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Effects of temperature, food quantity, and nymphal rearing density on life-history traits of a northern population of *Hexagenia* (Ephemeroptera:Ephemeridae)

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Abstract. *Hexagenia limbata* (Serville) and *H. rigida* McDunnough from Southern Indian Lake, Manitoba, were reared at 10°, 15°, and 20°C to assess temperature effects on growth. Subsequently, *H. limbata* was reared at two food levels and two or three nymphal densities (depending on size class), while temperature again was varied, to determine whether these factors affected life-history features such as growth, development, fecundity, and mortality. Nymphs of both species grew at 8°C, 2° below the published temperature threshold for development. At both limited and non-limited food levels, growth and development rates increased with increasing temperature, but food limitation slowed both growth and development within a temperature treatment and resulted in increased degree day requirements. Nymphs reared at 20°C at a non-limiting food level grew into significantly larger and more fecund adults than those reared at 20°C at a limiting food level, or at 15°C under non-limiting food conditions. Growth and development rates were not affected by rearing density. Thermal requirements observed in the laboratory were near the lower end of those observed in nature, and are probably a reflection of the effect of temperature on development under near-optimal conditions. Growth responses to temperature were similar for the two species.

Key words: *Hexagenia limbata, Hexagenia rigida,* growth and development, temperature responses.

Temperature and food are the factors most frequently reported to affect life-history traits in aquatic insects (Sweeney 1984). Many researchers have shown a strong relationship between growth or development rate and temperature (e.g., Brittain 1976, Vannote and Sweeney 1980, Humpesch 1981). Others have shown that growth may depend on the quality and quantity of food (Anderson and Cummins 1979, Fuller and Mackay 1981, Webb and Merritt 1987). In most studies on aquatic insect growth and development, the focus has been on one of these factors at a time, varying food quality while holding temperature constant (e.g., Fuller and Mackay 1981, Webb and Merritt 1987), or varying the rearing temperature while maintaining a constant food level (e.g., Humpesch 1981, Leggott and Pritchard 1985, Park 1988). The relative importance of each factor is difficult to evaluate, because temperature and food interact in nature. Temperature directly affects growth by influencing metabolic rates (Ward and Stanford 1982, Robinson et al. 1983) and feeding rates (Zimmerman and Wissing 1978, Wallace and Merritt 1980); it also affects food quantity and quality by influencing algal production and microbial growth rates on detritus (Anderson and Cummins 1979, Ward and Stanford 1982, Rempel and Carter 1986).

In spite of difficulties associated with separating temperature and food effects, several researchers have shown the importance of considering both of these factors simultaneously when evaluating growth and development of aquatic insects (e.g., Sweeney et al. 1986a, 1986b, Baker and Feltmate 1987, Rosillon 1988, Söderström 1988). Temperature appears to be the most important variable affecting the growth/development response, as long as there is no environmental cue controlling phenology (e.g., photoperiod). However, food can modify the response, resulting in changes in body weight, fecundity, and developmental timing.

A third factor, rearing density, is rarely considered when evaluating growth and development. At high densities, competitive interactions between organisms may affect feeding rates and growth. For example, Rosillon (1988) found that individually reared *Ephemerella ignita* nymphs were larger and more fecund as adults than those reared in groups.

In this paper, we focus on the relative im-

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portance of temperature, food quantity, and nymphal rearing density on some life-history traits of two species of burrowing mayfly, *Hexagenia limbata* and *H. rigida*, from Southern Indian Lake (SIL) in northern Manitoba (57°N, 99°W). *Hexagenia limbata* is widespread throughout much of North America; *H. rigida* is found mainly in the eastern part of the continent (McCafferty 1975). Both approach their northern range limits at SIL. *Hexagenia* development is known to be strongly temperature dependent, with life cycles ranging from <1 yr in the south (Carlander et al. 1967, Craven and Brown 1969) to 2 yr in mid-latitude locations (Neave 1932, Flannagan 1979) to several years in the extreme northern part of the range (Giberson 1991). The developmental threshold temperature (temperature below which no development occurs) was previously reported to be ~10°C for both species (Hunt 1953, Friesen et al. 1979), with an apparent requirement for ~1900–2500 degree days (dd) above 10°C to complete development (McCafferty and Pereira 1984, Heise et al. 1987). *Hexagenia* nymphs are filtering collectors, feeding primarily on detritus (Zimmerman and Wissing 1980), but little is known about the effect of food on their growth, fecundity, and development. Although reported to occur in very high densities (>1000/m^2) in some locations (Carlander et al. 1967, Hudson and Swanson 1972, Heise et al. 1988), it is not known whether density influences their life histories.

Methods

Study animals

Rearing trials were conducted on medium-sized (17.1 ± 1.4 mm, $\bar{x}$ ± 1 SD) *H. limbata* and *H. rigida* nymphs collected from Wupaw Bay, SIL (Giberson 1991), in September 1987, and on *H. limbata* nymphs reared from artificially fertilized eggs obtained from SIL adults in July 1987 (Giberson and Rosenberg 1992). Nymphs were collected by gently washing the contents of an Ekman grab through 400-μm mesh nets. Adults for fertilization were captured with sweep nets and by hand-picking from vegetation along shorelines at SIL; eggs from 20 females were combined and mixed with the macerated terminal segments of 20 adult males, and then were cooled for storage (Friesen 1981, Giberson and Rosenberg 1992). Subsequent hatching success for eggs treated in this fashion was 85–95%.

Experimental trials

All nymphs were maintained in continuously aerated 3-L plexiglas aquaria (7 cm wide × 30 cm long × 15 cm high) containing ~5 cm of sterilized SIL sediment and deionized tap water. The top 6 or 7 cm of water were decanted from each aquarium monthly during the experimental period, and replaced with deionized water. Trials were run in constant light in controlled environment chambers (temperature fluctuations <0.5°C).

Nymphs were fed a diet of ground Tetramin-B®. Food requirements varied between temperature treatments because of the effect of temperature on metabolic rates, and they also varied with time as nymphs grew larger. Because of this complexity, food was quantified as it was added for the purpose of comparison between aquaria, but not recorded for the whole study period. Aquaria in the preliminary temperature experiments were examined twice weekly, and additional food was added when necessary to maintain a barely visible layer of food on the surface of the mud, assessed as a "non-limiting" level of food. In experiments where food availability and rearing density were evaluated as well as temperature, non-limiting food requirements were determined twice weekly, as above, for the aquaria containing the highest nymphal density at each study temperature. Food amounts for the remaining aquaria in each temperature treatment were then calculated based on comparative nymphal densities and desired food treatment. For example, if the highest nymphal density in a treatment was 30 nymphs/aquarium, food rations for aquaria containing 20 (or 10) nymphs were calculated as ⅔ (or ⅓) of that needed for 30 nymphs. "Limiting food" was calculated as half the amount given in the non-limiting treatments.

Experiment 1 was a 3-way factorial experiment designed to evaluate temperature effects on growth of male and female nymphs of each species. Field-collected medium-sized nymphs (see Table 2 for sizes) of both sexes and both species were reared under non-limiting food conditions at 10°, 15°, and 20°C. Species were identified using characters given by McCafferty

(1975) and by J. F. Flannagan (Freshwater Institute, Winnipeg, personal communication). Sexes were separated (based on presence or absence of developing penes in the male) and individual body lengths were measured (using an ocular micrometer in a dissecting microscope) before nymphs were added to the aquaria. Each treatment consisted of two replicate aquaria, both containing eight individuals of one sex of one species. Sets of eight replicate aquaria (2 replicates × 2 sexes × 2 species) were monitored at three temperatures (10°, 15°, and 20°C) for a total of 24 aquaria. Growth (mm) was calculated as the difference between initial size and size at the end of the trial (120 d at 10°C, 80 d at 15°C, and 50 d at 20°C). Growth is usually exponential, and the relationship between body length (L, mm) and time (t, days) may be expressed as:

$$L = ae^{bt}$$

where a and b are empirically derived constants (Rosillon 1988). The instantaneous growth rate (G) was determined from:

$$G = 100b$$

(Rosillon 1988). Growth rates (%Length/d, after arcsine transformation) were compared for each sex and species at each temperature using a 3-way ANOVA.

Experiment 2 was designed to evaluate the relative importance of temperature, food, and nymphal density on instantaneous growth rates of *H. limbata*. Treatments were again set up in a 3-way factorial configuration. Because growth rates were dependent upon body size, two size classes were used: 1) small nymphs that were reared directly from fertilized eggs (initial size at hatch: 1.01 ± 0.02 mm; $\bar{x} \pm 1$ SD, determined by measuring 50 freshly killed hatchlings); and 2) large nymphs, which were reared at 20°C for 90 d prior to treatment (initial size: 20.0 ± 0.58 mm; $\bar{x} \pm 1$ SD, determined by direct measurement before placement into aquaria; Table 4). Each treatment consisted of two replicate aquaria containing 10, 20, or 30 small nymphs (430, 860, and 1290/m²) or 5 or 10 large nymphs (215 and 430/m²) at each of two food levels (nonlimiting and limiting, as above) and five constant temperature regimes (8°, 10°, 12.5°, 15°, and 20°C). Overall, 12 aquaria (6 at each food level) containing small nymphs and 8 containing large nymphs (4 at each food level) were monitored at each of the five temperatures. The amount of food/nymph was kept constant in each food treatment, regardless of density, to separate the potential effects of competition for food and competition for space. Each trial lasted 90 d or until all adults had emerged, and instantaneous growth rates were calculated as above. Growth rates at the different treatments (G, %Length/d, arcsine transformed) were compared using ANOVA.

In Experiment 3, *H. limbata* nymphs were reared from eggs to the emergence of adults to determine both the shape of the growth curve and the time required for development. Nymphs were reared at 8°, 15°, and 20°C at the non-limiting food level to evaluate temperature effects. Food effects were also monitored, but only at 20°C; growth was too slow at the cooler temperatures for us to be sure of obtaining complete data on development time. Fifty-five eggs were added to each of eight aquaria in each treatment; eggs were used at this stage because they suffered less handling mortality than small nymphs. To estimate the timing and success of egg hatch, three additional sets of 55 eggs, each from the same egg mass, were placed in dishes and checked daily; hatching in the dishes was completed within 7 d. All aquaria were held at 20°C for 10 d initially to ensure uniform hatching between treatments, because hatching success declines at low temperatures (Giberson and Rosenberg 1992). Aquaria in the 8° and 15°C treatments were then transferred to 15°C, and four days later, those in the 8°C treatment were transferred to 8°C; the transfer was staggered to avoid exposing newly hatched nymphs to abrupt temperature changes. The initial size of nymphs at the start of the experiment was 1.01 ± 0.02 mm. The size of the nymphs following placement at 8° and 15°C was inferred from growth in the dishes, and was 2.05 mm. The number of eggs that hatched in the dishes was 49–51 ($\bar{x} = 50$, or 91%).

One aquarium in each treatment was sacrificed at ~45-d intervals, and nymphs were gently washed from the mud through a 200-μm screen and measured. Survivorship was calculated on each sampling date as the percent remaining from the original number of eggs hatched, and mortality was expressed as total percent (%) and as percent/day (%/d). Screens on each aquarium prevented emerging adults from escaping. Adult (subimago) body size was

TABLE 1. ANOVA and regression statistics in growth studies on *Hexagenia* reared in the laboratory (see text). For each experiment, temperature was evaluated alone (ANOVA, simple regression) and in conjunction with other factors (3-way ANOVA, multiple regression); p = probability level, R^2 = correlation coefficient.

Expt.	Size	Treatment	df	F	p	R^2
1	Medium	Temp	2, 20	42.5	<0.001	0.81
		Temp (T)	2, 11	55.6	<0.001	0.92
		Species (Sp)	1, 11	4.6	0.055	
		Sex	1, 11	5.8	0.035	
		T × Sp	2, 11	1.3	0.307	
		T × Sex	2, 11	0.3	0.879	
		Sp × Sex	1, 11	0.04	0.830	
		T × Sp × Sec	2, 11	0.7	0.519	
2	Small	Temp	4, 41	240.6	<0.001	0.85
		Temp (T)	4, 16	147.8	<0.001	0.94
		Food (F)	1, 16	27.8	<0.001	
		Density (D)	2, 16	0.12	0.85	
		T × F	4, 16	1.09	0.36	
		T × D	8, 16	1.28	0.30	
		F × D	2, 16	1.31	0.27	
		T × F × D	8, 16	1.64	0.17	
2	Large	Temp	4, 35	175.2	<0.001	0.82
		Temp (T)	4, 20	93.3	<0.001	0.98
		Food (F)	1, 20	7.6	0.01	
		Density (D)	1, 20	0.01	0.93	
		T × F	4, 20	4.8	0.007	
		T × D	4, 20	0.25	0.91	
		F × D	1, 20	0.72	0.41	
		T × F × D	4, 20	0.61	0.66	

measured as body length (mm) between the tip of the frontal process and the end of the abdomen, excluding the caudal filaments. Fecundity was determined by dissecting female subimagos and counting the eggs. Development time was expressed as number of days from egg hatch to emergence, and also as degree days (dd) to emergence:

$$dd = (T - T_0)d$$

where T = study temperature, T_0 = threshold temperature, and d = number of days above threshold (Davidson 1944). Development rate (1/t) generally shows a logistic (sigmoidal) relationship with temperature, but for temperatures in the middle range of the development/temperature curve, the relationship is approximately linear and can be described by:

$$1/t = a + bT$$

where t = days required for development, T = temperature, and a and b are empirically derived constants. The threshold temperature was calculated by extrapolating the line to the point where the development rate (1/t) approaches zero; and dd = 1/b (Davidson 1944, Pruess 1983, Rosillon 1988).

Results

Growth rates

Temperature was the most important variable influencing growth and development in all experiments. Temperature alone accounted for >80% of the variability in growth for both species (Table 1, Figs. 1, 2), and also caused large differences in emergence timing in *H. limbata* (Fig. 3). Growth of large nymphs was observed at all experimental temperatures, although growth rates were very low at 8°C. Small *H. limbata* did not grow at 8°C (Fig. 2).

For medium-sized *Hexagenia* in Experiment 1, sex had a significant effect on growth in both species (Table 1, Fig. 1), so care was taken in later experiments to include equal proportions of males and females. *Hexagenia limbata* consistently grew faster than *H. rigida*, though this

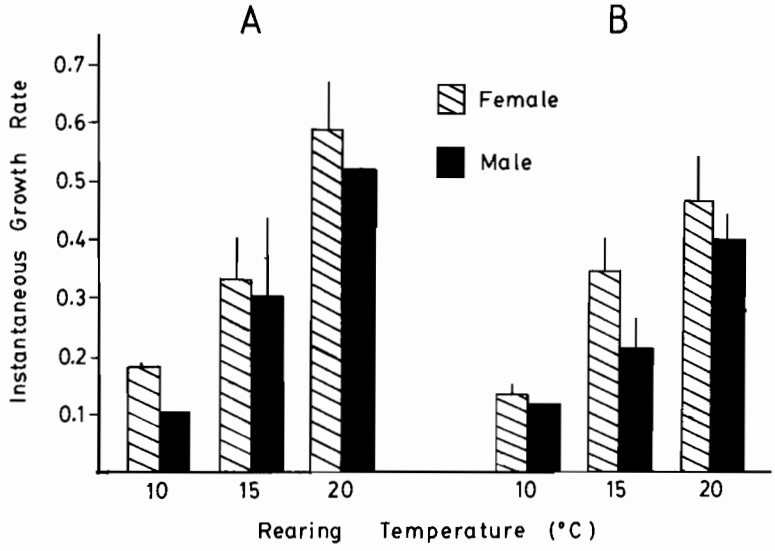

FIG. 1. Instantaneous growth rates (%Length/d ± 1 SD) of mid-sized, field-collected *Hexagenia* nymphs at a range of temperatures in the laboratory (Experiment 1). A: *Hexagenia limbata*. B: *Hexagenia rigida*.

trend was not quite statistically significant at $p < 0.05$ ($p = 0.055$; Table 1).

In Experiment 2, no significant density effect was observed on growth for either size class of *H. limbata* (Table 1), so the density treatments were combined to analyse the relative effects of temperature and food (Fig. 2). Nymphs given more food grew faster, and for large nymphs, the response to food was stronger at higher temperatures (Table 1, Fig. 2). No other significant interactions were observed.

Mortality

Nymphal mortality during rearing was related to rearing temperature and size class, but not to density, food level, species, or sex (Tables 2–4). Mortality was generally higher for small

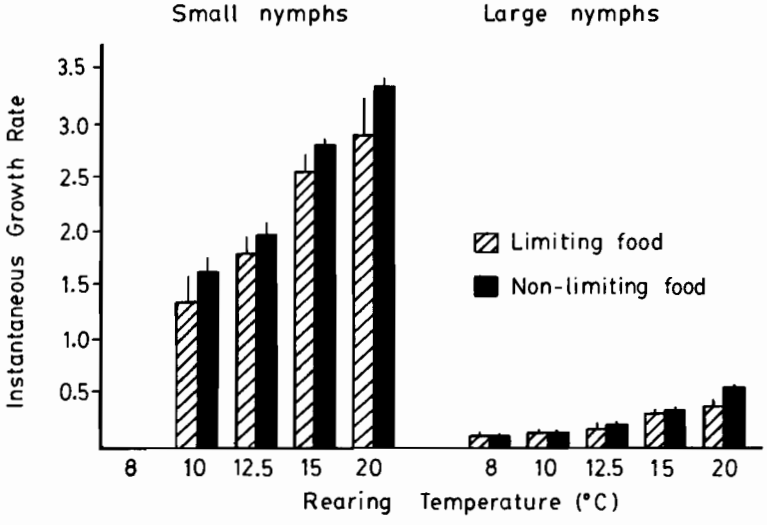

FIG. 2. Instantaneous growth rates (%Length/d ± 1 SD) of two size classes of *Hexagenia limbata* nymphs reared in the laboratory at two food levels over a range of temperatures (Experiment 2; density-treatment data were pooled).

TABLE 2. Initial and final sizes ($\bar{x} \pm 1$ SD) and mortality of field-collected medium-sized male and female *Hexagenia limbata* and *H. rigida* nymphs reared in the laboratory at 10° (120 d), 15° (80 d) and 20°C (50 d) (n = 16; Experiment 1).

			Initial size (mm)	Final size (mm)	Days	Mortality Total (%)	Mortality %/d
H. limbata	♀	10°C	15.83 ± 0.62	21.15 ± 0.71	120	17	0.14
		15°C	17.29 ± 0.28	22.71 ± 1.2	80	26	0.33
		20°C	16.50 ± 0.77	22.00 ± 0.2	50	30	0.60
	♂	10°C	17.51 ± 0.42	20.97 ± 0.26	120	17	0.14
		15°C	16.15 ± 0.80	20.64 ± 0.9	80	17	0.21
		20°C	13.78 ± 0.77	19.00 ± 1.3	50	17	0.34
H. rigida	♀	10°C	18.73 ± 0.59	22.84 ± 1.1	120	6	0.05
		15°C	17.29 ± 0.32	22.35 ± 0.4	80	0	0
		20°C	17.45 ± 0.40	22.00 ± 0.37	50	68	1.36
	♂	10°C	17.81 ± 0.80	21.21 ± 1.28	120	6	0.05
		15°C	17.47 ± 0.59	20.75 ± 0.12	80	12	0.15
		20°C	16.28 ± 0.51	19.80 ± 0.39	50	12	0.24

nymphs than for large ones (Tables 3, 4). Within size classes, mortality of large nymphs was highest at 20°C (Tables 2, 4), and mortality of small nymphs was highest at 8° and 10°C (Table 3).

Development

Development of *H. limbata* was related to both food and rearing temperature. In Experiment 3, nymphs grown at non-limiting food levels at 20°C began emerging in less than half the time (183 d) of those reared at 15°C (380 d; Fig. 3, Table 5). Temperature thresholds for developing nymphs varied with nymphal size; the large nymphs in Experiment 2 achieved measurable growth and development at 8°C (Fig. 2), but no growth or development was noted at 8°C in the small nymphs in either Experiment 2 (Fig. 2) or Experiment 3 (Fig. 3). The developmental threshold for *H. rigida* nymphs was not determined, but growth of medium-sized nymphs in Experiment 1 showed a similar pattern to *H. limbata*, with measurable growth at 10°C.

The derived threshold for total development for *H. limbata* nymphs in this study (extrapolation based on the dd model) was 10.4°C. Development (from egg hatch to start of emergence period) required ~1750 dd>10.4°C under non-limiting food conditions (Table 5). Virtually all emergence was complete within 60 d at 20°C (non-limiting food), resulting in a total dd range of 1745–2333 for all individuals at that temperature (Table 5). Only ~60% of nymphs had emerged by 60 d after the start of emergence at 15°C. Experiments were terminated at this time (because of an incubator failure), so the full dd range for emergence at 15°C is not known, but ranged from 1750 to >2100 dd>10.4°C.

Development of *H. limbata* was slowed by limiting the availability of food. The emergence period started later and extended for longer under limiting food levels at 20°C when compared with non-limiting levels (Fig. 3). However, there was a high degree of overlap between emergence periods for the two food treatments (Table 5).

Adult size and fecundity

Temperature and food quantity affected adult size and fecundity of *H. limbata*. Nymphs reared at a non-limiting food level but at 15°C grew into significantly smaller and less fecund adults (t-test, $p < 0.01$) than those reared at non-limiting levels and 20°C (Table 5). Similarly, those reared at 20°C but under limiting food conditions were smaller and less fecund as adults (t-test, $p < 0.01$) than those at 20°C at non-limiting food conditions (Table 5). No significant differences were seen in adult size or fecundity

TABLE 3. Final sizes ($\bar{x} \pm 1$ SD) and mortality of *Hexagenia limbata* nymphs reared from eggs in the laboratory at two food levels and a range of temperatures and nymphal densities for 90 d; initial size for all treatments: 1.01 ± 0.02 mm (Experiment 2).

		Limiting food			Non-limiting food		
			Mortality			Mortality	
Temp. (°C)	Density (no./ aquarium)	Final size (mm)	Total (%)	%/d	Final size (mm)	Total (%)	%/d
8	10	0.99 ± 0.06	68	0.76	1.21 ± 0.05	70	0.78
	20	1.10 ± 0.05	66	0.73	1.07 ± 0.06	74	0.82
	30	1.00 ± 0.04	77	0.85	1.12 ± 0.05	80	0.89
10	10	—	100	1.11	5.01 ± 0.30	66	0.73
	20	3.21 ± 0.47	70	0.78	4.04 ± 0.95	65	0.72
	30	3.56 ± 0.78	68	0.76	4.06 ± 0.65	67	0.74
12.5	10	5.92 ± 0.86	45	0.50	5.85 ± 0.01	25	0.28
	20	5.10 ± 0.02	35	0.39	6.02 ± 0.87	32	0.36
	30	4.11 ± 0.21	13	0.14	5.95 ± 0	3	0.03
15	10	10.48 ± 0.18	47	0.53	12.25 ± 0.25	50	0.56
	20	9.55 ± 0	50	0.56	12.00 ± 0.7	27	0.30
	30	9.62 ± 1.8	40	0.45	12.72 ± 1.8	40	0.45
20	10	10.05 ± 0.77	40	0.45	20.13 ± 0.67	55	0.61
	20	14.80 ± 6.8	55	0.61	21.64 ± 0	60	0.67
	30	17.05 ± 4.5	57	0.63	20.21 ± 0.25	51	0.57

between those reared at 15°C at non-limiting food levels and at 20°C at limiting food levels.

Discussion

Temperature and food are known to affect aquatic insect life histories through their effects on growth and development (Sweeney 1984). In our study, growth rates increased with increasing temperature for both *H. limbata* and *H. rigida*. Food availability also influenced growth rates of *H. limbata*, and increased growth rates resulted in shorter developmental periods, larger size at maturity, and higher adult fecundity. Although these traits were not investigated for *H. rigida*, the two species have virtually identical life cycles in Lake Winnipeg (Neave 1932) and SIL (Giberson 1991), and similar growth/temperature responses between 10° and 20°C. Therefore temperature and food probably have a similar influence on life-history traits of *H. rigida*. The effect of food quantity on growth of *H. limbata* was more pronounced at higher temperatures, particularly for the larger nymphs. Temperature may have affected the quality of the available food, perhaps by stimulating microbial growth. Rosillon (1988) noted a similar interaction between food and temperature for *Ephemerella ignita* and suggested that the rate of acceleration of growth with temperature may depend on diet.

Growth of *H. limbata* was not affected by nymphal rearing density, at least at the densities evaluated. In natural *Hexagenia* populations, densities may range from $<5/m^2$ to $>2000/m^2$, with averages generally from 60–300/m^2, depending upon location and time of year (Neave 1932, Carlander et al. 1967, Hudson and Swanson 1972, Heise et al. 1988). Aquarium densities of 430–1290/m^2 (small nymphs) and 215–430/m^2 (large nymphs) were therefore within the natural range. If density had an effect on growth, for example through interference competition, decreased growth rates or increased variability in the rates should have been noted with increasing density, particularly at the limiting food level. However, there was no consistent effect of density on growth pattern at any of the temperatures or food levels evaluated. Rosillon (1988) noted differences in growth rates and other life-history parameters in *Ephemerella ignita* nymphs that were reared individually and in groups, so it is likely that interactions may play a role in some cases. However, for *H. lim-*

TABLE 4. Initial and final sizes ($\bar{x} \pm 1$ SD) and mortality of large *Hexagenia limbata* nymphs reared in the laboratory at two food and nymphal density levels and a range of temperatures. Nymphs were measured

Temp. (°C)	Density (number/ aquarium)	Limiting food		
		Initial size (mm)	Final size (mm)	Mortality Total (%)
8	5	19.80 ± 2.2	22.44 ± 2.5	13
	10	20.01 ± 1.8	22.89 ± 2.6	7
10	5	19.59 ± 1.9	23.40 ± 3.0	26
	10	19.01 ± 1.8	22.45 ± 2.8	13
12.5	5	20.88 ± 2.2	24.96 ± 3.8	0
	10	20.01 ± 1.9	25.73 ± 3.4	13
15	5	20.13 ± 1.8	26.31 ± 3.6	5
	10	20.03 ± 1.7	27.31 ± 2.9	5
20	5	20.63 ± 2.2	24.64 ± 2.6	40
	10	21.24 ± 1.6	25.55 ± 6.4	12

bata, competitive interactions may be unimportant or only become important at extremely high densities or low food levels.

When insect development is strictly temperature dependent, development can be predicted using a dd model. The relationship between temperature and the time required for insects to complete development usually follows a sigmoidal, or logistic pattern, although it can be described by a linear relationship to temperature in the middle part of the range (Davidson 1944, Pruess 1983, Rosillon 1988). Total development (egg to adult) was followed for *H. limbata* at 15° and 20°C, and the linear equation describing development between those temperatures was used to derive the lower developmental threshold temperature (by extrapolating the line to the point where the development rate approached 0). Even though only two study temperatures were evaluated, the derived threshold of 10.4°C for the SIL population was near the published value of 10.0°C for *H. limbata* (Hunt 1953, Heise et al. 1987). However, it is difficult to make comparisons with southern populations since the literature value is based on field data (Hunt 1953) and has not been determined experimentally.

The derived threshold temperature is usually higher than the actual threshold. Because of the sigmoidal relationship of development rate and temperature, development is faster at temperatures at the low end of the temperature range than predicted from the linear model, and some development occurs at temperatures below the predicted developmental zero (Davidson 1944, Pruess 1983, Higley et al. 1986). For the SIL population of *H. limbata*, the actual developmental threshold probably lies near 8°C, although some variability was noted among developmental stages. Large nymphs showed slow but measurable growth at 8°C, indicating a lower threshold <8°, while the threshold for small nymphs may be nearer to 9°C. In a concurrent study (Giberson and Rosenberg 1992), the egg

TABLE 5. Adult body length and fecundity ($\bar{x} \pm 1$ SE), development time (egg hatch to emergence), and mortality (%/d) of *Hexagenia limbata* reared in the laboratory at 15° and 20°C; N = Non-limiting food, L = Limiting food; see text for explanation (Experiment 3).

Food	Temp (°C)	Body length (mm)			
		Females	*n*	Males	*n*
N	15	23.71 ± 1.8	14	18.20 ± 1.3	12
N	20	25.34 ± 2.1	15	20.24 ± 1.7	11
L	20	22.64 ± 2.2	16	17.76 ± 1.1	14

TABLE 4. Extended.

after 120 d at 8°, 10°, and 12.5°, after 90 d at 15°C, and at the time of first emergence from aquaria at 20°C (35–70 d after start of experiment) (Experiment 2).

Limiting food	Non-limiting food			
Mortality	Initial size	Final size	Mortality	
%/d	(mm)	(mm)	Total (%)	%/d
0.11	19.71 ± 2.3	22.48 ± 1.7	0	0
0.06	20.32 ± 1.9	23.20 ± 1.8	13	0.11
0.22	19.32 ± 2.4	22.39 ± 1.4	0	0
0.11	20.64 ± 2.1	24.37 ± 0.35	0	0
0	19.78 ± 2.0	24.13 ± 1.8	13	0.11
0.11	20.21 ± 2.4	26.02 ± 3.4	26	0.22
0.06	20.63 ± 2.3	28.20 ± 1.7	10	0.11
0.06	20.04 ± 2.1	29.01 ± 4.2	20	0.22
0.44	19.45 ± 2.2	26.35 ± 3.6	11	0.22
0.22	19.15 ± 2.2	25.79 ± 2.6	29	0.78

developmental threshold for SIL *H. limbata* was also determined to be near 8°C. Development of *H. rigida* was not evaluated below 10°C in our study, but its growth response at 10°C was similar to *H. limbata*, so its lower threshold is probably also similar.

The variability in reported threshold temperatures between northern and southern populations, or even between developmental stages within a single population, suggests that degree day analysis may be inappropriate for predicting developmental timing of *Hexagenia* in the field. However, in populations where temperatures in spring and fall change rapidly, and little time is spent at temperatures near the actual threshold, the linear model provides a good predictor of insect development (Pruess 1983, Higley et al. 1986). In contrast, in situations where populations spend more time at or near the developmental threshold, the ~2°C difference between the actual and derived developmental threshold temperatures means that development will proceed more rapidly than predicted from a linear degree day model, resulting in lower-than-expected apparent dd requirements (e.g., Giberson 1991).

Life-cycle duration varies in *H. limbata* depending on environmental temperatures, although temperature may not be the only factor controlling life-cycle length. Over most of the geographic range of *H. limbata*, ~1900–2500 dd > 10°C are required to complete development from egg through to adult (McCafferty and Pereira 1984, Heise et al. 1987; and calculated from data in Hunt 1953, Hudson and Swanson 1972, Welch and Vodopich 1989). The reported 10°C threshold has become accepted because of generally good agreement between dd accumulations for widely separated populations. Latitudinal differences in the thermal regime then result in variations in life-cycle length; these range from <1 yr in the southern part of the

TABLE 5. Extended.

Fecundity	Development time		Mortality
(No. eggs/♀)	(Days)	(dd>10.4°C)	(%/d)
2873 ± 633	380–?	1750 → >2100	0.27
3433 ± 520	183–243	1745 → 2333	0.07
2506 ± 515	220–310	2111 → 2976	0.18

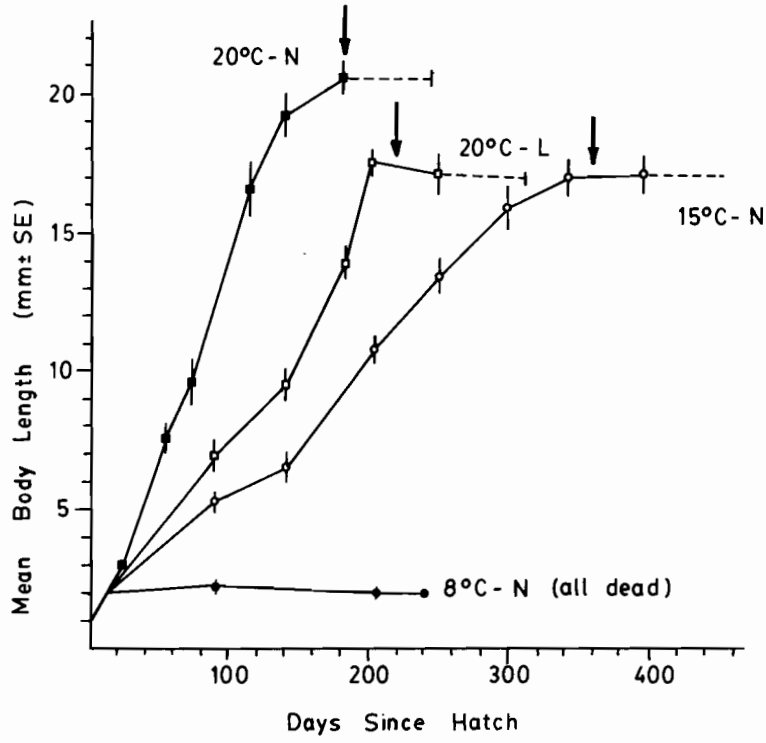

FIG. 3. Growth pattern (body length, mm ± 1 SE, over time) of *Hexagenia limbata* at three constant temperatures (8°, 15°, and 20°C) and two food levels (L = limiting, N = non-limiting; Experiment 3, see text for explanation). Arrows point to time of first emergence; dashed lines represent continuation of emergence period following last sampling date (only ~60% of emergence was complete in the 15°C trial at the time of termination (450 d), but the entire emergence period is shown for the 20°C treatments).

range (Hunt 1953, Craven and Brown 1969) to 2 yr in mid-latitude locations (Flannagan 1979, Heise et al. 1987), and at least 3 yr in the far northern part of the range (Giberson 1991).

Laboratory dd accumulations for SIL *H. limbata* at non-limiting food levels were only ~1750 dd above the derived threshold (10.4°C, compared with the 1900–2500 indicated above. Most researchers (e.g., Heise et al. 1987) have assumed a 10°C threshold for calculations of *H. limbata* dd requirements, rather than deriving the threshold experimentally. Assuming a 10°C threshold temperature for the SIL lab population, a minimum of ~1830–1900 dd>10°C was required to complete development at 15° and 20°C. These values are similar to those reported by Heise et al. (1987) for *H. limbata* in Dauphin Lake, Manitoba, but are generally lower than those reported for southern populations.

Degree day accumulations for field populations, however, vary both between sites and within sites on an annual basis. Heise et al. (1987) noted a trend for dd requirements to decline with increasing latitude over the geographic range of *H. limbata*. Within sites, variations of nearly 300 dd>10°C have been reported between cohorts emerging in different years (Hudson and Swanson 1972, Heise et al. 1987). The trend for lower apparent dd requirements at higher latitudes is probably related to increased time spent near the lower developmental threshold by northern populations, as discussed above. Within-site differences, however, may be related to food availability. In this study, nymphs reared at limiting food levels required 20–30% more dd to complete development than those grown at non-limiting food levels. Furthermore, although density had no effect on growth in this study, it is possible that, at higher densities and lower food levels, competition for

food might limit growth and result in greater dd differences. Hunt (1953) hypothesized that slower growth of a laboratory population of *H. limbata* compared with a field population under similar temperatures may have been due to overcrowding and food limitation.

Fecundity and adult size are also important life-history parameters that affect overall success of field populations. Fecundity is directly related to female body size in *Hexagenia* (Clifford and Boerger 1974, Giberson 1991), so differences in fecundity with temperature should correlate with differences in adult size. For hemimetabolous aquatic insects, the temperature regime for nymphs that produces the largest and most fecund adults has been viewed as optimal (Sweeney and Vannote 1978). The optimum temperature for growth of *H. limbata* is probably between 20° and 25°C (Zimmerman and Wissing 1978, McCafferty and Pereira 1984), and both size and fecundity of adults reared from SIL eggs were significantly greater at 20°C than at 15°C. Limiting food quantity could have effects similar to rearing at lower temperatures: at 15°C, under non-limiting food conditions, emerging adults were similar in size and fecundity to those grown at 20°C under limiting food conditions. Therefore, food quantity must also be considered when comparing growth/temperature responses in different populations.

Mortality of laboratory-reared *Hexagenia* nymphs appeared unrelated to density or food level, but was related to temperature and body size. The high mortality of large nymphs observed at 20°C may have been caused by accumulations of metabolic wastes in aquarium water at that temperature, since growth rates were highest at 20°C and the water in each aquarium was only renewed monthly. It is not clear why survival of small nymphs should have been low at low temperatures, but newly hatched nymphs may first need to accumulate food reserves to survive a long period of dormancy or low-temperature quiescence.

Temperature and food availability during nymphal development had an important effect on life-history traits such as growth and development rates and adult size and fecundity of *Hexagenia*. Reducing the quantity of food available generally modified the temperature response by reducing growth rates, and ultimately adult size and fecundity, by approximately the same magnitude as lowering temperatures by 5°C. Fecundity, and its effect on reproductive effort, is an important factor in the success of natural populations, so food and temperature effects on this parameter may have important implications for field populations. Differences in food availability may account for some of the reported variability in dd accumulations for *Hexagenia* development. Rearing density, at least within the range of 430–1290 nymphs/m^2 (small nymphs) and 215–430 nymphs/m^2 (large nymphs), had no effect on growth or development.

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