOVA) was used to examine the effects of initial size, temperature, sex, and all possible interactions on growth rate, size at large wing-pad stage, and nymph survival to emergence. ANOVA was also used to help evaluate effects of temperature and sex on adult size and survival.

RESULTS

Growth of H. bilineata nymphs was reasonably described by a linear function at all temperature-sex-size combinations for the

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[a] Hartz Mountain Corporation, Harrison, New Jersey 07029.

49-day period (Fig. 1). The coefficient of determination (r^2) of all but 3 of 36 regressions of length against day was 0.90 or greater and the lowest r^2 value was 0.81. The slope was significant for all replicates; of 36 replicates, 28 were significant at $P < 0.01$ and all at $P < 0.05$.

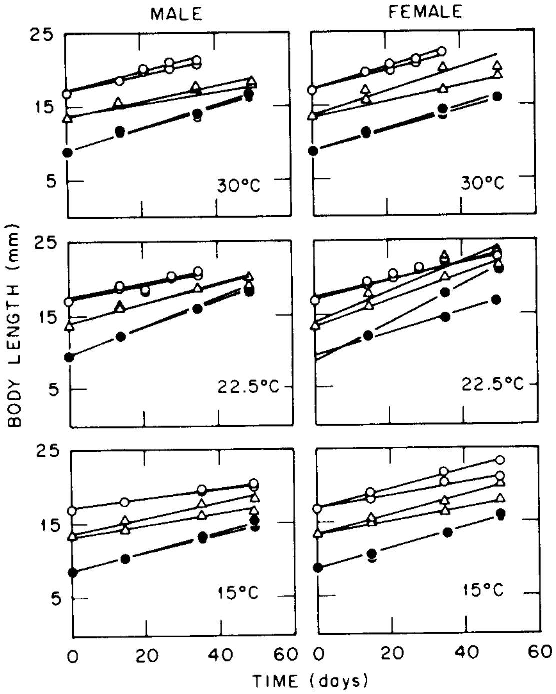

Fig. 1. The regression of body length (●—● small, △—△ medium, ○—○ large initial size) against time. Two groups were run at each size class, temperature and sex combination.

Growth rate estimates (equal to regression slopes) were significantly affected by the main effects of temperature, size, and sex ($P < 0.01$, Table 2). There also was an indication of a significant size-temperature interaction ($P = 0.0623$). All other interactions were not significant ($P > 0.25$) (Table 2). The pattern of temperature and initial size effects on growth rate was similar for both sexes (Fig. 2). Duncan's multiple range test indicated that growth rate of the smallest size group (9 mm) differed significantly from that of the two larger size groups ($P < 0.05$). However, at 30°C the data did not demonstrate that trend very well, perhaps contributing to the suggested interaction between temperature and size. Duncan's test separated the 15°C temperature effect from the other two temperatures, and male and female growth rates were also shown to differ significantly ($P < 0.05$).

Table 2. Results of analysis-of-variance testing for the effect of size, temperature, sex, and all possible interactions on growth rate and length at large wing-pad (LWP) stage, and for the effect of temperature and sex on length of adults.

Source of Variation	$P > F^a$		
	Growth rate	Length LWP stage	Length of adults
SIZE	0.0006	0.0001	----
TEMP	0.0016	0.0001	0.0001
SEX	0.0066	0.0001	0.0001
SIZE - TEMP	0.0623	0.0006	----
SIZE - SEX	0.3375	0.9889	----
TEMP - SEX	0.7005	0.0344	0.20
SIZE - TEMP - SEX	0.8254	0.7139	----

[a] Probability of a greater F value.

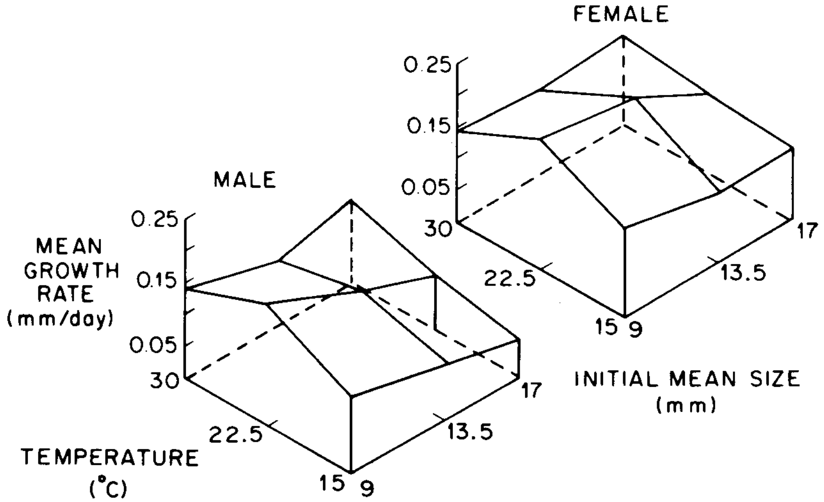

Fig. 2. The mean growth rate (equivalent to the mean slope of the two regressions of body length against time within each sex, size, and temperature combination), displayed for each sex separately as a function of both temperature and initial size on a three-dimensional scale.

Size of nymphs at the large-wing-pad (LWP) stage was affected by interactions between both temperature-size and temperature-sex ($P < 0.05$), as well as by all three individual variables ($P < 0.001$) (Table 2, Fig. 3). Female body lengths were consistently larger than male body lengths at the LWP stage (Table 3). Length at the LWP stage was positively correlated with initial mean size at all three temperatures; but at 15 and 22.5°C mean differences between the smallest and largest size classes were only 1 to 2-mm, whereas at 30°C the mean difference was about 4-mm.

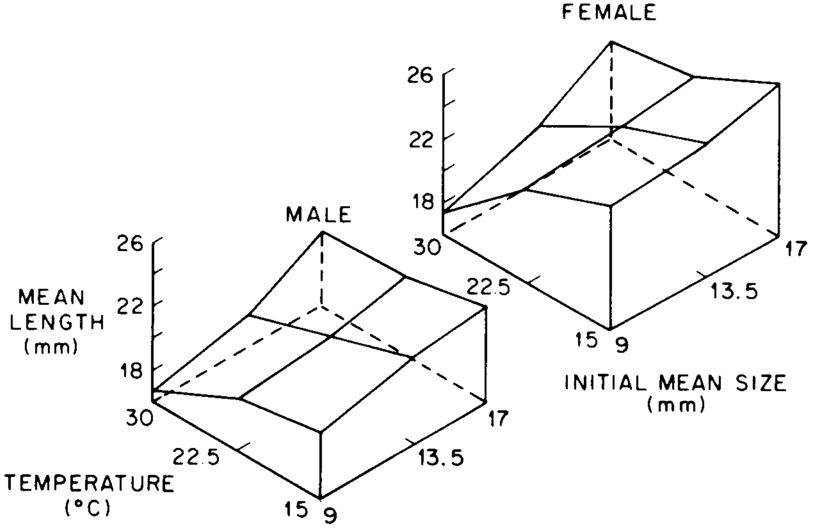

Fig. 3. The mean length of large wind-pad nymphs displayed for each sex separately as a function of both temperature and initial size.

Table 3. Mean length in mm ± 95% C.L. of nymphs measured at the large wing-pad stage with sample size shown in parentheses.

Mean Initial Size (mm)	Temperature					
	15°C		22.5°C		30°C	
	♂	♀	♂	♀	♂	♀
9.1	20.1 ± 1.0 (3)	23.7 ± 1.6 (3)	19.2 ± 0.8 (11)	21.9 ± 0.8 (11)	16.8 ± 1.2 (5)	17.5 ± 1.4 (4)
13.5	21.5 ± 1.4 (4)	24.3 ± 1.6 (3)	19.7 ± 0.8 (11)	22.4 ± 0.9 (9)	18.0 ± 1.1 (6)	19.5 ± 1.4 (4)
17.0	21.9 ± 1.1 (7)	25.5 ± 0.8 (12)	20.9 ± 1.1 (7)	22.8 ± 0.8 (13)	20.6 ± 0.9 (9)	22.1 ± 0.7 (14)

We had assumed that the response of body length to the effect of temperature, sex, and initial size would be the same for both LWP nymphs and adults. Thus, our experimental setup was designed to capture adults in order to determine emergence success but no effort was made to maintain separation between the size classes. This was unfortunate since analysis of body lengths of adults captured suggested that our a priori assumption had not been entirely correct. Adult body length was significantly affected by both sex and temperature (P = 0.001), with no apparent interaction between temperature and sex (P = 0.20, Table 2). Although this agrees in general with the LWP data, the temperature effect was apparently much greater for the adults than for the LWP nymphs, since the reduction in mean body length was greater at 30°C than at 22.5°C (Table 4). It appears that nymphs at the LWP stage do not provide an adequate substitute for analysis of temperature effects on adult size.

Table 4. Difference between mean size of large wing-pad nymphs and adults. Nymph data are pooled over initial size classes for this comparison.

Category		Mean Body Length 95% C.L. (mm)				Difference
Temp	Sex	Nymph	n	Adult	n	(mm)
30°C	♀	21.65 ± 0.87	(18)	16.42 ± 0.73	(12)	5.23
30°C	♂	19.29 ± 0.92	(20)	15.29 ± 0.90	(17)	4.00
22.5°C	♀	22.39 ± 0.64	(33)	19.48 ± 0.78	(29)	2.91
22.5°C	♂	19.79 ± 0.56	(29)	17.32 ± 0.60	(22)	2.47
15°C	♀	24.83 ± 0.78	(18)	---		--
15°C	♂	21.00 ± 0.60	(14)	---		--

Nymph survival to emergence differed significantly between the 22.5 and 30°C temperature groups (P < 0.05, Table 5). Initial size and sex did not have a significant effect on survival to emergence at these two temperatures. Survival of nymphs at 15°C to the subadult stage was not statistically compared with that at the other two temperature groups because metamorphosis to the adult stage was rare during 151 days of observation. Nymph survival at 15°C during the 151 days of observation appeared to be affected by size since it was relatively high for the 17-mm and 9-mm group, but considerably lower for the 13.5-mm group (Table 5). Since size was not a factor in survival at 22.5 and 30°C, we doubt if size per se was responsible for the high mortality in both replicates of the 13.5-mm group at 15°C.

Success of metamorphosis into the subimago and imago stage was also affected by temperature. Most obvious was the almost total lack of success at 15°C. Individuals at 15°C reached various stages of metamorphosis. Some nymphs were found dead on the water surface with engorged wing-pads but unbroken thorax;

Table 5. Numbers of nymphs surviving to emergence at 22.5°C and 30°C and number surviving to day 151 at 15°C. Twenty nymphs were initially introduced into each replicate.

Temperature °C	Initial size mm	Number surviving Replicates	Number surviving Mean ± 95% C.L.
	9.0	6, 4	
30	13.5	6, 3	5.50 ± 1.72
	17.0	7, 7	
22.5	9.0	8, 15	
	13.5	10, 7	9.33 ± 3.16
	17.0	7, 9	
	9.0	14, 13	
15	13.5	4, 2	9.33 ± 5.38
	17.0	13, 10	

other individuals partially escaped from the nymph exuviae before dying; and others escaped the exuvia, but died on the surface as subimagos. Only one individual reached the imago stage. In contrast, all but three nymphs successfully reached the imago stage at 30°C, and all nymphs were successful at 22.5°C.

Timing of metamorphosis was affected by both initial size and temperature, but the effects were not clearcut (Table 6). For instance, more than 50% of the nymphs at both 30°C and 22.5°C in the 17-mm and 13.5-mm size groups had completed metamorphosis by June 5 or 6, and the rest metamorphosed on June 15. A few individuals in the 17 mm size group transformed prior to June 5 at both 22.5°C and 30°C. The biggest difference in timing of metamorphosis between the 22.5°C to 30°C groups was among the 9-mm nymphs, where metamorphosis at 22.5°C occurred up to one month later than that at 30°C. Attempts to metamorphose occurred considerably later at 15°C, although the first attempt occurred on June 5 (the same day that a major portion of nymphs metamorphosed at the other temperatures). Only nymphs from the large size group at 15°C metamorphosed during the 151 days of observation.

Temperature also affected fecundity. Four of 11 female subimagos emerging from the 30°C aquaria did not have eggs. All female subimagos emerging from the 22.5°C temperature appeared to have eggs.

DISCUSSION

The results of our experiment suggest that growth rate, size, and fecundity of Hexagenia bilineata are functions of temperature regime. At a constant temperature of 30°C, emergent adults were significantly smaller than at lower temperatures and

Table 6. Number of *Hexagenia bilineata* metamorphosing under constant temperature laboratory conditions. Initial number of nymphs at each size group equaled 40 on April 14, 1978.

Temp (°C)	Initial size (mm)	Dates of Emergences												
		5/8	5/16	5/24	5/30	6/5&6	6/15	6/28	7/3	7/13	8/16	8/24	9/11	
30.0	17.0		2	1	1	9	1							
	13.5					9								
	9.0					1	9							
22.5	17.0	1			1	10	5							
	13.5					8	7							
	9.0					1	4	7	8	3				
15.0	17.0					2		2		0	2	1	2	→
	13.5													→
	9.0													→

several of the females did not contain eggs. Additional evidence of a suboptimal environment for life cycle completion at 30°C included lower survival of nymphs to the stage of metamorphosis and a slightly lower success of metamorphosis from the nymph through the subimago and imago stage. Thus, at a temperature assumed to be above the "optimum," the physiological responses of *H. bilineata* were in agreement with the predictions of the thermal equilibrium hypothesis proposed by Sweeney and Vannote (1978) and Vannote and Sweeney (1980). This is an example of the more general hypothesis of all poikilotherms having species-specific optimal thermal ranges (Wieser 1973). The three temperatures studied did not, however, allow us to determine the optimal thermal range.

The observed effects of temperature, size, and sex on growth rates appear to generally support the conclusions arrived at in investigations of the growth pattern of *H. bilineata* from egg stage to adult (Wright and Mattice, *unpublished* manuscript). For instance, the data demonstrate that growth rate of females is normally faster than that of males at all three temperatures, regardless of initial size, and that growth rates at the two higher temperatures were similar and much greater than growth rates at 15°C. In general, growth rate tended to be highest for nymphs of smaller initial size and lowest for the larger initial sizes. This is in accord with the growth patterns of most organisms (Weatherly 1972). The 30°C data appear to be an exception to that trend. Both the general trend and the exception at 30°C agree with the findings reported in Wright and Mattice (unpublished manuscript).

An uncontrollable factor in the experiment was food quality. Although we believe food addition was sufficient to provide a layer of food material on the substrate surface at all times, no measurements of organic carbon, nitrogen or caloric content of the sediments were made to confirm the amount or type of nutrients actually available. Since the type and amount of microbiota supported could differ with temperature, it cannot be assumed that food quality was similar at all temperatures. The microbial community associated with detritus is potentially an important component in the diet of Hexagenia under natural conditions (Zimmerman and Wissing, 1980). The action of temperature on the microbial community could be one mechanism by which temperature affects growth and maturation in Hexagenia.

Based on Sweeney and Vannote's (1978) major criterion for determining an "optimum" temperature regime (i.e., maximized body size), it appears that 15°C would be closest to "optimum" of the three temperatures compared. The mean body length of LWP nymphs was significantly larger at 15°C than at 22.5 or 30°C, particularly among the females where fecundity is directly related to size when effects of temperature are normalized (Clifford and Boerger 1974). Length of LWP nymphs may not be an accurate indicator of adult size (see Table 4), but we do not have measurements on adults at 15°C. However, the lack of successful metamorphosis would seem to rule out 15°C as an optimal constant temperature for population success. The constant temperature condition optimizing size of adults may be at the lowest temperature allowing successful metamorphosis, which Fremling (1973) has suggested is around 19°C based on field observations. Since nymph size does appear to be maximized at 15°C, it seems reasonable that the mean temperature of an "optimum" seasonally fluctuating temperature regime could be around 15°C, if temperatures warmed to 19°C or greater during the period of emergence.

Different size groups were used in the experiment primarily because the size distribution of the field populations from which the experimental nymphs were collected varied over a wide range. Use of a range of sizes allowed us to determine the effect of temperature on the initiation of adult tissue development. The nearly synchronous emergences and the short period of emergence of nymphs from 13.5-mm and 17-mm size groups at both 22.5°C and 30°C suggest that the period of initiation of adult tissue development was short. Vannote and Sweeney (1980) observed that the critical threshold for adult tissue development of Ephemerella subvaria, a cool-water mayfly, was about 9°C. Fremling (1973) concluded that a critical threshold of 19°C was necessary to permit development of the final instar of H. bilineata based on field data. We found that adult tissue development could occur at 15°C; however, the emergence period was protracted with no peaks, indicating that simultaneous initiation of tissue maturation had not occurred. The nearly simultaneous emergences of the larger size classes at both 22.5°C and 30°C were probably a function of both sets of nymphs exceeding the critical threshold for adult tissue development at the same time they were moved from 15°C lake temperatures and quickly acclimated to the test temperatures.

However, the lack of complete synchrony of emergence for all size classes indicates that development of adult tissue was not entirely size independent. Although nymphs at 13.5-mm and 17-mm initial size groups did seem to mature simultaneously, the nymphs in the 9-mm size group appear to lag somewhat even at the 30°C temperature. Thus we hypothesize that a size threshold exists between 9 mm and 13.5 mm for development of adult tissue in H. bilineata.

In summary, our laboratory data indicate that H. bilineata generally do conform to the expected responses to a suboptimal temperature treatment as proposed by the thermal equilibrium hypothesis of Vannote and Sweeney (1980). In particular, adult size and fecundity and nymph survival were all reduced at 30°C, which is a much higher temperature than would naturally be encountered for long periods. The 15°C treatment did not appear to be suboptimal for the nymphs when judged by the nymphal size attained, but adult metamorphosis was mostly unsuccessful. Our hypothesis is that the "optimal" constant temperature regime for H. bilineata is between 15°C. and 22.5°C, but a seasonally fluctuating regime with summer temperatures above 19°C and a yearly mean of 15°C may provide nearly optimal conditions.

Since H. bilineata did respond to temperature differences in the laboratory by emerging at different sizes, as hypothesized for all aquatic insects by Vannote and Sweeney (1980), size differences might be expected between the sub-populations in the two coves which we found to differ by about 5°C year-round. However, the size differences among the 9-mm nymphs in the laboratory were more pronounced between 30°C and 22.5°C than between 22.5°C and 15°C. Both 30°C and 22.5°C are high when compared to the mean annual temperatures of the natural aquatic environments of Hexagenia bilineata. Even the cove receiving the heated discharge water of a power plant only occasionally reached a bottom temperature of 30°C, and the mean annual temperature was only about 20°C. The mean annual temperature of the cove used for comparison was about 15°C. The most logical reason for the lack of observed size differences between the populations emerging from the two coves is that both are reasonably near the theoretical "optimum" thermal regimes. Thus size differences may be small even if they occur and may be difficult to detect due to large variation in adult size.

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