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# Life histories of two *Ameletus* mayflies (Ephemeroptera) in two mountain streams: the influence of temperature, body size, and parasitism

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*Abstract.* The life histories of *Ameletus celer* and *A. similior* were compared at Ford Creek and Elbow Creek, two sites in the same drainage system that differed in their annual temperature regimes. We used electrophoresis to identify very small larvae and to determine parasitism by a mermithid nematode. *Ameletus similior* had an overwintering egg diapause and a univoltine summer cycle at both sites. *Ameletus celer* eggs appeared to take about 1 yr to hatch and larvae took a further year to develop at the warmer site but 2 yr at the colder site. Both species were parasitized by a mermithid nematode, the prevalence of parasitism reaching ~10% in the *A. celer* population at the colder site. Parasitism had the effect of prolonging larval life by 2 mo, and parasitized mayflies laid no eggs, even if they survived to adulthood. Temperature and larval size had significant effects on growth rates in the laboratory but larvae of both species of mayfly grew at the same rate at each temperature between 0.6°C and 18°C. The number of day-degrees at the colder site was sufficient to allow the smaller *A. similior* to mature in one year, but not the larger *A. celer*. Extension of physiological time to complete the life cycle at the colder site could not be satisfactorily explained by delayed development, a thermal minimum above 0°C, a nonlinear relationship between temperature and growth rate, placement of recruitment within the thermal cycle, food supply, or parasitism. However, some extension of the life cycle in *A. celer* may be achieved by increase in final size.

*Key words:* life history, mayflies, temperature, growth rate, physiological time, parasitism.

Temperature, acting directly on development rates or indirectly through the food supply, may be the prime factor determining the length of the life cycle in mayflies (e.g., Ide 1935, Brittain 1982, Clifford 1982, Sweeney 1984, Tokeshi 1985, Sweeney et al. 1986, Perry et al. 1986, Thorup et al. 1987, Rader and Ward 1989, Takemon 1990, Benke et al. 1992, Giberson and Rosenberg 1992). Thus, the thermal sum (day-degrees) accumulated in a stream should determine the generation time (Ward and Stanford 1982). However, there are at least five reasons why temperature accumulation may not accurately predict generation time. 1) Developmental delays (diapause) under the influence of thermal or photoperiodic cues may be present. For example, Rader and Ward (1989) showed that the life cycle of *Baetis tricaudatus* was shortened at warmer sites below a dam, but three other species at that site showed no change in voltinism. However, there were changes in synchrony of development and length of the emergence period, and Rader and Ward (1989) implied that a response to thermal or photoperiodic cues constrained development in these three other species. 2) Site temperature accumulations are expressed as thermal sums above 0°C, but the thermal minimum for development may be above 0°C. Benton (1988, 1989) worked at two sites, one of which was warmer in winter than the other and accumulated a significant proportion of its annual thermal sum at this time. However, this differential would be inconsequential if the temperature were still below the lower thermal threshold for mayfly growth. Furthermore, a warm site loses relatively fewer effective day-degrees than a cool site as the thermal minimum rises. Perry et al. (1986) used a thermal minimum of 0°C and cite some good agreements between sites for the temperature accumulations required to complete development in two ephemerellid mayflies. However, Giberson and Rosenberg (1992) used 10°C as the thermal minimum to give satisfactory thermal sums for *Hexagenia limbata*. 3) Giberson and Rosenberg (1992) discuss the unreliability of correlations between thermal sum and growth rate when the temperature is just above the thermal minimum for long periods. This results because an organism's developmental response to temperature is usually sigmoid up to a temperature at which growth is maximal. So one day-degree just above the minimum will have less effect than one at a higher temperature. Beyond the

maximum, the effect of temperature decreases again. Thus, it is necessary to know how growth is related to temperature over the whole range experienced in the field. 4) Hawkins (1986) pointed out that the effect of temperature on individual growth rates in natural populations of mayflies may be obscured by the relationship between body size and growth rate. Thus, if an insect grows at a greater rate when it is small than when it is large, and the effect is most marked at low temperatures, the life cycle should be completed faster if it starts in the low-temperature portion of an annual thermal regime than if it starts in the high-temperature portion. 5) Intra- and inter-specific interactions such as competition and parasitism may slow development.

*Ameletus celer* McDunnough and *A. similior* McD. co-occur and are parasitized by a mermithid nematode at two sites in the Rocky Mountain foothills of southwestern Alberta which have different temperature patterns during the year. Thus we had the opportunity to examine the roles of temperature and parasitism in the regulation of mayfly life cycles. Benton (1988, 1989) has previously worked on the same system. He reported *A. celer* only in the stream with a fluctuating temperature (Ford Creek), where he determined a one-year life cycle; in contrast, he reported *A. similior* only in the colder, less variable stream (Elbow Creek), where he determined a semivoltine life cycle. However, improved methods for species identification (Zloty et al. 1993) cast doubt on Benton's results and we have reexamined the life histories of the two species at these sites. In addition, we examined growth rates of the two species at several temperatures in the laboratory to address the following questions: 1) Are the responses of the two species' growth rates to temperature the same, and do the two species have the same low and high temperature thresholds for growth? 2) Can the length of the life cycle be attributed solely to the thermal sum available in the habitat?

## Methods

### *Study sites*

Two study sites were in the Elbow River drainage in the Bow-Crow Forest in the eastern Rocky Mountain foothills of southwestern Alberta, Canada, and were the same as those used by Benton (1988, 1989). The site on Elbow Creek (50°38'N, 115°00'W, elevation 2103 m) is 50 m downstream from the confluence of a spring source of the Elbow River and outflow from the Elbow Lake (which is much reduced in dry years). The site on Ford Creek is a 2nd-order stream, about 1 km above its confluence with the Elbow River, 25 km downstream from the first site (50°48'N, 114°41'W, elevation 1707 m). Continuously recording Ryan submersible thermographs were placed in both streams from May 1992 to September 1993. However, the Ford Creek thermograph did not record over winter and we estimated below-ice temperatures. The Elbow Creek thermograph did not record during the summer of 1993.

During 1992–1993, temperatures in the two streams were lower than those reported by Benton (1987) (Table 1), and the relative difference between the two streams was greater. Whereas Benton's study showed a 6.6% difference in annual thermal sums for the two sites (Elbow Creek: 1304 day-degrees; Ford Creek: 1390 day-degrees), our measurements from June 1992 through May 1993 showed that Ford Creek was 10.3% warmer (Elbow Creek: 784 day-degrees; Ford Creek: 865 day-degrees). Spring and summer temperatures were lower in both streams in 1993 than in 1992, so the annual sum in Ford Creek from September 1992 through August 1993 was only 771 day-degrees. Elbow Creek showed a similar proportional reduction. Clearly, there is variability among years and Ford Creek's temperatures are particularly affected if ice removal is delayed by a late spring as it was in 1993. The temperature at Elbow Creek is somewhat buffered by its proximity to the spring source.

### *Collecting methods*

Larval samples were taken at monthly intervals from June 1988 to April 1990 (biweekly in Ford Creek in 1988), except for the period from December through March at Elbow Creek and November through April at Ford Creek, when the sites were inaccessible due to road closures and heavy snowfall. Additional samples were taken from Elbow Creek in late August 1990 and 1992 and late July and late August of 1993, and from Ford Creek in mid-May, late July, and late August of 1993. We sampled with a D-frame

net with an inner bag of 0.8 mm mesh and (except in 1988) an outer bag with 0.2 mm mesh. Pools and riffles were sampled at both sites. Samples were taken in water-filled plastic bags on ice to the laboratory, where we sorted, identified, and counted larvae and measured their head widths under 25× or 50× magnification.

*Species identification*

The taxonomy of the North American species of *Ameletus* is in some confusion. Currently, 33 species are recognized (Burrows 1987). Needham et al. (1935) published the only key (to 19 of the 33 species), but it contained numerous shortcomings that were quickly pointed out by McDunnough (1936). In our study, we compared our adult specimens with descriptions of all 33 described species and examined all type specimens. We have identified seven species of *Ameletus* from the two sites—*A. celer* McD., *A. cooki* McD., *A. similior* McD., *A. validus* McD., and three undescribed species. All seven species were found at Ford Creek and four (including *A. celer* and *A. similior*) were common, whereas only two (*A. celer* and *A. similior*) were common at the Elbow Creek site.

A major problem in the elucidation of aquatic insect life histories is the identification of larvae. We approached this problem by rearing adults from late instar larvae and identifying morphological characters of the larvae that would separate the species. We were able to extend these characters back through the life cycle and identify species in all but the first few instars. For these we resorted to Cellulose Acetate Gel Electrophoresis. Of 29 enzymes tested, we used seven (Aconitase hydratase 1 and 2, Adenylate kinase, Esterase, Lactate dehydrogenase, Glucose-6-phosphate isomerase, and Phosphoglucomutase) to separate the species of *Ameletus* present in the study area; Glucose-6-phosphate isomerase also differentiated parasitized larvae (Zloty et al. 1993).

Having characterized the species, we could then apply the electrophoretic technique to life-history elucidation. For each of our field samples containing small larvae, we electrophoretically determined the proportional representation of each species in a subsample of 150 small, unidentifiable larvae. Then we multiplied appropriately to obtain the number of each species in the whole sample.

TABLE 1. Day-degrees above 0°C recorded in Benton's (1987) study and ours. Temperatures were estimated during ice cover at Ford Creek in 1992–1993. "nd" = no data.

| | Benton (1987) | | This study | | | |
|---|---|---|---|---|---|---|
| Month | Ford | Elbow | Ford 1992 | Ford 1993 | Elbow 1992 | Elbow 1993 |
| Jan | 31 | 62 | nd | 16 | nd | 30 |
| Feb | 28 | 56 | nd | 14 | nd | 25 |
| Mar | 70 | 62 | nd | 16 | nd | 31 |
| Apr | 100 | 75 | 31 | 15 | nd | 45 |
| May | 100 | 108 | 106 | 30 | nd | 62 |
| Jun | 182 | 135 | 171 | 125 | 105 | nd |
| Jul | 287 | 172 | 175 | 151 | 108 | nd |
| Aug | 254 | 169 | 191 | 167 | 117 | 107 |
| Sep | 138 | 144 | 118 | nd | 94 | nd |
| Oct | 101 | 132 | 66 | nd | 81 | nd |
| Nov | 68 | 111 | 37 | nd | 52 | nd |
| Dec | 31 | 78 | 16 | nd | 34 | nd |
| Sum | 1390 | 1304 | 957[a]/771[b] | | 784[c] | |

[a] April 1992–March 1993.
[b] September 1992–August 1993.
[c] June 1992–May 1993.

*Laboratory rearing*

Larvae of *Ameletus celer* and *A. similior* were collected from both sites during the summer of 1992 and reared through at least two moults at a light:dark photoperiod of 16:8 and constant temperatures ranging from 0.06°C to 20°C in approximately 2.5°C intervals. Temperatures <10°C were maintained in water baths housed in cold rooms where larvae were kept in 60-mL containers fixed into styrofoam floats. Temperatures >10°C were maintained in small incubator cabinets, where larvae were kept in aerated water in 200-mL jars. Larvae were provided with diatom-encrusted stones which were replaced weekly. Temperatures were recorded twice a day (at about 09:00 and 18:00), and at the end of each experiment the mean temperature to which each individual had been subjected was determined. Temperature variation during the experiment was always <10% of the mean. We recorded dates on which larvae moulted and we measured larval head widths after the first moult and again when a larva died or after it entered the final instar

Growth rates of the two species were compared with a 2-factor ANCOVA, with temperature and species as categorical variables and size as a covariate. Neither logarithmic nor in-

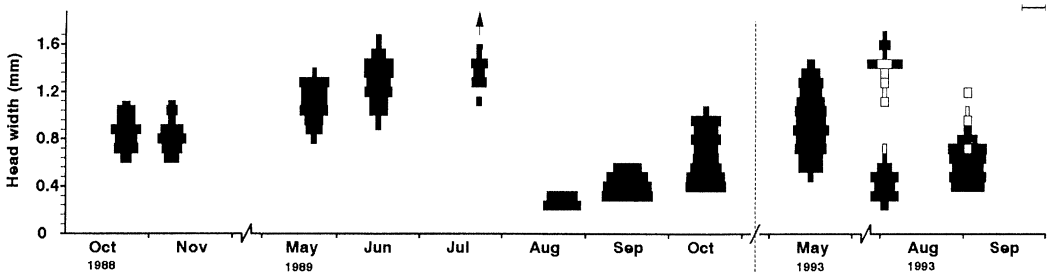


FIG. 1. Size structure of the larval *Ameletus celer* population in Ford Creek in 1988, 1989 and 1993. Larvae <0.4 mm head width were not identified in 1988. Larvae were visually examined for parasitism by a mermithid nematode (open bars) in 1993. The length of the scale line indicates $\log_{10}10$ individuals. The arrow shows adult emergence.

verse transformations corrected for heteroscedasticity, and so the data were weighted before analysis. Trend Analysis (Kirk 1982) was used to test the linearity of the relationship between growth rate and temperature. Because only five obviously parasitized *A. celer* larvae were available, the effect of parasitism on growth rate was not addressed in this experiment.

## Results

### *Growth patterns in the field*

*Ameletus celer at Ford Creek.*—Head width data (Fig. 1) showed that larvae take about one year to develop into adults, with only a single cohort evident except for a very brief period of overlap between generations (e.g.,early August 1993). Eggs hatched in August, and larvae attained a mean head width of 0.6 mm in October, with some growing to 1 mm. Further growth occurred in spring, and adult emergence took place in July. It is unlikely that there were enough day degrees in July for eggs to hatch in the same year in which they were laid. Eggs of *A. celer* took 110 d to hatch in the laboratory at 7°C and hatching continued for another 4 mo. Apparently, eggs took about 1 yr to hatch in the field. It is impossible to accurately determine the physiological time required for growth and development in the field because we do not know the mean dates of egg hatch and adult emergence. However, the site accumulated about 795 day-degrees above 0°C from the estimated date of first hatch in 1992 to the estimated date of first emergence in 1993.

*Ameletus similior at Ford Creek.*—A univoltine life cycle was evident in 1989 and 1993 (Fig. 2). Eggs were laid in late summer but did not hatch before ice formed in November. Small larvae first appeared in May samples. Larvae grew rapidly in May and June and adults emerged from mid-July to mid-September. From April to mid-July 1993, 245 day-degrees above 0°C accumulated at the site.

*Ameletus celer at Elbow Creek.*—Two distinct

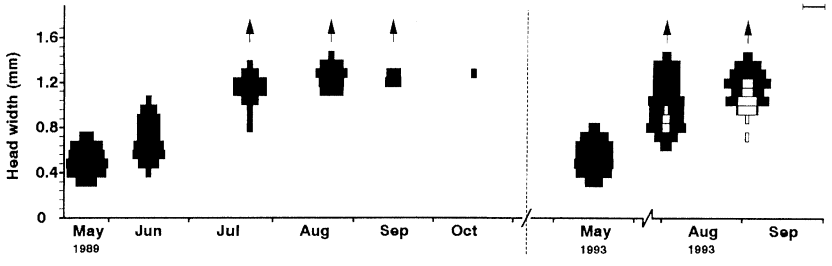


FIG. 2. Size structure of the larval *Ameletus similior* population in Ford Creek in 1989 and 1993. Larvae were visually examined for parasitism by a mermithid nematode (open bars) in 1993. The length of the scale line indicates $\log_{10}10$ individuals. The arrows show adult emergence.

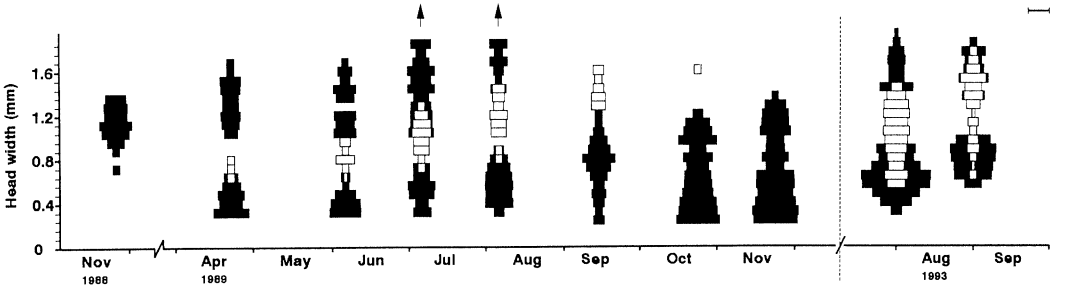


FIG. 3. Size structure of the larval *Ameletus celer* population in Elbow Creek in 1988, 1989 and 1993. Larvae <0.4 mm head width were not identified in 1988. Larvae in the senior cohort were visually examined for parasitism by a mermithid nematode (open bars) in 1989 and 1993. Parasitism was also detected by electrophoresis in the junior cohort but the effects of parasitism at this stage were not advanced enough to clearly separate the cohort into fast- and slow-growing groups. The length of the scale line indicates $\log_{10}10$ individuals. The arrows show adult emergence.

larval cohorts were evident throughout the year, suggesting that *A. celer* larvae took two years to develop at the Elbow Creek site (Fig. 3). As in Ford Creek, eggs appeared to take about a year to hatch. Larvae were recruited into the population from at least September to November when sample collection ended for the year, and some larvae attained a head width of >0.6 mm at this time. We do not have data over the winter for this cohort because we were unable to identify *A. celer* larvae in the autumn of 1988; but a comparison of larval size in April and November 1989 suggests that there was little growth over winter. Larvae grew again in their first summer and in the following year before emergence, but more slowly than in Ford Creek. The largest final instar larvae of *A. celer* in Elbow Creek had head widths 15% larger than final instar larvae in Ford Creek. Adults emerged from Elbow Creek from July to September, somewhat later than in Ford Creek. Based on 1992–1993 temperatures, the Elbow Creek site accumulated about 1343 degree-days above 0°C during the larval life-time of *A. celer*.

A notable features of *A. celer* at Elbow Creek was a high level of infection of larvae by a mermithid parasitoid. These parasitoids can be detected by electrophoresis in small larvae and become visible under the microscope in second year larvae; about 10% of these older larvae were parasitized in April. Parasitized larvae were smaller than non-parasitized larvae of the same cohort (Fig. 3), and the proportion parasitized increased over the summer until all final-year *A. celer* larvae in September were parasitized. This pattern suggests that development was slowed and adult emergence delayed or prevented by parasitism. In parasitized final instar larvae kept in the laboratory, the nematode completely filled the body cavity of the mayfly

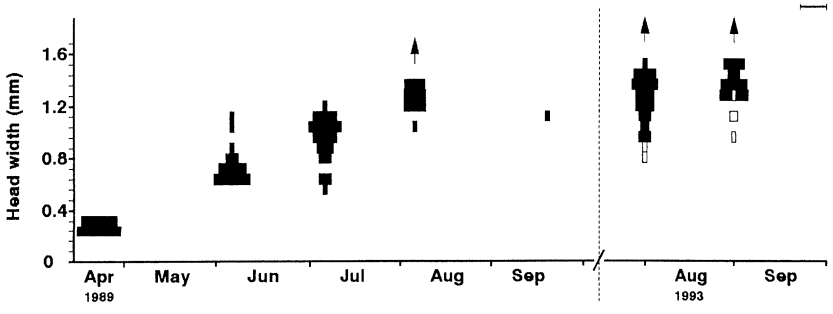


FIG. 4. Size structure of the larval *Ameletus similior* population in Ford Creek in 1989 and 1993. Larvae were visually examined for parasitism by a mermithid nematode (open bars) in 1993. The length of the scale line indicates $\log_{10}10$ individuals. The arrows show adult emergence.

TABLE 2. Analysis of variance table for growth rates of *Ameletus celer* and *A. similior*.

| Source of variation | df | *F* | *p* |
|---|---|---|---|
| Species | 1 | 1.30 | 0.2554 |
| Temperature | 7 | 11.98 | 0.0001 |
| Species × temperature | 7 | 1.03 | 0.4102 |
| Size | 1 | 13.23 | 0.0004 |

larva and usually exited, leaving an empty larval shell. Occasionally, a parasitized, but infertile, adult would emerge. Parasitized male mayfly larvae developed a female-like appearance of the eyes and abdominal sternites 9 and 10.

*Ameletus similior at Elbow Creek.*—The life history of *A. similior* at Elbow Creek (Fig. 4) was univoltine, as at Ford Creek, but emergence occurred later in the year and more day-degrees above 0°C (320) were accumulated during the larval period than in Ford Creek.

### *Growth patterns in the laboratory*

Larvae of both species grew at all temperatures between 0.5°C and 17.7°C and some *A. celer* grew at 20°C. Interactions between the covariate and the main effects (temp × species × size, species × size, temp × size) were non-significant and were successively removed from the covariance analysis. The conclusions (Table 2) were the same from both weighted and unweighted analyses. There was no difference between the growth rates of the two species ($p > 0.2$), but growth rate increased significantly with temperature (Fig. 5) and decreased significantly with size (Figs. 6 and 7) ($p < 0.001$). Linear regression analysis gave the following relationships:

*A. celer*:

$$GR = 0.0064 + 0.0005T - 0.0038S \quad (1)$$

*A. similior*:

$$GR = 0.0115 + 0.0006T - 0.0119S \quad (2)$$

where GR = % growth rate (mm mm$^{-1}$ d$^{-1}$), T = temperature (°C), and S = size (mm).

Trend Analysis showed that 82% of the temperature effect was explained by a linear relationship, but a significant amount of variation was unexplained ($p < 0.05$). Addition of a quadratic component increased the value to 85%, but the difference was not significant ($p > 0.1$).

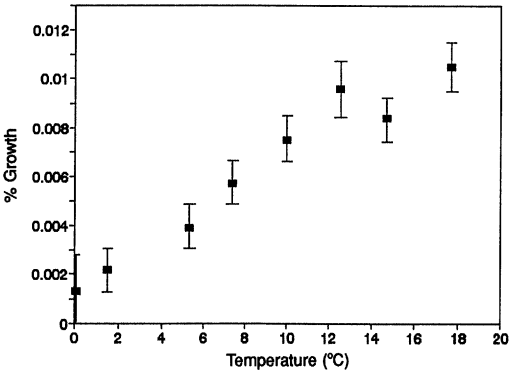


FIG. 5. The effect of fixed temperatures on growth rate (mm/mm/day expressed as %) of larval *Ameletus celer* and *A. similior* in the laboratory. Data from both species are pooled and adjusted for larval size. Means ± 1 SE are shown.

## Discussion

Benton (1988) argued that although Elbow Creek was warmer than Ford Creek during the winter, these temperatures were too low to allow significant growth at either site during the winter, whereas the summer temperatures led to a univoltine life cycle in *Ameletus celer* in Ford Creek but a semivoltine life cycle in *A. similior* at Elbow Creek. Benton (1988) then went on to argue against the use of annual thermal sums above 0°C in predicting aquatic insect growth patterns, given that two similarly-sized mayflies that lived in streams in the same catchment with similar thermal sums had very different life cycles. However, further sampling from the two sites used by Benton suggested that this explanation may not be correct. First, the annual thermal sums of the two sites were different in the years of our study and very different from those reported by Benton (1987). Second, the two species are not of similar size,

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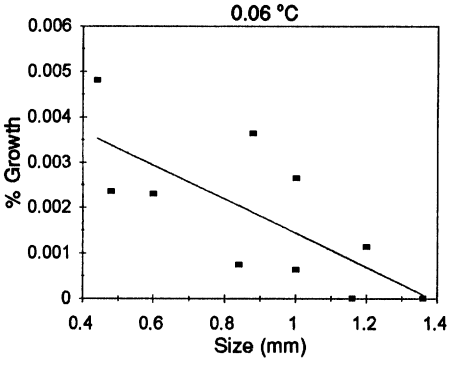
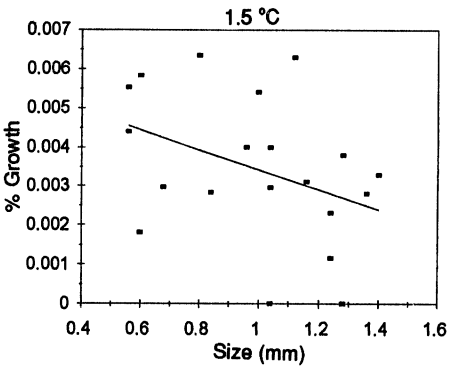
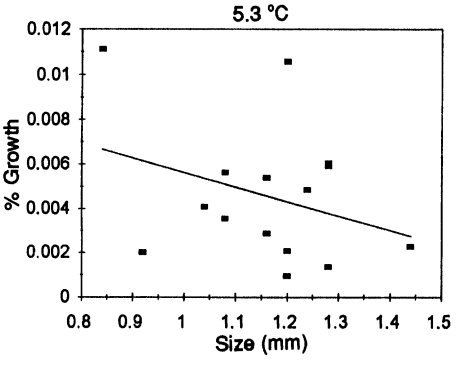
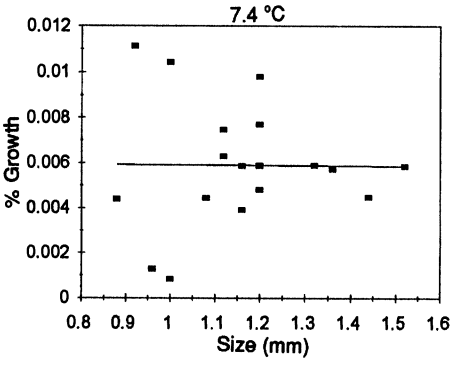
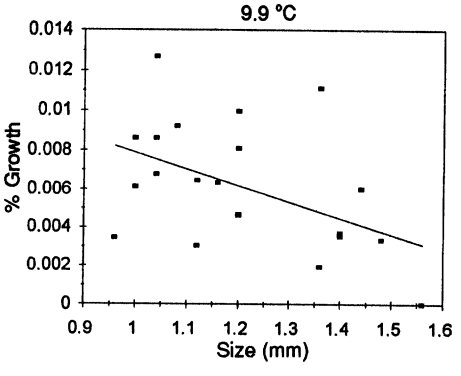
FIG. 6. The effect of *Ameletus celer* larval size on growth rate (mm/mm/day) at fixed temperatures in the laboratory. Least square linear regression lines are shown.

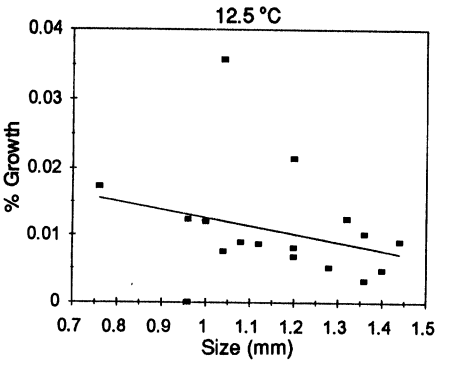

0.06 °C
% Growth
Size (mm)

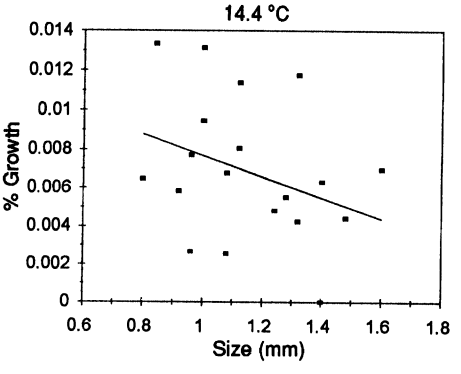

1.5 °C
% Growth
Size (mm)

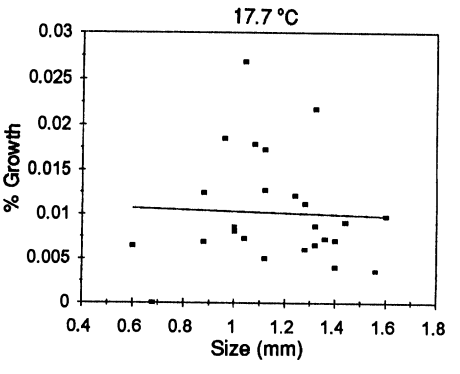

5.3 °C
% Growth
Size (mm)

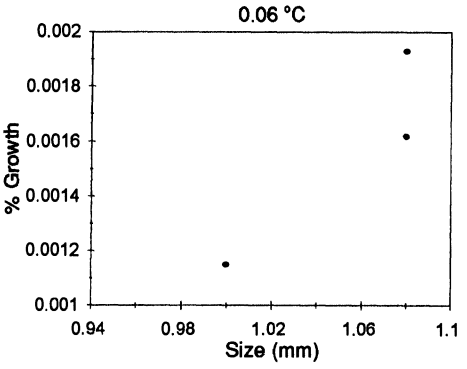

7.4 °C
% Growth
Size (mm)

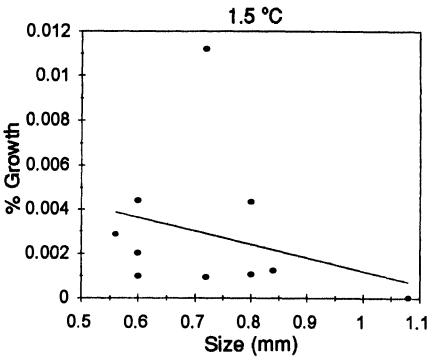

9.9 °C
% Growth
Size (mm)

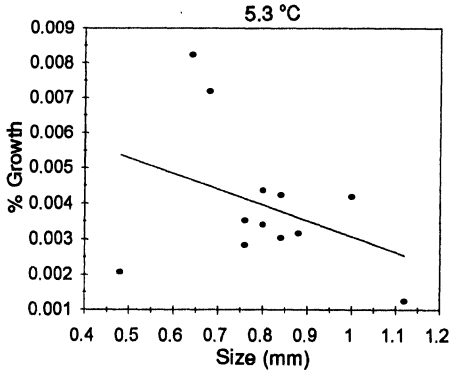

12.5 °C
% Growth
Size (mm)

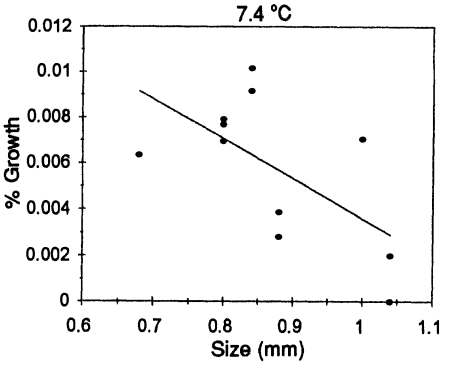

14.4 °C
% Growth
Size (mm)

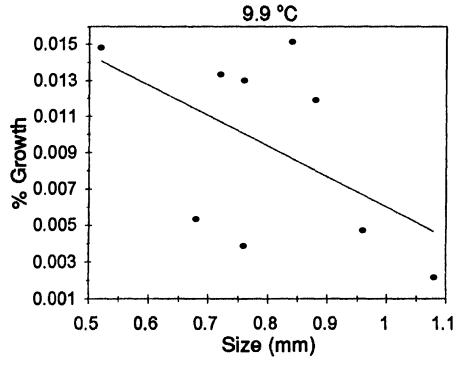

17.7 °C
% Growth
Size (mm)

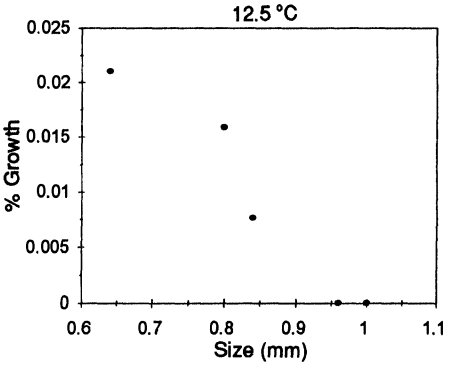

0.06 °C
% Growth
0.002
0.0018
0.0016
0.0014
0.0012
0.001
0.94
0.98
1.02
1.06
1.1
Size (mm)

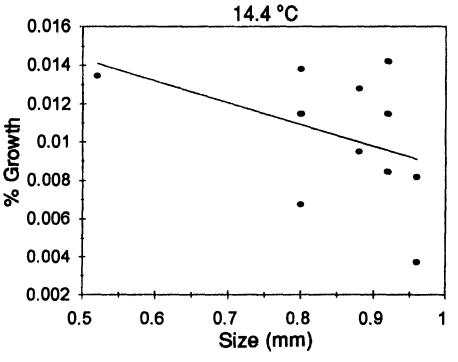

1.5 °C
% Growth
0.012
0.01
0.008
0.006
0.004
0.002
0
0.5
0.6
0.7
0.8
0.9
1
1.1
Size (mm)

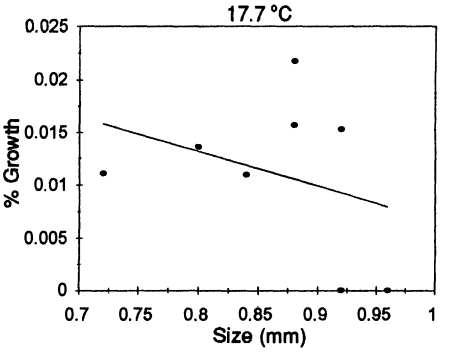

5.3 °C
% Growth
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
Size (mm)


7.4 °C
% Growth
0.012
0.01
0.008
0.006
0.004
0.002
0
0.6
0.7
0.8
0.9
1
1.1
Size (mm)


9.9 °C
% Growth
0.015
0.013
0.011
0.009
0.007
0.005
0.003
0.001
0.5
0.6
0.7
0.8
0.9
1
1.1
Size (mm)


12.5 °C
% Growth
0.025
0.02
0.015
0.01
0.005
0
0.6
0.7
0.8
0.9
1
1.1
Size (mm)


14.4 °C
% Growth
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0.5
0.6
0.7
0.8
0.9
1
Size (mm)


17.7 °C
% Growth
0.025
0.02
0.015
0.01
0.005
0
0.7
0.75
0.8
0.85
0.9
0.95
1
Size (mm)

*A. celer* being considerably larger than *A. similior*. And third, there are several species of *Ameletus* at the two sites and they are not easily distinguished as small larvae (Zloty et al. 1993). In fact, *A. celer* and *A. similior* were common at both sites and not restricted to single sites. Thus, whereas Benton (1988, 1989) reported a one-year life cycle for *A. celer* in Ford Creek and a two-year life cycle for *A. similior* in Elbow Creek, our results show that *A. similior* has a one-year larval life at both sites, whereas *A. celer* has a one-year larval life at the warmer site but a two year larval life at the cooler site.

In Clifford's (1982) review, 92% of the Siphlonuridae (to which *Ameletus* had been assigned until recently; see McCafferty [1991] for an alternative classification) were univoltine, with a roughly equal split between summer cycles (eggs over-winter) and winter cycles (larvae over-winter). The nine populations of the only *Ameletus* species then studied (*A. inopinatus*) all had univoltine winter cycles. Since then, univoltine life cycles have also been described for the eastern North American species *A. tarteri* (Matthews and Tarter 1989) and *A. ludens* (Giberson and Mackay 1991). If we modify the definition of univoltine to discount the year spent in the egg stage by *A. celer*, all seven species in Ford Creek are univoltine (Zloty and Pritchard, unpublished data). Four species (*A. cooki, A. similior, A. validus* and one undescribed species) have summer cycles and three (*A. celer* and two undescribed species) have winter cycles. The summer species completed larval growth within about 4 or 5 mo, and eggs were presumably in diapause for the other 7 mo of the year. The larger winter species required 11–12 mo to complete larval growth at Ford Creek, although little growth occurred during the four winter months.

The summer species are all much the same size (body length of final-instar larva, excluding the caudal filaments, is 8–10 mm), and much smaller than the winter species (which are 16–20 mm). Thus, the larger the species the longer period of time taken to reach emergence size. Given that the growth rates of *A. celer* and *A. similior* in the laboratory respond in the same way to fixed temperatures, it might appear that at Ford Creek larvae grow until they reach some final size and then emerge. Thus, the length of the larval period is determined by final larval size. Also, if the relationship between growth rate and temperature is linear from 0°C over the whole range experienced in the field, we would expect that a thermal sum above 0°C would determine the length of the life cycle. Thus, *A. similior*, which is small and is estimated to complete its life cycle in about 245 degree-days above 0°C in Ford Creek, should also be able to complete its larval development in Elbow Creek between March and July, even in the cold year of 1993. However, on the basis of 795 day-degrees accumulated over its larval growth period in Ford Creek, *A. celer* should not be able to quite complete its life cycle in one year in the years of our study at Elbow Creek (although it should have been able to do it in one year in Benton's study). This was indeed the case, but the explanation is not quite so simple. Not only is there an extension of real time to complete the life cycle in Elbow Creek but also an extension of physiological time. *Ameletus similior* requires possibly 30% more degree-days to complete development in Elbow Creek, and *A. celer* requires perhaps 70% more.

Of the factors listed in the Introduction that may limit the use of thermal sums as predictors of voltinism, most can probably be ruled out. There is no indication of developmental delays unrelated to the direct effects of temperature on metabolism (point 1) in the growth patterns of either species. Also, it is unlikely that the placement of recruitment in the annual thermal cycle (point 4) provides an explanation for the slower growth in Elbow Creek. Although small larvae of both species grew faster than large larvae, recruitment was at much the same relative time in the thermal cycle in both streams.

Diet can interact with temperature to influence aquatic insect life histories (e.g., Anderson and Cummins 1979, Sweeney 1984, Söderström 1988). A possible explanation for the extension of life cycles in physiological time at Elbow Creek is that food supply is limited (point 5) in the spring-fed water of this site. We have no

←

FIG. 7. The effect of *Ameletus similior* larval size on growth rate (mm/mm/day) at fixed temperatures in the laboratory. Least square linear regression lines are shown where >5 data points are available.

data on food, but Hawkins (1986) found little evidence that food supply affected growth rates of ephemerellids in streams in Oregon and Alberta. Food supply was more likely correlated with population density, and the only possibly food-related decrease in growth rates occurred in sites with prolonged ice-cover. If this was true of our sites, we would have seen slower growth rates in Ford Creek.

Parasitism (point 5) is not a reason for the 2-yr life cycle of *A. celer* at Elbow Creek, even though growth rate of parasitized mayflies is slowed. Most individuals in the population are not parasitized and it is these individuals that have the 2-yr life cycle. Also, *A. celer* larvae are parasitized in Ford Creek, although prevalence is much lower, and univoltine *A. similior* are sporadically parasitized as well. The lower prevalence of parasitism in *A. similior* may be related to mayfly size (see e.g., Calentine et al. 1970). The host must be large enough to support the parasitoid throughout its parasitic stage, and *A. similior* may simply not be large enough to support this large nematode. However, we do not know whether the same species of nematode occurs in both mayflies nor do we know anything about the life history of the parasitoids. A similar situation was observed in northern Sweden by Söderström and Johansson (1988), where only one of two coexisting species of *Parameletus* was parasitized by a mermithid. Again it was the larger species that was infected, but both mayflies had similar life histories and the authors were at a loss to explain the specificity.

This leaves a thermal minimum above 0°C (point 2) and a sigmoid relationship between growth rate and temperature (point 3) as possible explanations for the extra day-degrees required by both species in Elbow Creek. Determinations of thermal minima by extrapolation of Equations 1 and 2 show an increase with larval size, but only in large *A. similior* does it rise above zero. However, some larvae of all sizes of both species did grow, albeit very slowly, at 0.06°C in the laboratory, and there is no noticeable drop in growth rate of large larvae in the field. Thus it seems unlikely that the use of a thermal minimum of 0°C is a major source of error in the measurement of physiological time in the field. And the departure from linearity of the relationship between growth rate and temperature appears insufficient to account for the discrepancy.

There is, however, one further phenomenon which might provide at least a partial explanation for the extension of physiological time in *Ameletus celer* in Elbow Creek. Clearly, in this highly seasonal environment larvae cannot simply grow to mature size and then emerge; adult emergence has to be precisely timed. This problem is not restricted to *A. celer* in the headwaters of the Elbow River; it is a general question that can be asked of all life cycles that are exactly one year or multiples thereof in length. Developmental delays that are cued by photoperiod during the larval stage are usually implicated (Norling 1984, Tauber et al. 1986, Danks 1987, 1991), but these delays have never been reported in mayflies and our data do not show developmental delays when temperature is favorable for growth. Newbold et al. (1994) have developed a two-stage model, based solely on degree-days, that explains emergence synchrony in mayflies and univoltinism over a wide range of thermal regimes. They propose that a summer dormancy when temperatures are above a maximum temperature for growth can lead to an equivalent effective day-degree exposure under a variety of thermal regimes. This prepares individuals for the second phase, namely development to emergence, which requires a fixed amount of physiological time.

However, the observation that final instar *A. celer* larvae are larger in Elbow Creek than in Ford Creek suggests that the extended life history of *A. celer* in Elbow Creek might be explained in part by a different process; they simply keep growing under the direct effect of temperature until the appropriate cues for emergence occur. That is, development time is not determined by final size, but vice versa. Although larvae cannot achieve minimum size for emergence in their first year of growth, they do not require a whole extra year to attain that size. So, just as Sweeney and Vannote (1986) showed a correlation between female size and degree-days in the stonefly *Soyedina carolinensis*, larvae of *A. celer* in Elbow Creek may use the additional day-degrees that are available to grow to a larger size. This would lead to increased fecundity which might offset the extra mortality experienced in a 2-yr larval life.

However, this is only a partial explanation.

We do not know what the cues for adult emergence are. Neither do we know why *A. similior*'s life cycle is also extended in physiological time in Elbow Creek, nor why *A. celer* grows so slowly in its first year of life. Probably the difficulty of measuring physiological time accurately in the field and the problems in applying laboratory data directly to the field situation (Hawkins 1986) provide the rest of the explanation.

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