Biology of the mayfly *Leptophlebia cupida* (Say) (Ephemeroptera:Leptophlebiidae)

HUGH F. CLIFFORD, HAL HAMILTON, AND BRIAN A. KILLINS
Department of Zoology, University of Alberta, Edmonton, Alta., Canada T6G 2E9

Received October 30, 1978

CLIFFORD, H. F., H. HAMILTON, and B. A. KILLINS. 1979. Biology of the mayfly *Leptophlebia cupida* (Say) (Ephemeroptera:Leptophlebiidae). Can. J. Zool. **57**: 1026–1045.

Leptophlebia cupida (Say) is a widely distributed and extensively reported mayfly species of North America. In the Bigoray River, a brown-water stream of Alberta, Canada, *L. cupida* is a univoltine species. The rate of progressing through the life cycle, i.e. predicted instar intervals, was found to be better correlated with the amount of cumulative degree days (water temperature) that the nymphs receive than with the cumulative calendar days of the life cycle. *Leptophlebia* subimagoes had a higher total potential fecundity per unit body size than any of the other Bigoray River mayflies.

Nymphs are fine particle detritivores, ingesting about 96% detritus and 4% diatoms. Average particle size ingested was 38 μm. In the laboratory at 20°C, there were 34 nymphal instars; but there is probably no fixed number of nymphal instars, the 34th being just one of several instars in which the nymphs, given the proper environmental cues, might transform. The first nine instars are figured.

Using mainly laboratory data, we investigated the relative contribution that small individuals (< 1.5 mm) make to cohort production. This theoretical exercise indicated that the error which occurs in cohort production estimates by not adequately sampling the small individuals could be less than 10%.

CLIFFORD, H. F., H. HAMILTON et B. A. KILLINS. 1979. Biology of the mayfly *Leptophlebia cupida* (Say) (Ephemeroptera:Leptophlebiidae). Can. J. Zool. **57**: 1026–1045.

Leptophlebia cupida (Say) est une éphémère très répandue et très connu en Amérique du Nord. Dans la rivière Bigoray, ruisseau d'eau colorée de l'Alberta, Canada, *L. cupida* est une espèce univoltine. Le taux du déroulement du cycle biologique, i.e. les intervalles prévus entre les stades, est relié plus directement au nombre cumulatif de jours-degrés (température de l'eau) que reçoit la larve qu'au nombre absolu de jours écoulés dans le cycle. Les subimagos de *Leptophlebia cupida* ont, par unité de taille, une fécondité totale possible plus élevée que toutes les autres éphémères de la rivière Bigoray.

Les larves sont des détritivores consommatrices de fines particules, leur diète étant composée de 96% de détritus et de 4% de diatomées. La taille moyenne d'une particule consommée est de 38 μm. En laboratoire, à 20°C, il y a 34 stades larvaires; en réalité, il n'y a probablement pas de nombre fixe de stades, le trente-quatrième stade étant seulement l'un des nombreux stades pendant lequel peut se produire la métamorphose si la larve se trouve dans des conditions favorables à sa transformation. On trouvera ici l'illustration des neuf premiers stades.

La contribution relative des petits individus (< 1.5 mm) à la production de la cohorte a pu être estimée à partir surtout des données de laboratoire. Cet exercice théorique indique que, dans le calcul de la production, l'erreur entraînée par l'abandon des petits individus pourrait être de moins de 10%.

[Traduit par le journal]

Introduction

Leptophlebia cupida (Say), formerly *Blasturus cupidus* Say, is probably the most widely distributed of the 10 North American *Leptophlebia* species. In Canada, *L. cupida*'s range extends almost from the Atlantic to the Pacific, the species having been reported from Nova Scotia (Clemens 1915) to British Columbia (Scudder 1975). Generally, in North America, *L. cupida* is confined to east of the Cordillera; its southern limits appear to be Georgia (Berner 1977), and the species occurs at least as far north as the Great Slave Lake region in the Northwest Territories. A very closely related species, *Leptophlebia nebulosa* (Walker), has been collected in the Mackenzie River system at latitude 68° (Wiens *et al.* 1975). Possibly *L. cupida* and *L. nebulosa* are variants of the same species. The single distinguishing feature of the two is the brown cloud covering the outer third of the forewing in *L. nebulosa* males; the cloud is absent in *L. cupida* males. It is presently impossible to distinguish the nymphs and adult females of the two species; several workers have commented on this. Also, especially in north temperate regions, the two species usually occur together and emerge at about the same time.

Leptophlebia cupida nymphs are generally found on soft, small-particle substrate of lakes and

0008-4301/79/051026-20$01.00/0

slow-moving streams. In spring, stream populations often migrate via vernal tributaries into marshes and other lentic habitats. At times, the nymphs can occur in very large numbers; and, being large nymphs when mature (female nymphs sometimes weigh in excess of 7 mg dry weight), they can contribute significantly to the standing crop of aquatic systems. The species is common enough in North America to be given the name black quills by fly fishermen (Swisher and Richards 1971; Schwiebert 1973).

In terms of biomass, *L. cupida* nymphs are the single most important species of the benthos for much of the year in a brown-water stream of Alberta, Canada. In this stream, various aspects of *L. cupida*'s biology have been studied specifically or as part of other investigations. Recently, we completed studies on the nymphs' food habits and laboratory growth and development. The purpose of this report is to give a comprehensive life cycle description of this widely distributed species by (1) bringing together the large amount of data available on *L. cupida*'s biology in the brown-water stream and from other geographical areas, (2) reporting on its food habits and laboratory growth, and (3) relating the laboratory study to field phenomena.

Study Area

The Bigoray River (53°31′ N, 115°26′ W), part of the Arctic Ocean Drainage, is a slow-moving meandering type stream located in west-central Alberta, Canada. At the study site, average winter base flow is 0.14 m^3/s and average summer base flow is 0.80 m^3/s. Average gradient to the study site is 3.1 m/km. The stream is completely ice-covered from mid November until early April. Maximum water temperatures rarely exceed 18°C and daily fluctuations are never greater than 3°C. Average total degree days of water temperature (based on mean daily water temperatures) per year is only 2190. Chironomidae larvae (35%), Ephemeroptera nymphs (17%), and Ostracoda (13%) account for 65% of the total yearly fauna by numbers, when averaged over several years. The white sucker *Catostomus commersoni* (Lacépède) is the only abundant fish and then only during its spring spawning run from the Pembina River. A detailed limnological description of the stream is given by Clifford (1978).

Life Cycle

Nymphs

Autumn and Winter Populations

Based on 5 years of bottom fauna samples, *L. cupida* nymphs accounted for 6.6% of the total yearly bottom fauna by numbers. It was the most abundant mayfly species, accounting for 39% of the total yearly mayfly fauna (Clifford 1978). The life cycle is univoltine (Fig. 1). New generation nymphs first appear in late July, and they grow rapidly during the remainder of the ice-free season. Based on compound eye development and the appearance of the forceps, some male nymphs could first be distinguished in August, when the nymphs were about 6 mm in length. By early November, at which time the stream freezes over, some nymphs appear to be fully grown. There is a continuous influx of small individuals into the population in autumn, indicating extensive delayed hatching.

The developmental stages of Fig. 1 are based on mesothoracic wing pad development: stage I nymphs lacked wing pads; stage II nymphs had wing pads whose lengths were shorter than the distance separating the two wing pads; stage III nymphs had wing pad lengths that were greater than the distance separating the two pads; stage IV nymphs had darkened wing pads, indicating a discrete instar, the last nymphal instar. One can recognize three growth and development phases: (1) rapid growth and development in autumn, especially early autumn, when water temperatures are high; (2) slow growth and very little development in winter when water temperatures are constant and near 0°C; and (3) resumption of growth and rapid development in spring when water temperatures are exhibiting maximum rate increase.

Spring Migration

In April, during and shortly after the ice goes out, nymphs move to the shore and then start following the shoreline upstream. All sizes of nymphs participate in the migration. The nymphs follow the main stream's shoreline upstream until it bends in towards a tributary, usually small vernal tributaries. This leads the nymphs out of the main stream and into the tributaries and subsequently into the marshy regions drained by the tributaries. Most tributaries have about the same water temperature as does the main stream. The nymphs move up all tributaries with seemingly no preference. Usually by 1 June, most of the population is extensively dispersed in the marshy areas drained by the tributaries. However a residual population remains in (and emerges from) the main stream. The growth and development features for the June dates of Fig. 1 are based on the combined main stream and tributary–marsh populations, whereas the water temperature data are from only the main stream.

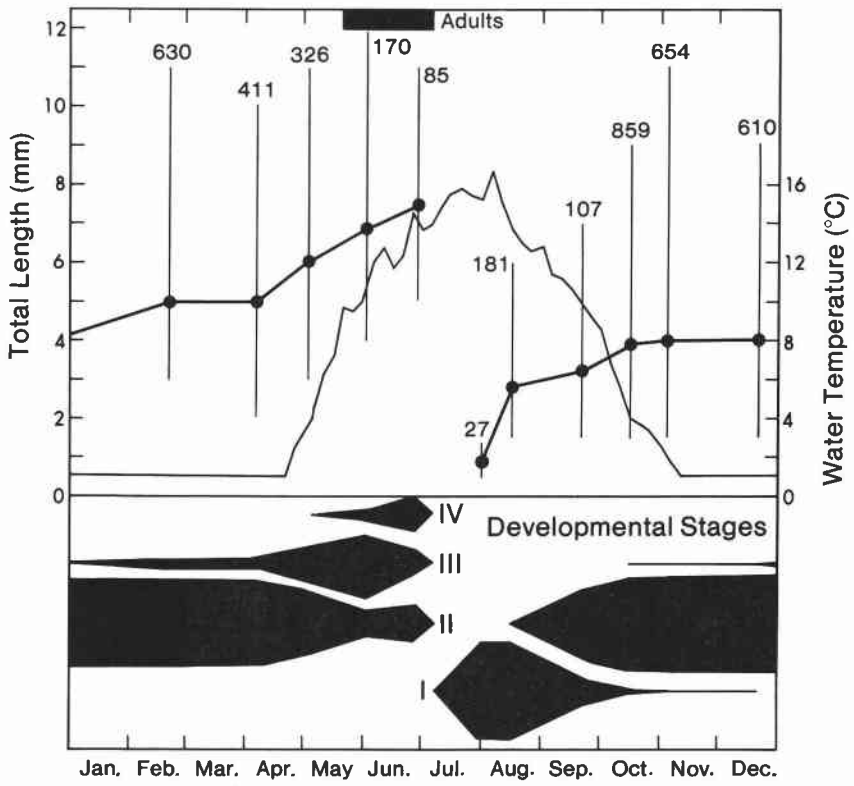

FIG. 1. Growth, developmental stages, and emergence of the *L. cupida* population, 1966–1967, as related to average 5-day water temperatures of main stream. Mean size per sampling date derived from number of nymphs indicated above range. For developmental stages (see text for further explanation), width of the spindle is proportional to the number of nymphs per sampling date; greatest width, e.g. August, equals 100%. Adults include both main stream and marsh specimens.

Traver (1925) was the first to report the migratory behavior of *L. cupida* nymphs (Ithaca, New York), and Neave (1930) gave a detailed description of the migration in Manitoba. Subsequently, observations on the migration were made by Ide (1935) in Ontario, Lyman (1955, 1956) for a lake population of *L. nebulosa* in Michigan, Prather (1969) in Ohio, and Coleman and Hynes (1970) in Ontario. In the Bigoray River, the initial movements in the main stream were associated with a rise in water level (Hayden and Clifford 1974). Upstream movements are much more pronounced during daylight hours than at night. This is in contrast with the typical night-active drift pattern of this species and of mayflies in general. The immediate adaptive value of the movements would seem to allow the nymphs to escape from the springtime turbulence of the main stream. Other workers have suggested that the springtime movements into the marshes allow the nymphs to exploit a more plentiful food supply or expose the nymphs to warmer water and hence accelerate development. As indicated in the next section, there was no evidence that marsh nymphs ingested greater amounts of detritus and diatoms than did nymphs of the main stream. Also, the marsh that we studied accumulated fewer degree days than did the main stream, e.g. between 15 April and 31 May 1970, the marsh accumulated 260 degree days as opposed to 280 for the main stream.

Allometric Growth

During and after the migration, as water temperatures rise, there is a springtime acceleration of nymphal growth and development. The nymphs change shape as they mature. An allometry of size study indicated that none of the body part dimensions (head length, pronotum width, mesonotum width, length, and abdomen width) exhibited isometric growth with total length (Clifford 1970). All female nymphs' dimensions and some male nymphs' dimensions deviated from simple size allometry when the nymphs were about 7 to 9 mm in total length. It is as if the nymphs were passing from an 'immature' to 'mature' stage. Generally, there are accelerated changes in the percentage composition of the development stages when the nymphs

are about 7 to 9 mm in total length; this takes place in late April, May, and June. If the deviations are not due simply to random size variations of the body parts as the nymphs increase in total length, it is possible that the deviations reflect the changing ratios of the parts' specific growth rates, due to the induction or accelerated development of adult structures. Hence, the suggestion is that once the nymph achieves the 'mature' stage, it will very shortly be able to transform regardless of size. Just how long it remains in the mature stage (and continues to grow) before emerging will depend on the proper exogenous cues necessary for this phenomenon. The net result would be to accumulate nymphs in a 'potential-emerging' stage and hence synchronize emergence.

Two power functions best expressed the length–weight relationship of *L. cupida* nymphs. For specimens (male and female nymphs combined) less than 6.0 mm:

$$W = 0.0045L^{2.23}, \quad r^2 = 0.99$$

for specimens 6.00 mm and larger:

$$W = 0.0002L^{4.01}, \quad r^2 = 0.99$$

where W is dry weight in milligrams and L is body length in millimetres. These functions are based on field-preserved specimens and 103 first and second instar nymphs of the laboratory cultures. Empirically determined individual dry weight of first and second instar nymphs combined (from laboratory cultures) (average total length = 0.49 mm) was 0.00093 mg. We could not detect ash in nymphs smaller than 2.0 mm. For size classes 2 to 14 mm, the average ash content of *L. cupida* nymphs was 12.4%, SD = ±5.7%, SE = 0.91, with no tendency for larger nymphs to have relatively more or less ash than smaller nymphs. We did not determine caloric content. Kelso (1973) reported 5453 calories/g dry wt. for *L. cupida* nymphs.

Standing Crop, Drift, Fish Food

Using a coring device that sampled 70 cm^2 of substrate to a depth of ca. 20 cm, we determined that in 1974 the average monthly abundance of *L. cupida* nymphs in the main stream was the following: July, 93/m^2; August, 1210/m^2; September, 3884/m^2; and October, 1984/m^2. Density estimates were considerably lower when a box-type sampler, enclosing an 0.08-m^2 area and having a mesh size of 320 μm, was used; for comparable months in 1971, densities per square metre were 34 (July), 275 (August), and 119 (October) (Clifford 1972*b*).

During a 1-year drift study, *L. cupida* nymphs comprised a total yearly drift density of 1195/100 m^3 of water filtered by the nets (Clifford 1972*a*). *Leptophlebia cupida* nymphs accounted for 15% of all mayflies found in the drift. (In contrast, they accounted for 39% of yearly benthic mayflies; this discrepancy is due in part to the much greater propensity of *Baetis* to drift. *Baetis* accounted for 33% of the benthic mayflies, but 72% of the drifting mayflies.) The nymphs exhibit a night-active behavioral drift pattern typical of most mayflies. They retained the night-active pattern even during the long winter (December and March samples), when the stream was completely frozen over and covered by a thick layer of snow. Most of the *L. cupida* population have moved out of the main stream when the white suckers make their spawning run up the Bigoray River, usually in early May. However, those nymphs remaining in the main stream are an important food item of the suckers. Bond (1972) found that *Leptophlebia* nymphs occurred in the stomachs of 44% of the upstream moving suckers (mainly between 10 May and 26 May 1969), the nymphs comprising 10% of the total weight of the stomach contents. Nymphs were found in only 23% of the postspawning, downstream-moving suckers (20 May to 4 June), accounting for 3% by weight of total stomach contents. *Leptophlebia cupida* nymphs are not important in the diet of young-of-the-year suckers, which start hatching in late June and have moved out of the stream by early August.

Adults

Emergence

Emergence in the main stream usually starts in mid-May, when early-hatching nymphs would have accumulated about 2000 degree days. In 1973, *L. cupida*'s (including four *L. nebulosa* males) cumulative emergence percentages for the main stream were the following: 18 May, 0%; 21 May, 4%; 28 May, 52%; 4 June, 69%; 7 June, 73%; 14 June, 88%; 18 June, 95%; and 25 June, 100%. These data were derived from an emergence study (Boerger and Clifford 1975), where 16 floating box traps, each 0.1 m^2 in area, were arranged in four transects across the stream. The traps were operated continuously from 25 April to 23 October 1973. Total yearly emergence for *L. cupida* in the main stream was 28.5 (43% males, 57% females) per square metre. This was only 8% of all mayflies emerging in 1973, indicating (re *L. cupida* nymphs' 39% benthic composition) that probably 75 to 85% of the *L. cupida* population moves into the marshes during the spring migration.

In a 1969 quantitative emergence study of the marsh (Hayden 1971), where 12 traps were extensively dispersed throughout the marsh, emergence

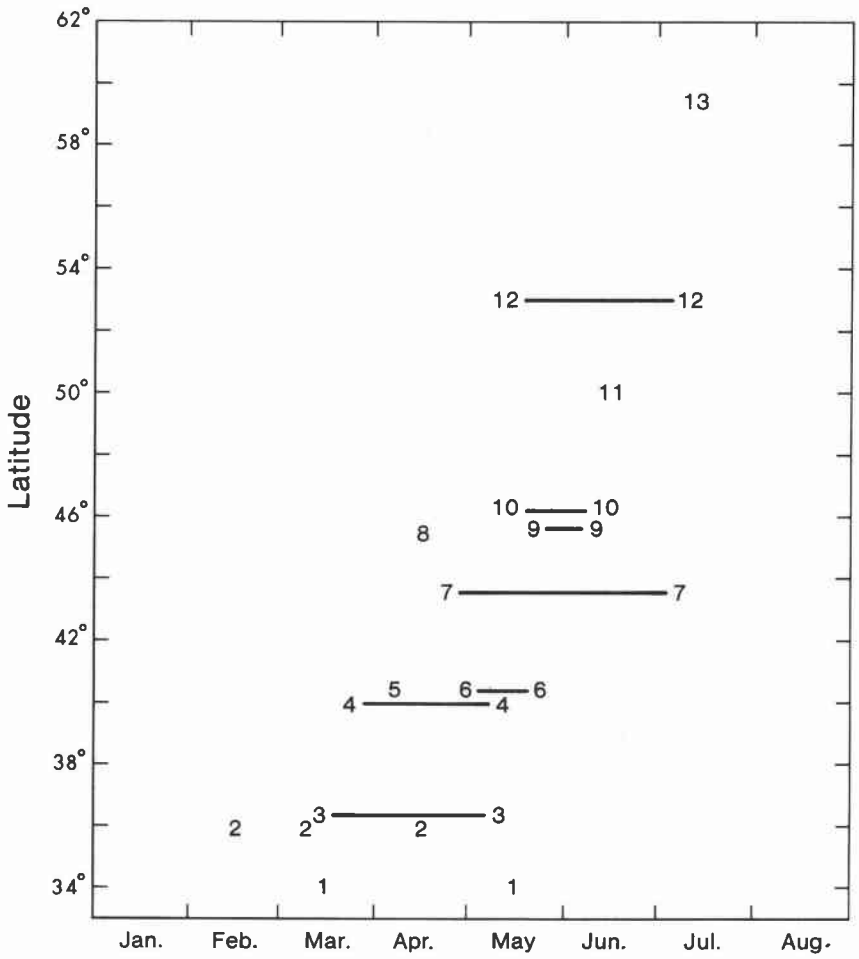

FIG. 2. Emergence of *L. cupida* (and *L. nebulosa*) in North America as related to latitude. A horizontal line indicates extensive records throughout the emergence period; others are mainly spot collections. Source: 1, 2, Berner (1977); 3, Peters and Warren (1966); 4, Burks (1953); 5, 13, Spieth (1938); 6, Traver (1925); 7, Coleman and Hynes (1970); 8, Hilsenhoff *et al.* (1972); 9, Lyman (1955); 10, Harper and Magnin (1971); 11, Harper *et al.* (1975); 12, present study.

started on 20 May. Maximum emergence occurred in the last week of May and first week of June, with a few specimens continuing to emerge through July, especially in marshy areas that warmed slowly. Based on these two studies and 9 years of field notes, over 95% of the *L. cupida*'s marsh-plus-mainstream population emergences during a 50-day period (mid-May to early July), with maximum emergence about 1 June. In other geographical areas, *L. cupida* adults have been reported 'on the wing' as early as February (Berner 1977). There is a definite tendency for the emergence period to start later in the year as one proceeds northward (Fig. 2). And, even considering all the vagaries of associating temperature with latitude, this would appear to be mainly a response to water temperatures. It would be instructive to know whether *L. cupida* is univoltine throughout its range. The emergence data for this species in northwestern Arkansas (Peters and Warren 1966) would indicate that it has a univoltine cycle at latitude 36°.

Penultimate and Ultimate Molts

Emergence from both the main stream and the marshes was essentially an afternoon event, mainly between 1200 and 1600 hours. Emergence was also confined to the afternoon in laboratory cultures, where we observed the males invariably emerging before the females. The penultimate molt has been described by several workers. These reports (Morgan 1911; Traver 1925; Lyman 1955; Leonard and Leonard 1962) and our observations indicate a fairly consistent pattern of three phases. (1) There is a period of vigorous swimming, lasting anywhere from a few minutes to over an hour. (2) This culminates in the nymph being at the water's surface and grasping vegetation or stones that project out of the water; the nymph first breaks the surface film with

its head and the dorsal part of the thorax. (3) The ecdysis per se usually takes less than 5 min. The subimago escapes from the old nymphal exoskeleton via a medial suture, appearing first on the mesonotum and quickly widening to encompass the pronotum and metanotum. Once free of the old exoskeleton, the subimagoes are usually airborne in 2 or 3 min. Our laboratory observations and reports from other workers indicate that the subimago stage lasts about 20–24 h.

Transformation to the imago has been described in detail by Traver (1925). This ultimate molt is similar to the penultimate molt. However, at least in our laboratory cultures, it appears to be a much more precarious molt, as the majority of the imagoes were never able to completely extricate their wings from the subimago exuviae. We have never observed mating flights for Bigoray River populations of *L. cupida*. Other reports indicate that the swarms are small (seldom consisting of over 30 individuals and usually less than 10), take place about 3 to 10 m over either water or open areas some distance from the water, and occur during daylight, usually in the late afternoon (Morgan 1913; Traver 1925; Lyman 1955; Leonard and Leonard 1962). Morgan (1911) observed aerial copulation as taking but a fraction of a minute, with the female imago dropping to the water's surface to oviposit as soon as the couple separated.

For Bigoray River populations, the female imagoes returned to the main stream; we have never observed them ovipositing in the marshes or other lentic habitat. The ovipositing female released a few eggs at a time by repeatedly dipping the abdomen into the water while flying low over the water, either upstream or downstream. It was difficult to keep track of individual females, but it appears that the time from when ovipositing started until the spent female had perished (usually by alighting on the water and not being able to become airborne again) was less than 5 min. Oviposition occurred during daylight hours, in the morning as well as the afternoon, indicating that the imago stage can be considerably less than 24 h. Regardless, the maximum longevity for *L. cupida* combined subimago and imago stages is about 48 h.

Size of Adults, Fecundity

Both male and female adults vary greatly in size. For example, since 1966 we have collected male subimagoes ranging in total length from 7.9 to 11.9 mm and dry weight from 1.41 to 3.72 mg. Female subimagoes can be slightly larger (8.0 to 14.5 mm) and, because of egg biomass, considerably heavier (1.36 to 7.10 mg). The length–weight relationship for female subimagoes (18 preserved specimens) can be expressed by the power function:

$$W = 0.0015L^{3.3}, \quad r^2 = 0.77$$

where W is dry weight in milligrams and L is body length in millimetres. Size of *L. cupida* adults (and hence fecundity, see below) tend to decrease as the emergence season progresses (Clifford 1969), a phenomenon that has been reported for several species of mayflies having extensive emergence periods (e.g., Rawlinson 1939; Ide 1940; Gledhill 1959, Brown 1961). Recently Sweeney and Vannote (1978) studied this phenomenon for several hemimetabolous aquatic insects, including *L. cupida*, in respect to thermal conditions. They found that small adult size results when water temperatures are either raised or lowered with respect to the insect's optimal thermal conditions, suggesting that temperature affects adult size by changing the nymphal growth rate and also the induction and rate of adult tissue development for each nymph.

The ovoid shaped egg of *L. cupida* has been figured by Morgan (1913) and Smith (1935), and Koss (1968) described the egg of *Leptophlebia* sp. including the micropylar device. A characteristic feature of *L. cupida*'s eggs is numerous peglike attachment structures projecting from the egg's surface; these spring out within a minute of the egg contacting water and serve to anchor the egg. They account for *L. cupida*'s eggs being easy to locate in culture dishes. Of 30 eggs measured, average length × width was 0.23 × 0.12 mm, with a range of 0.16 × 0.11 mm to 0.24 × 0.16 mm. The average individual dry weight of unfertilized eggs (weighed in four lots, each containing over 2000 eggs) was 0.00067 mg.

For subimagoes, the average number of eggs produced by an 11 mm, 4.10 mg female (about average size for the Bigoray River population) was 2959 (Clifford and Boerger 1974). Morgan (1913) reported 3700 eggs for a single *L. cupida* imago (New York State), and Sweeney and Vannote (1978) give fecundity values of between 1072 and 2065 for adult *L. cupida* from a southeastern Pennsylvania stream. Total potential fecundity of course varies with the size of the female, and fecundity for both length and weight of *L. cupida* subimagoes (preserved specimens) for this univoltine population can be adequately expressed by power functions:

$$F = 2.02L^{3.04}, \quad r^2 = 0.76$$

$$F = 753W^{0.97}, \quad r^2 = 0.76$$

where F is number of eggs and L and W are length

and dry weight respectively. (The linear regression for fecundity – dry weight was $F = 694W + 182$, $r^2 = 0.73$). *Leptophlebia* has a higher total potential fecundity per unit body length than any of the other Bigoray River mayflies. For example, average numbers of eggs per millimetre of subimago body length for the five more fecund species were the following: *L. cupida*, 269; *Baetis tricaudatus*, 184; *Centroptilum* sp., 140; *Siphloplecton basale*, 133; and *Paraleptophlebia debilis*, 133.

Unlike those of long-lived adult insects, mayfly eggs are fully developed when the nymph transforms, and almost all eggs produced are oviposited. Because of the brevity of the adult stages, egg resorption by adults is not a factor. Hence, one can estimate the average weight of eggs deposited by a female and determine how much the egg mass contributes to the female's total body weight. An *L. cupida* female of 11 mm, 4.10 mg would oviposit about 1.98 mg (2959 eggs × 0.00067 mg dry wt. of individual egg) of eggs. This is about 48% of the female's body weight. An 11 mm male weighs about 2.7 mg, which is comparable with the weight of the female (ca. 2.1 mg) minus the eggs.

We never encountered evidence of parthenogenesis, gynandromorphism, or internal parasites in the Bigoray River population of *L. cupida*. Daggy (1944) recorded an adult *L. cupida* gynandromorph from Minnesota. Cooper (1915) found the metacercarial stage of an allocreadiid trematode in *L. cupida* nymphs.

Food Habits

Methods

Nymphs were collected on nine sampling dates during 1975–1976; they were fixed in 75% ethanol and preserved in 15% formalin. Depending on the amount of material available, from 2 to 20 specimens were used for each size class per sampling date. For nymphs 2 mm or less, the pooled data were always derived from at least 20 guts. Last instar nymphs do not feed, and their guts were not included in the pooled data. Stomach contents (the foregut, midgut, and as much of the hindgut as could be dissected) were separated into four components: plant detritus, diatoms, filamentous algae, and mineral matter (there was no animal material in the guts of *L. cupida*). One fraction of the sample was passed through a 45-μm membrane filter, the residue being used for identification and enumeration of diatoms, filamentous algae, and mineral matter. The other fraction was analyzed in a Coulter Particle Size Counter (model TAII) to determine number of particles, volume of each particle, and total volume of particles. Resulting data from the two fractions were then entered in a computer program that expressed results per stomach as follows: (1) particle size distribution, (2) total volume of detritus, (3) total volume of mineral matter, (4) total volume of filamentous algae, (5) total volume of diatoms, (6) total number of diatoms, and (7) number and volume of each diatom species. Standing crop by numbers of epilithic diatoms was determined from five rocks collected during each sampling date. After brushing, clearing, and sedimentation, identification and counts were made on two subsamples for each date and equated to calculated exposed surface area of rocks.

Results

On a yearly basis, when stomach content volumes of all size classes were considered, *L. cupida* nymphs ingested 95.6% detritus and 3.9% diatoms. (The percent volume of filamentous algae and mineral matter never made up more than 0.8% of the total stomach content volume for any size class, and henceforth will not be included in the results.) Nymphs fed on small particles, up to 160 μm in diameter. The average particle size of all food components was 37.5 μm. There was a tendency for larger nymphs to ingest larger particles, although some small nymphs ingested particles as large as those found in the stomach of large nymphs (Fig. 3). In Cummins's (1973) functional classification, *L. cupida* nymphs would be fine-particle detritivores in the collector category, the large nymphs, at least, being found on depositional type substrate.

Although *L. cupida* nymphs ingest small particles, H. R. Hamilton (unpublished data) studied the food habits of 15 mayfly species from three Alberta streams and found that *L. cupida* nymphs ingested larger size particles than 11 of the other 14 species. Petersen and Cummins (1974) found *Leptophlebia* sp. (prob. *cupida*) nymphs associated with natural leaf packs; they considered the nymphs to be fine-particle feeders, i.e. feeding on the surface of leaves in contrast with large particle feeders, which eat chunks of the leaf material.

There were no large consistent changes in the detritus:diatom stomach content ratio throughout the year; however, nymphs tended to ingest relatively fewer diatoms by volume as the nymphs increased in size (Table 1), but this could be a function of seasonal availability (see below). Do nymphs eat less food in winter when there appears to be little growth for most individuals? Table 2 shows absolute gut volumes for the 4 to 6 mm size class, this size class being present from September to May. Nymphs of this size class did ingest less food in winter, the average total food volume per gut in winter (December and March) being 0.0563 mm^3 compared with 0.0886 mm^3 during the ice-free season. However, the means of the winter and ice-free seasons are not significantly different at $p = 0.05$ level; they are at $p = 0.10$ level. Perhaps a slower assimilation rate in winter could also be important.

We compared the food habits of the 9 mm size classes collected in the marsh and the main stream for 24 April 1975. The average total food volume per gut was 0.185 mm^3 (95% detritus, 5% diatoms) for marsh nymphs and 0.295 mm^3 (98% detritus, 2% diatoms) for main stream nymphs. Certainly from these data, there are no indications that the annual migration into the marsh is advantageous in allow-

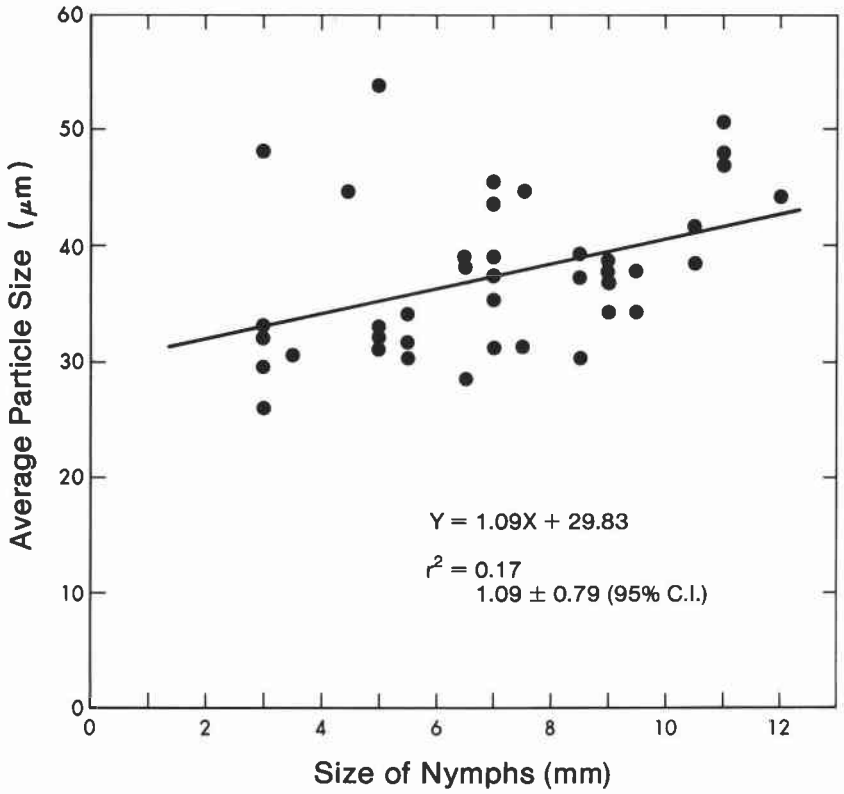

FIG. 3. Average size of particles ingested by *L. cupida* nymphs of various sizes. C.I., confidence interval.

TABLE 1. Average percent volume of diatoms and detritus per gut for (1) each sampling date, 1975–1976, and (2) for each size class of nymph

Sampling date	% vol. diatoms	% vol. detritus
20 August	5.2	94.4
10 September	5.9	93.7
10 October	2.6	96.9
17 December	5.3	94.2
20 March	2.1	97.5
24 April	2.2	97.4
14 May	2.2	97.1
4 June	5.3	94.2
6 August	3.9	95.3
Size class, mm	**% vol. diatoms**	**% vol. detritus**
2–4	3.7	95.9
4–6	3.7	95.7
6–8	2.9	96.5
8–10	2.8	96.7
10–12	2.5	97.1
12–14	1.6	97.9

TABLE 2. Stomach content volumes of 4–6 mm size class nymphs

	Average volume ($mm^3 \times 10^4$) per gut		
Date	Diatoms	Detritus	Total
10 September 1975	42	628	670
10 October	48	1037	1085
17 December	28	447	475
20 March 1976	12	639	651
24 April	22	777	799
14 May	26	962	988

ing nymphs to exploit a more plentiful food supply in the marsh. Both populations consumed relatively more *Achnanthes* spp. than other diatom species (marsh population 28%, main stream population 35%); but for other diatom species, as one might expect, the percent composition was quite different.

It is unlikely that nymphs select detritus over diatoms. The relative amounts of detritus and diatoms in the guts probably simply reflect the relative amount of these small particle food items accessible on the substrate. *Leptophlebia*'s food habits were also studied in another stream, Stauffer Creek, where the standing crop of diatoms was higher. The yearly average for this stream's population was 92% detritus and 8% diatom by volume; and in November, diatoms accounted for 20% of the stomach contents by volume. Shapas and Hilsenhoff (1976) found that the gut contents of

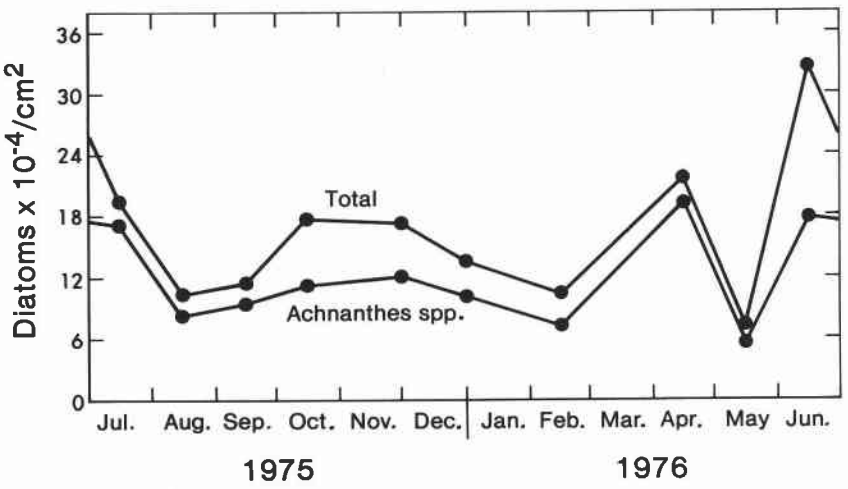

FIG. 4. Total diatom standing crop and standing crop of *Achnanthes* spp., 1975 and 1976.

Leptophlebia sp. nymphs of Wisconsin, U.S.A., streams contained 26% diatoms by volume; and Moore (1977) in a summer study, Northwest Territories, Canada, determined that algae, mainly fragments of the green filamentous *Bulbochaete*, represented up to 65% of *L. nebulosa*'s stomach contents.

In the Bigoray River, *L. cupida* nymphs are small-particle detritivores, and the amount of diatoms in the diet is generally insignificant. Nevertheless, it is instructive to look at the diatom data in more detail, because it is possible to distinguish different kinds of diatoms, something one can not usually do for detritus. Also, yearly standing crop data of epilithic diatoms are available (Fig. 4). Two *Achnanthes* species made up at least 70% of the diatom standing crop on all sampling dates, except June when they accounted for 55% of the total standing crop. The percent composition of diatoms (by number) in the guts reflects the large *Achnanthes* standing crop (Table 3), and the relative importance of the other diatom species in guts can be accounted for, within limits, by the large absolute standing crop values of these species. Possibly a more instructive picture of standing crop diatoms in terms of their accessibility would be data on epipelic diatoms, since in the Bigoray River, large nymphs are often found on fine sediment.

We did not do a detailed study of *L. cupida*'s feeding mechanism. Field and laboratory observations indicate that nymphs often move across and presumably feed on the surface of fine sediment. If rocks were present, the nymphs were found on top of the rocks and presumably fed only on material that was on top of rocks. Traver (1925) mentions that *L. cupida*'s mouthparts are rather generalized, and she suggests that all the mouthparts and especially the maxillae are used to rake in food material. Berner (1975) presents photographs of *L. bradleyi*'s mouthparts that would indicate the functional importance of maxilla setae in gathering small particle food items. We found that when large numbers of nymphs were placed in the same container they would feed on the tissues of the leaves, shredding them in a matter of hours. The nymphs could also subsist in the laboratory by feeding on frog eggs. In fact, large numbers of nymphs are very easy to maintain in standing-water aquaria and require little attention. Nymphs of *L. cupida* have been used as bioassay organisms in the study of the herbicide atrazine (Farleigh 1976), and *L. nebulosa* nymphs were one of several aquatic insects used in a laboratory study of oxygen stress (Nebecker 1972).

TABLE 3. Yearly percentage numbers of standing crop diatoms and yearly percentage numbers of diatom species per gut for all size classes

Standing crop	%	Gut	%
Achnanthes spp.	76	*Achnanthes* spp.	39
Cocconeis placentula	4	*Synedra* sp. A	6
Rhoicosphenia curvata	2	*Synedra* sp. B	5
Synedra sp. B	2	*Navicula* sp. A	4
Nitzschia sp. B	2	*Nitzschia* sp. C	4
Cymbella sinuata	2	*Nitzschia* sp. A	3
Nitzschia sp. C	1	*Nitzschia* sp. B	3
Nitzschia sp. A	1	*Cocconeis placentula*	2
Epithemia sp. A	1	*Rhopalodia gibberula*	2
Epithemia sp. B	1	*Cyclotella* sp. B	2
Gomphonema sp. A	1	*Gomphonema* sp. A	2
Others	7	Others	28

Laboratory Growth and Development

Methods

The *L. cupida* parental stock was collected as nymphs from the marsh on 29 May 1974. Nymphs started transforming in the laboratory on 30 May. Artificial fertilization was accomplished

by either mixing imago eggs and sperm directly or by placing the eggs in a drop of water and then applying the sperm. For the direct method, 67% of the cultured egg masses hatched and half of these produced at least 50 nymphs each. For the water method, 50% of the egg masses hatched, but no more than 10 nymphs hatched from any one culture. The fertilized eggs, in water, were maintained at 20°C or held at 2°C. Fertilized eggs could be held at 2°C without developing; when transferred to 12 or 20°C, they would then hatch. In one 20-day experiment, we found no correlation between time of eggs at 2°C and hatching time at 20°C. Unfertilized eggs were also cultured, but these never hatched.

After hatching at 20°C, each nymph was placed in a separate 60 × 15 mm dish containing water and cultured at 20°C or 12 ± 2°C. As the nymphs increased in size, they were transferred to larger dishes. When the nymphs were small their water did not require aeration, but about half the water of each dish was removed and then replaced with aerated water each time the dish was checked, usually daily. As a precaution, we aerated the dishes containing nymphs of ca. 4 mm or larger. Photoperiod was not rigidly controlled. Average daily light (fluorescent ceiling fixtures) and dark periods for the 20°C cultures were roughly 16 and 8 h, respectively. Oatmeal, plant detritus, and TetraMin (a fish food containing greater than 46% crude protein, 5% crude fat, and less than 8% crude fiber, manufactured by TetraWerke, W. Germany) were used as food. The type of food has no effect on molting rate, longevity, or increase in total length of nymphs. Because it was difficult to find small nymphs and their exuviae in the plant detritus cultures, most of the growth and development data were obtained from oatmeal- and TetraMin-fed nymphs.

Results

Incubation Time

Egg development was not followed in detail. Cleaving eggs were first apparent 3 days after fertilization. The nymph escaped from the egg shell via a longitudinal fissure in the chorion. At 20°C, hatching started between 10 and 14 days after fertilization. For each egg mass, over 50% of the nymphs usually hatched during the first day of hatching; however, delayed hatching continued from some egg masses for at least 43 days, at which time observations were terminated. For the Palearctic *L. vespertina*, Brittain (1976) found that 90% of the nymphs hatched during the first 2 days of hatching.

Development

There were 34 nymphal instars. A brief description of some of the instars' prominent anatomical features as observed in the 20°C culture follows.

First instar (Fig. 5A)—Nymphs were without pigmentation, gills, and compound eyes. Tracheae were not evident. The gut was visible and there were three prominent ocelli. Each antenna and caudal filament had five relatively long articles. Average instar duration (AID) was 2.0 days; average total length (ATL) of live specimens, not including caudal filaments and antennae, was 0.48 mm. The first instar nymphs were positively phototrophic and they actively ingested all three food types.

Second instar (Fig. 5B)—This instar appeared morphologically identical to the first, except for a pair of untracheated gills on abdominal segment 7 and the addition of one article (a total of six) to each caudal filament and antenna. There were no indications that nymphs of this instar or any other instar ate their shed exuviae. AID: 3.4 days; ATL: 0.50 mm.

Third instar (Fig. 5C)—Very light and sparse pigmentation was evident. Each caudal filament and antenna now had seven articles. A pair of gills had developed on abdominal segment 6. The longitudinal tracheal trunks became visible and a branch ran to the now elongated gills of segment 7. The primordia of the compound eyes appeared behind the two posteriolateral ocelli. AID: 4.0 days; ATL: 0.57 mm.

Fourth instar (Fig. 5D)—Gills appeared on abdominal segment 5. Spines on legs became more abundant and prominent. Antennae and caudal filaments of most nymphs had eight articles, but some nymphs had up to 10 articles. There was a tendency for the caudal filaments' distal articles to break off, resulting in an uneven number of filaments for some nymphs. Hence, after the third instar, the number of articles is not a good criterion of instar number for *L. cupida*. AID: 4.8 days; ATL: 0.73 mm.

Fifth instar (Fig. 5E)—Gills first appeared on abdominal segments 1 and 4. Pigmentation was still sparse, and compound eyes were still small, being less than one-third as large as the ocelli. AID: 4.9 days; ATL: 0.88 mm.

Sixth instar (Fig. 5F)—Distinct pigmented patches appeared on the head and thorax. Gut was barely visible through the integument. Gills first appeared on abdominal segment 3. The compound eyes were about one-half the size of the ocelli. AID: 3.8 days; ATL: 0.93 mm.

Seventh instar (Fig. 5G)—Pigmentation was much more prominent. Gills appeared on abdominal segment 2; hence the full complement of seven pairs of gills was now present. Compound eyes were about the same size as the ocelli. AID: 4.0 days; ATL: 1.06 mm.

Eighth instar (Fig. 5H)—Pigmentation was extensive. Compound eyes were larger than ocelli. Gills (all still uniramous) were becoming very long relative to body size. AID: 4.4 days; ATL: 1.33 mm.

Ninth instar (Fig. 5I)—Nymphs were distinctly dark by this instar. All gills had a single tracheal branch. Small buds, the beginning of the inner, or secondary, gill rami, appeared at the base of the primary ramus of gills on segments 3 through 7. Fanning of gills was first observed. Compound eyes

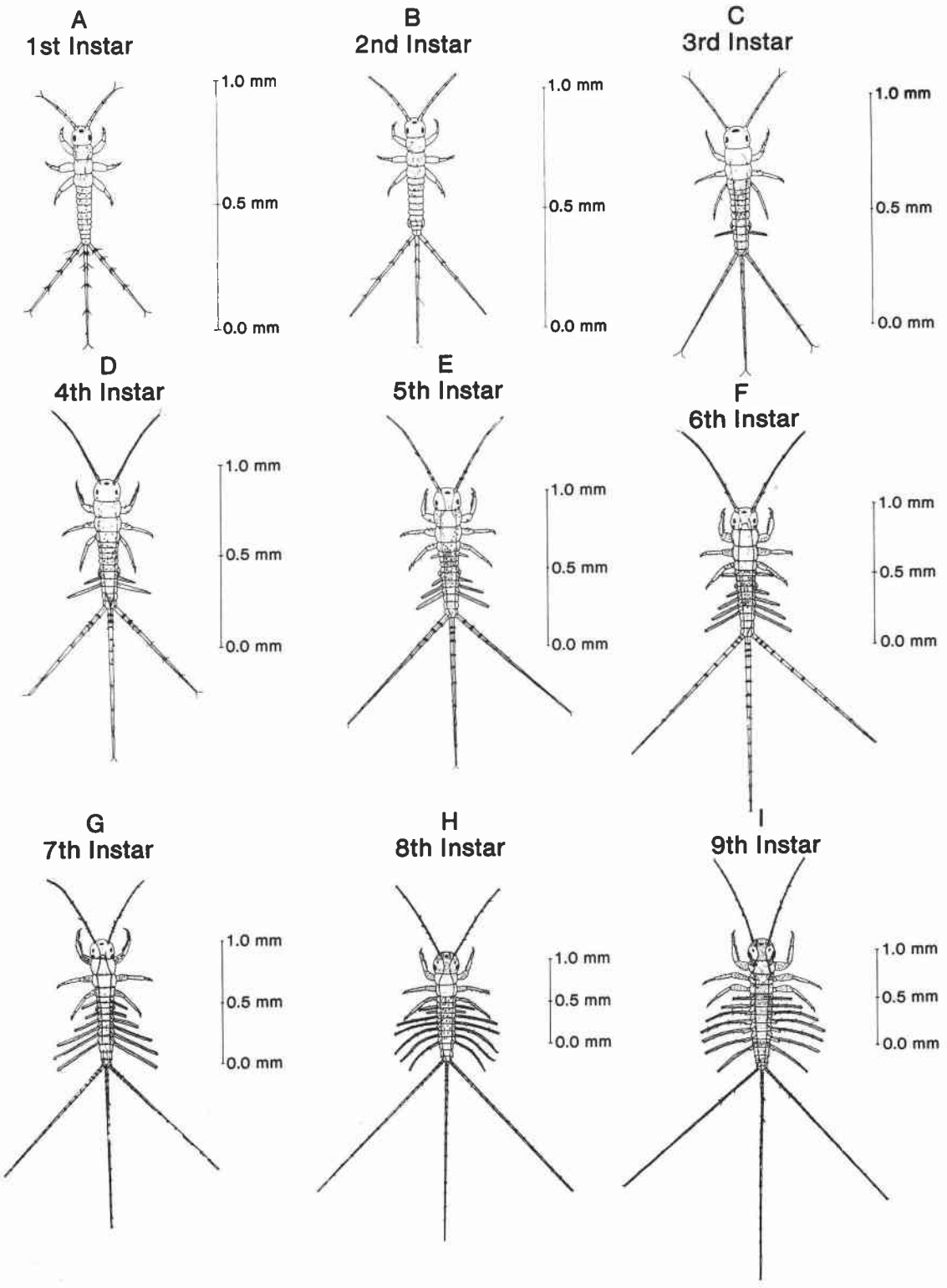

FIG. 5. Camera lucida drawings of the first nine instars of *L. cupida* nymphs.

were twice as large as the ocelli. AID: 4.5 days; ATL: 1.39 mm.

Subsequent instars (gills)—The inner gill rami of segments 3 through 7 were about one-third as long as the primary rami during the 10th instar; at this time buds of the inner rami first appeared on gills of segment 2. The inner gill rami of segment 1 were apparent at the 12th instar. Most of the inner gill rami exhibited tracheae (extending from a tracheal trunk of the primary ramus) by the 13th instar. By the 15th instar, the inner gill rami of segment 7 were equal in length to the primary rami, and the other inner gill rami were almost as long as their primary counterparts. By the 16th instar, gills on all segments except 1 and only slightly so on segment 7 were becoming somewhat lamelliform, the characteristic gill shape of mature nymphs. By the 27th instar, gills of segments 2 through 6 had become strongly lamelliform and notched. Gills of segment 7 remained only weakly lamelliform and without distinctive notches. Gills of segment 1 remained forked throughout. There were no further obvious changes in gill morphology in subsequent instars. The morphological features most often used to distinguish *Leptophlebia* nymphs are the simple forked gills of segment 1 contrasting with the bilamelliform gills of segments 2–7. This condition is not at all apparent until about the 16th instar and not obvious until about the 20th instar, by which time the nymph has gone through more than half its total number of nymphal instars. This certainly points up the possible pitfalls that might be encountered when attempting to identify mayfly nymphs that are not mature (e.g. see Edmunds *et al.* 1976, p. 34).

Subsequent instars (other structures)—The compound eyes continued to increase in size much faster than did the ocelli. By the 23rd instar, the upper frontal component of the male compound eye was first visible. The male forceps first appeared at the 24th instar. Either compound eye development or male genitalia can be used to separate male and female nymphs. By the 27th instar, the male's frontal eye component was larger than the lateral component. The mesothoracic wing pads were first visible at the 17th instar, and by the 21st instar they extended to the end of the mesonotum. The wing pads had reached the posterior edge of abdominal segment 2 by the 27th instar and the posterior edge of segment 3 by the 30th instar. In regards to developmental stages of field specimens, stage I would encompass nymphs through about the first 16 instars, stage II from instar 17 through about instar 25, and stage III from instar 26 to instars of the low thirties (see below).

Final nymphal instars—There were at least 10 and usually about 20 nymphs available for developmental observations through the first 28 instars at 20°C. At the 29th instar, only three nymphs had survived, and at the 30th instar there was only one. This nymph, a male, achieved the 33rd instar and finally perished without molting again. Except for its wing pads not being darkened, it appeared morphologically identical (e.g. forceps size and shape, compound eye structure, pigmentation) to ultimate instar nymphs (developmental stage IV), which are easily recognized as such by their darkened wing pads. We had ample material of ultimate instar nymphs from field collections and from a 75-L aquarium, in which we communally raised large numbers of nymphs from the original artificial fertilizations. We believe therefore that the 33rd instar is the penultimate nymphal instar. Hence the 20°C nymphs would have 34 instars; and for the entire life cycle (i.e. including the two adult instars), the *L. cupida* population would have 36 instars.

Probably, however, there is no fixed number of nymphal instars for *L. cupida*, the 34th being just one of several instars in which the nymphs, given the proper environmental cues, might transform. Other workers have reported a variable number of nymphal molts for different generations of certain multivoltine mayfly species and sometimes a variable number of molts within the single generation of univoltine species. Benech (1972) and Svensson (1977) suggest that the number of nymphal instars for *Baetis rhodani* and *Ephemera danica* respectively is influenced by the rate of nymphal development. Cianciaria (1979) was able to vary the number of *Cloeon dipterum* instars by providing the nymphs with different foods. We know of over 20 studies where there have been serious attempts to determine the total number of mayfly instars, usually by some sort of size–frequency analysis, less often by Palmen organ examination or culturing. Instar numbers of mayflies, including the adult stages, range from 11 for *Callibaetis floridanus* (Trost and Berner 1963) and *Baetis rhodani* (Benech 1972) to about 52 for *Ecdyonurus forcipula* (Gros 1925). The majority of species are in the range of 15 to 25 instars. Mayflies generally would appear to have a larger number and much more variable number of instars than most if not all other extant pterygote orders.

Growth

We measured body length, head width, pronotum width, and abdomen width of live nymphs (at 20°C) for each instar; we measured interocular

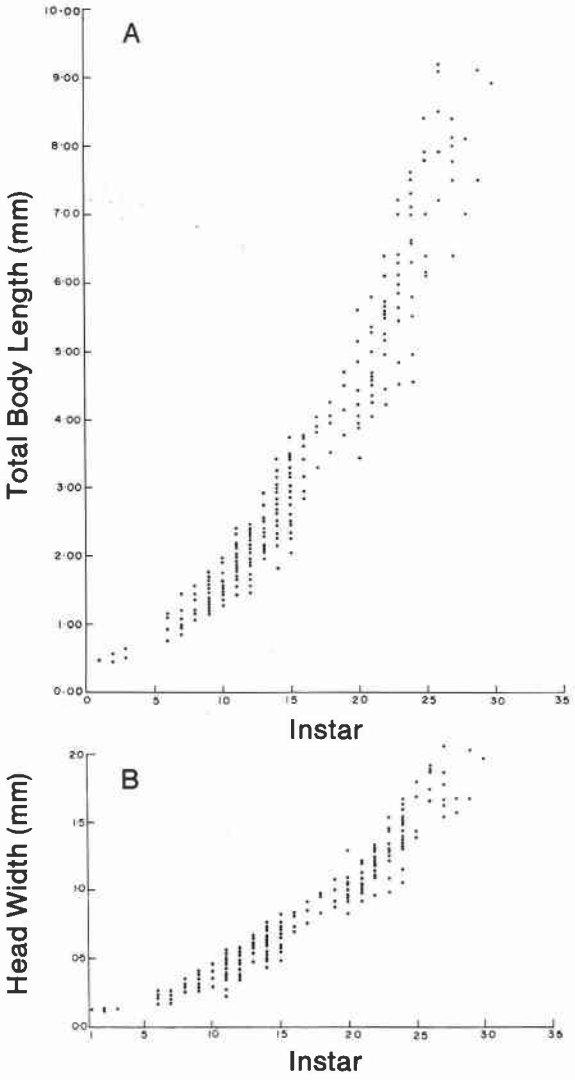

FIG. 6. Body length (A) and head width (B) of nymphs at each instar, 20°C. Values are superimposed when measurements coincide; hence some points represent more than one measurement.

distance of compound eyes starting at instar 6. The arithmetic plots of all dimensions versus instar number were curvilinear. The tendency was for size ranges of all dimensions per instar to become progressively greater at each instar. A nymph of a given size could usually be in one of several instars. For example, a nymph that was 2.00 mm in body length could have been in the 11th to 15th instar (Fig. 6A); a nymph having a head width of 1.00 mm could have been in the 19th to 23rd instar (Fig. 6B). Analysis indicated that it would be difficult to determine accurately most of the discrete instars of *L. cupida* by usual size–frequency techniques. Certainly our data confirm the observation of McClure and Stewart (1976) (for the leptophlebiid *Choroterpes*) that, without supplemented rearing of nymphs, determining discrete instars from size frequency data can be very difficult. For field populations of *L. cupida*, a compounding effect is the lengthy period of delayed hatching, up to 6 months.

The average body length growth curve at 20°C was curvilinear in respect to absolute time as well as in respect to evenly spaced instars (Fig. 7). There were no obvious growth stanzas; but from instar 26, when there were large size ranges for each instar, the exponential pattern breaks down. In terms of biomass (dry weights being calculated from *L. cupida*'s length–weight formulas), over 95% of weight gain takes place during the last 15 or so instars. The instantaneous growth rate G (based on dry weight) fluctuated considerably between instars when the nymphs were small (0.48 to 3.5 mm) and instar intervals were short, was fairly constant for the middle instars (3.5 to 7.0 mm), and was most variable for the older nymphs (7.0 to 9.0 mm). Obviously no single G value would accurately describe *L. cupida*'s growth rate for each instar interval, even though the nymphs were maintained at a constant 20°C. The figure also indicates that instar durations tend to become progressively longer as the nymphs become older.

Nymphs growing at 12 ± 2°C exhibited a slower rate of molting than those at 20°C (Fig. 8) and a greater percentage survival per time (Fig. 9), but not per instar. For example, at 20°C, nymphs were in instars 9 to 13 after 2 weeks; at 12 ± 2°C, nymphs were in these instars after 9 weeks. Hence, lower temperatures slowed the molting rate, but did not improve the percent survival at each instar.

We assessed the relative variability (V) of the various linear measurements for each instar (Fig. 10). The greatest variability, as indicated by coefficients of variation, for all dimensions occurred in the early instars and for some dimensions in the very late instars. The relatively high V values for all dimensions in instars 5 and 6 is inexplicable. Because the variates are homologous and absolute age is known, these values are useful and valid expressions of the dimensions' variability for this invertebrate. Most V values, and the interpretation of their meaning, have pertained to vertebrates. Simpson *et al.* (1960) compared numerous linear dimension V values for mammals and observed that most were between 4 and 10. They suggested that much lower values would probably indicate an inadequate sample and that higher values might indicate impure samples, for example, in respect to age. The higher variability in the early instars of *L. cupida* might in part be due to our measuring tech-

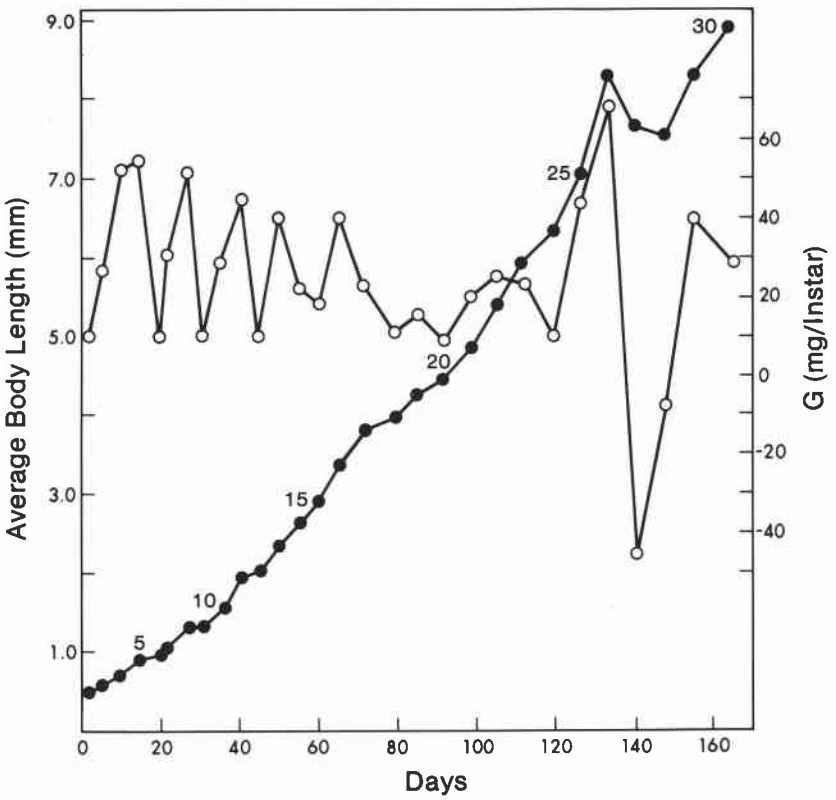

FIG. 7. Instantaneous growth rate (*G*) per instar interval and increase in total body length for nymphs at 20°C. Instar numbers are given above the total length line.

nique. We made measurements only on live nymphs, and when they were very small, perhaps even slightly different orientations of the nymphs could result in detectable errors when the units are small to start with.

Although no single dimension was least variable for all instars, head width consistently had the lowest *V* values. Grand averages (with total number of measurements per dimension in parentheses) of *V*'s for all instars were the following: head width, 3.42 (341); pronotum width, 3.73 (320); total body length, 4.35 (349); abdomen width, 4.38 (320); and interocular distance of compound eye, 5.26 (264). Total length variability was certainly not appreciably greater than the other dimensions' variability. Possibly, if the nymphs had been preserved in alcohol or formalin, variability, due to shrinking, would have been greater for body length.

Discussion

Instars of the Field Population

By combining laboratory and field data (see especially Fig. 1), one can obtain a good sequential picture of *L. cupida*'s life stages, including an estimate of the nymphs' instar numbers throughout the cycle. It is useful to associate the life cycle features with water temperature, a major driving variable of any aquatic system. We compiled the cumulative water degree days (based on mean daily water temperatures and averaged over 7 years) of *L. cupida*'s life cycle, i.e. starting the plot when the eggs are deposited (Fig. 11). The resulting curve is convenient for describing the various phenophases of the *L. cupida* population.

Maximum oviposition occurs ca. 1 June. Nymphs of the new generation first appear in late July. By this time, the eggs would have received about 700 degree days. This is considerably more than the 200 received by the 20°C laboratory population (average incubation time, 10 days). However, most of the tiny nymphs collected in the stream in July had the full complement of gills, indicating that some had already gone through at least seven instars. Sweeney (1978) also found that hatching of *Isonychia bicolor* eggs occurred later in a southeastern Pennsylvania stream than would be predicted from average temperatures in experimental regimes, suggesting that maximum diel temperatures should be given more importance in characterizing incubation time. Instar intervals of *L.*

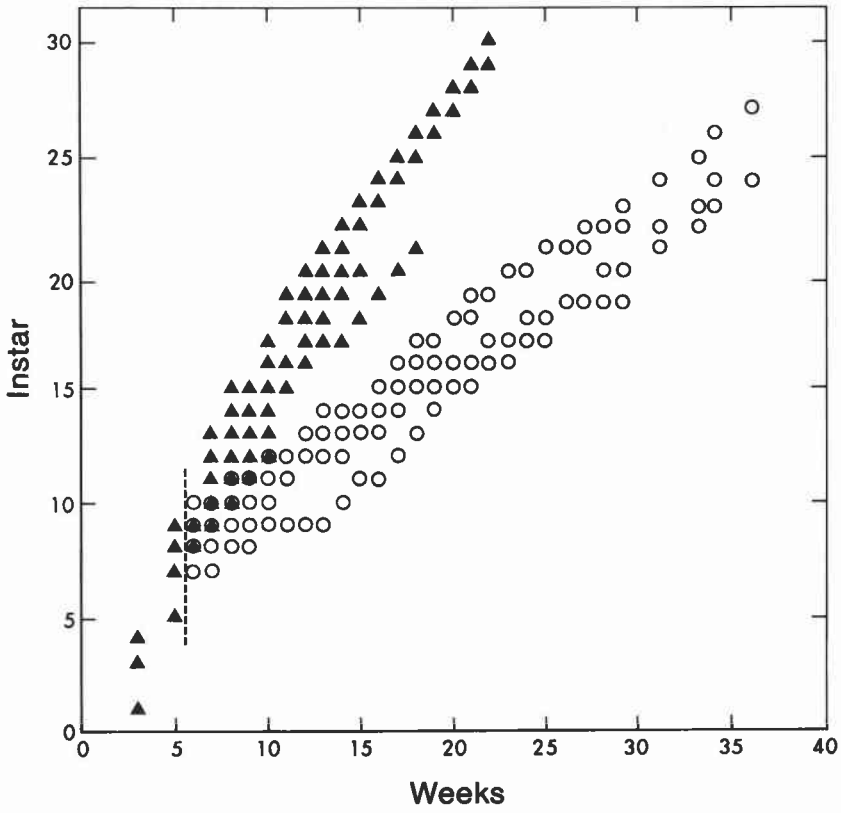

FIG. 8. Effect of temperature on molting rate of *L. cupida* nymphs. Triangles (▲) indicate nymphs at 20°C; open circles (○) represent nymphs transferred from 20°C to 12 ± 2°C at time indicated by vertical dashed line. Symbols are superimposed when measurements coincide.

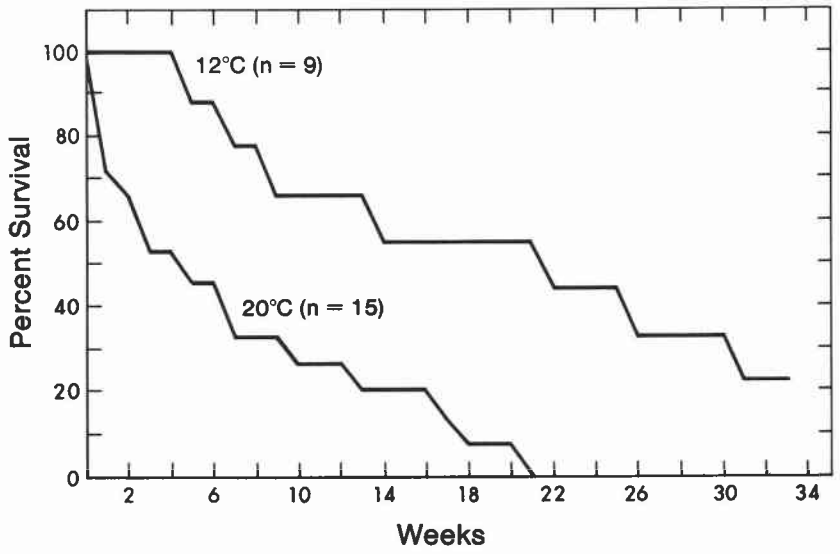

FIG. 9. Survival of *L. cupida* nymphs at 20°C and 12 ± 2°C. Nymphs of both temperature regimes were from the same egg mass, kept under the same light conditions, and fed oatmeal.

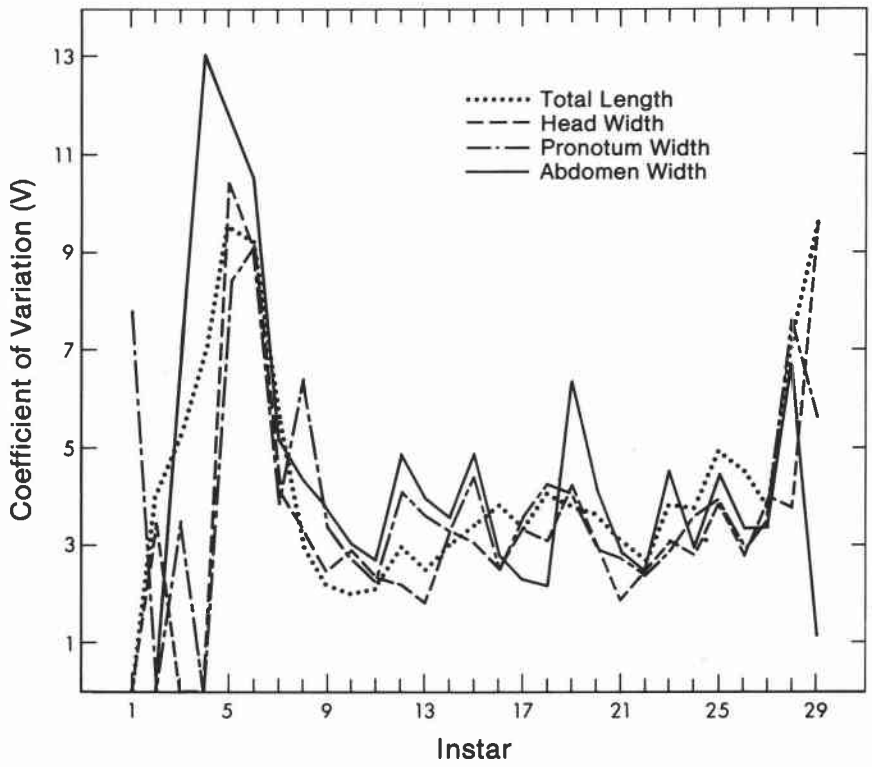

FIG. 10. Relative variability of linear measurements for each nymphal instar. Coefficient of variation (V) is standard deviation divided by mean times 100.

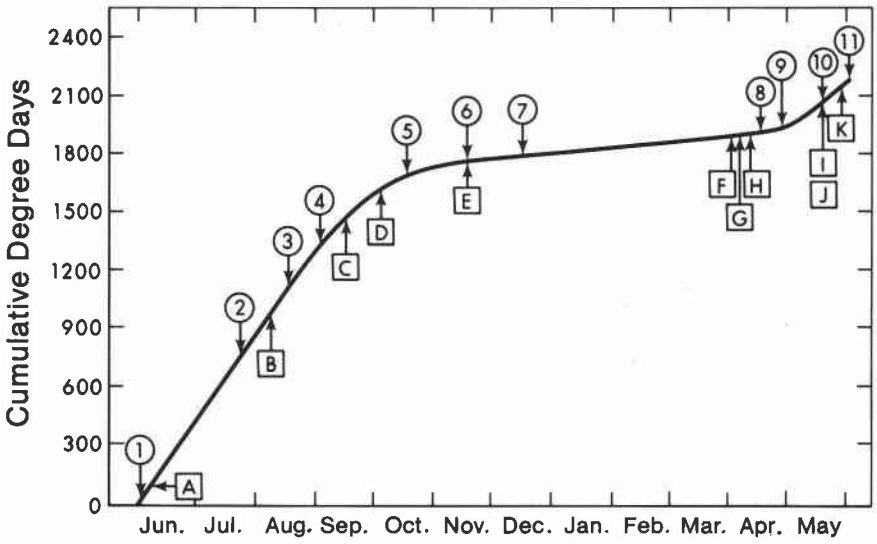

FIG. 11. Life cycle phenophases of the *L. cupida* population as related to the population's cumulative degree days of water temperature. 1, maximum oviposition; 2, first nymphs of new generation; 3, first appearance of developmental stage II nymphs; 4, sexes distinguishable in some nymphs; 5, first appearance of stage III nymphs; 6, some nymphs appear fully grown; 7, all nymphs have achieved at least stage II; 8, start of migration into tributaries; 9, resumption of rapid development; 10, start of emergence; 11, maximum emergence; A, end of downstream run of adult white suckers; B, end of downstream run of sucker fry; C, maximum litter fall rate; D, largest rate decrease of water temperature; E, stream completely freezes over; F, ice break-up, effective day lengths begin; G, tributaries start flowing; H, maximum main stream flow; I, largest rate increase of water temperature; J, start of upstream run of adult suckers; K, beginning of downstream run of sucker fry.

cupida are apparently very short in late summer when water temperatures are high. By the end of August, a few male nymphs were distinguishable, indicating that some of the early hatching individuals must have gone through at least 20 instars (in the laboratory cultures, anatomical features necessary for distinguishing males first were apparent in the 23rd instar). By the end of August, some of the new generation nymphs (including egg stage) would have received an average of 1300 degree days, or 60% of the total yearly degree days for *L. cupida*. Water temperatures, although still relatively high, decline rapidly in September and October, but at least part of the nymphal population continues rapid development and growth. By mid-November, when the stream freezes over, the degree day curve is approaching an asymptote. At this time some of the nymphs appear fully grown, are in developmental stage III, and apparently have gone through at least the 25th instar (or about 75% of the total number of nymphal instars).

Most nymphs appear to overwinter in instars between 17 and 26. By mid-December, even the late hatching nymphs apparently have achieved at least the 17th instar. However, extensive delayed hatching of *L. cupida* eggs in the Bigoray River makes it impossible to follow the population as a clear-cut cohort. Nymphal development and growth slow considerably during the long winter, but apparently neither completely stops for all members of the population. The stream becomes ice-free in early April; maximum flow occurs in middle or late April, and it is at this time that the nymphs, most being in the 17th to 26th instar, move into the tributaries and marshes. In late April, water temperatures (both main stream and marshes) start to rise, and the nymphs resume rapid development. By mid-May most of the population would have received about 2000 degree days, and most of the nymphs would be instars of the high twenties and low thirties. It is at this time that emergence starts with maximum emergence at the end of May.

Early hatching nymphs of the new generation would average about 23 instars during the remainder of the ice-free season, 2 or 3 instars during the long winter, and between 4 to 8 instars in spring. This means that at least 50% and probably closer to 75% of the instars are passed during the first 3 months, or 25%, of nymphal life. In short, the rate of progressing through the life cycle, re instar intervals, is certainly correlated better with the amount of cumulative degree days that the nymphs receive than with the cumulative calendar days of the life cycle.

Relative Production of Small Size Classes

A *Leptophlebia cupida* nymph goes through about nine instars (close to 30% of the total number of nymphal instars) before it achieves a total length of 1.5 mm. Immature hemimetabolous insects of this size and smaller often appear underrepresented in samples from streams, especially when the sampling device features some sort of net, even a fine-meshed net. In fact, quite often in single species production studies the very small size classes are from necessity ignored in the production estimate; or the small individuals are included, but it is pointed out that production is probably underestimated by an unknown factor because of underrepresentation of the small size classes. It would be instructive to know the contribution these small individuals make to cohort production.

For the *L. cupida* populations, empirical data are available for dry weight of individual (unfertilized) eggs, and weights of individual 1st and 2nd instar nymphs. For the 20°C laboratory cultures, information is available for average instar duration, mortality over time and for each instar, and average total length and weight (via length–weight functions) of individual nymphs at each instar. Utilizing these data, we investigated the relative contribution that small individuals make to the cohort production estimate by calculating production per instar. For calculation purposes, we assumed an initial cohort number of 100. Of course, the absolute cohort production value and absolute values for each instar are meaningless, but the relative production contribution of each instar, regardless of the initial cohort number, should be valid. Production per instar was determined by calculating daily mortality of each instar (re 20°C survivorship curve, Fig. 9) and then summing daily the individual increase in weight of all survivors of that instar. Summing the daily increases in weight until the designated emergence gives the theoretical cohort production value (Cushman *et al.* 1978).

For example, the production value of the 1st instar was calculated as follows: initial cohort number = 100, average duration of 1st instar = 2 days, cohort number at end of 1st instar (as extrapolated from the 20°C survivorship curve) = 95, total mortality through 1st instar = 5, daily mortality = $5 \div 2 = 2.5$, average dry weight of a 1st instar nymph = 0.00090 mg, average dry weight of a 2nd instar nymph = 0.00099 mg, individual weight gain from 1st to 2nd instar = 0.00009 mg, daily weight gain = $0.00009 \div 2 = 0.000045$ mg, day 1 production = 97.5 (survivors) × 0.000045 mg = 0.0044 mg, day 2 production = 95.0 (survivors) × 0.000045 mg = 0.0043 mg, production between 1st and 2nd in-

TABLE 4. Relative contribution to production by the various nymphal instars. See text for further explanation

Instar	Average total length, mm	Cumulative % production per instar interval: Emergence 1	Emergence 2	Emergence 3	Instar	Average total length, mm	Cumulative % production per instar interval: Emergence 1	Emergence 2	Emergence 3
1	0.48	0.1	0.1	0.1	14	2.64	26.5	22.1	16.3
2	0.50	0.4	0.4	0.3	15	2.86	36.9	30.9	22.8
3	0.57	1.2	1.0	0.8	16	3.42	44.1	36.9	27.2
4	0.71	2.5	2.1	1.6	17	3.78	48.0	40.2	29.6
5	0.90	2.7	2.3	1.7	18	3.97	54.4	45.6	33.6
6	0.93	3.7	3.1	2.3	19	4.26	58.2	48.7	35.9
7	1.06	6.2	5.2	3.8	20	4.44	66.4	55.6	41.0
8	1.34	6.8	5.7	4.2	21	4.85	79.4	66.5	49.0
9	1.39	8.8	7.4	5.4	22	5.42	94.4	79.0	58.2
10	1.58	13.2	11.1	8.2	23	5.99	100.0	87.7	61.7
11	1.92	14.3	12.0	8.9	24	6.36	—	100.0	73.7
12	2.01	19.3	16.2	11.9	25	7.10	—	—	100.0
13	2.39	22.9	19.2	14.1					

star = 0.0044 + 0.0043 = 0.0087 mg. We assumed three possible emergence times: (1) immediately after the nymphs had passed through the 23rd instar; this would be at end of day 119, when 11% of the initial population would have survived and hence emerged; (2) immediately after nymphs had passed through the 24th instar, the end of day 126, when 7% of the initial population would have emerged; and (3) immediately after nymphs had passed through the 25th instar, the end of day 133, when, because of no mortality during this interval, 7% of initial population would still emerge. The decision as to when to assume emergence is quite important in this type of analysis, because of the relatively large weight gains per instar of large nymphs. The total cohort production values were 8.23 mg for emergence 1, 9.83 mg for emergence 2, and 13.34 mg for emergence 3. We also calculated production using the instantaneous-growth (per instar) method (Waters and Crawford 1973). The results were almost identical to the above daily summation method, the cohort production values being 8.27 mg (1), 9.86 mg (2), and 13.49 mg (3).

Relative contribution to production by the various instars is shown in Table 4. The small size classes, i.e. nymphs of less than 1.5 mm in total length, contributed less than 10% to cohort production, even when we assumed 'early emergence' (emergence 1). Including production contributed by the egg stage does not appreciably increase the relative production contribution of the small size classes. Even assuming no mortality for an initial 100 eggs during the average 10 day incubation period at 20°C, egg stage production contribution would be only 0.023 mg (average individual dry weight of eggs = 0.00067 mg, average individual dry weight of 1st instar nymphs = 0.00090 mg). This would raise the total relative contribution of the first nine instars only to 9.1% (emergence 1), 7.6% (emergence 2), and 5.6% (emergence 3).

Although our production estimates were based on empirical data, one must use much caution in relating these results to what is actually happening in the field. Some major ponderables in our exercise were the artificiality of the laboratory survivorship curve, weights being based on preserved specimens, and the 20°C laboratory specimens undoubtedly being smaller than field specimens of the same age, at least for the older nymphs. Nevertheless, our assumptions were such as to give maximum production input to the small size classes. Hence, we cautiously suggest that for immature amphibiotic insects the error in cohort production estimates by not adequately sampling small individuals could be less than 10% and perhaps considerably less than 10%.

Acknowledgements

We wish to thank Mr. Allen Nip for technical assistance and Dr. Lynda D. Corkum for critically reading the manuscript. The studies were supported by grants from the National Research Council of Canada.

BENECH, V. 1972. Le polyvoltinisme chez *Baetis rhodani* Pictet (Insecta, Ephemeroptera) dans un ruisseau à truites des Pyrenées-Atlantiques, le Lissuraga. Ann. Hydrobiol. **3**: 141–171.

BERNER, L. 1975. The mayfly family Leptophlebiidae in the southeastern United States. Fla. Entomol. **58**: 137–156.

——— 1977. Distributional patterns of southeastern mayflies (Ephemeroptera). Bull. Fla. State Mus., Biol. Sci. **22**: 1–56.

BOERGER, H., and CLIFFORD, H. F. 1975. Emergence of mayflies (Ephemeroptera) from a northern brown-water

stream of Alberta, Canada. Verh. Int. Ver. Limnol. **19**: 3022–3028.

BOND, W. A. 1972. Spawning migration, age, growth, and food habits of the white sucker, *Catostomus commersoni* (Lacépède), in the Bigoray River, Alberta. M.Sc. Thesis, University of Alberta, Edmonton.

BRITTAIN, J. E. 1976. Experimental studies on nymphal growth in *Leptophlebia vespertina* (L.) (Ephemeroptera) Freshwater Biol. **6**: 445–449.

BROWN, D. S. 1961. The life cycle of *Chloeon dipterum* L. (Ephemeroptera, Baetidae). Entomologist, **94**: 114–120.

BURKS, B. D. 1953. The mayflies, or Ephemeroptera, of Illinois. Ill. Nat. Hist. Surv. Bull. 26.

CIANCIARIA, S. 1979. Studies on the biology and bioenergetics of *Cloeon dipterum* (L.) Proc. 2nd Int. Conf. Ephemeroptera, 1975.

CLEMENS, W. A. 1915. Rearing experiments and ecology of Georgian Bay Ephemeridae. Contrib. Can. Biol. Sess. Pap. **39***b*: 113–128.

CLIFFORD, H. F. 1969. Limnological features of a northern brown-water stream, with special reference to the life histories of the aquatic insects. Am. Midl. Nat. **82**: 578–597.

——— 1970. Analysis of a northern mayfly (Ephemeroptera) population, with special reference to allometry of size. Can. J. Zool. **48**: 305–316.

——— 1972*a*. A year's study of the drifting organisms in a brown-water stream of Alberta, Canada. Can. J. Zool. **50**: 975–983.

——— 1972*b*. Comparison of stream bottom fauna samples collected during the day and night. Limnol. Oceanogr. **17**: 479–481.

——— 1978. Descriptive phenology and seasonality of a Canadian brown-water stream. Hydrobiologia, **58**: 213–231.

CLIFFORD, H. F., and H. BOERGER. 1974. Fecundity of mayflies (Ephemeroptera) with special reference to mayflies of a brownwater stream of Alberta, Canada. Can. Entomol. **106**: 1111–1119.

COLEMAN, M. J., and H. B. N. HYNES. 1970. The life histories of some Plecoptera and Ephemeroptera in a southern Ontario stream. Can. J. Zool. **48**: 1333–1339.

COOPER, A. R. 1915. Trematodes from marine and freshwater fishes, including one species of ectoparasitic turbellarian. Trans. R. Soc. Can., Ser. 3, **9**: 181–205.

CUMMINS, K. W. 1973. Trophic relations of aquatic insects. Annu. Rev. Entomol. **18**: 183–206.

CUSHMAN, R. M., H. H. SHUGART, JR., S. G. HILDEBRAND, and J. W. ELWOOD. 1978. The effect of growth curve and sampling regime on instantaneous-growth, removal-summation, and Hynes/Hamilton estimates of aquatic insect production: a computer simulation. Limnol. Oceanogr. **23**: 184–189.

DAGGY, R. H. 1944. Two mayfly gynandromorphs (Ephemeroptera). Proc. Entomol. Soc. Wash. **46**: 256–259.

EDMUNDS, G. F., JR., S. L. JENSEN, and L. BERNER. 1976. The mayflies of North and Central America. University of Minnesota Press, Minneapolis.

FARLEIGH, W. J. 1976. Effects of the herbicide atrazine on the aquatic stages of Ephemeroptera (Arthropoda: Insecta). *In* 9th Annu. Rep. (1976). State University of New York, Oneonta Field Station, Oneonta. pp. 70–84.

GLEDHILL, T. 1959. The life history of *Ameletus inopinatus* (Siphlonuridae, Ephemeroptera). Hydrobiologia, **14**: 85–90.

GROS, A. J. 1925. Etudes sur les premiers stades des Ephémères du Jura Français. Ann. Biol. Lacustrine Brussels, **14**: 53–87.

HARPER, F., and E. MAGNIN. 1971. Emergence saisonière de quelques ephéméroptères d'un ruisseau des Laurentides. Can. J. Zool. **49**: 1209–1221.

HARPER, P. P., J.-G. PILON, and J.-M. PERRON. 1975. Insectes aquatiques du nord du Québec (Ephéméroptères, Odonates, Plecoptères et Trichoptères). Ann. Soc. Entomol. **20**: 33–43.

HAYDEN, W. 1971. Upstream migration of *Leptophlebia cupida* nymphs (Ephemeroptera: Leptophlebiidae) in the Bigoray River, Alberta. M.Sc. Thesis, University of Alberta, Edmonton.

HAYDEN, W., and H. F. CLIFFORD. 1974. Seasonal movements of the mayfly *Leptophlebia cupida* (Say) in a brown-water stream of Alberta, Canada. Am. Midl. Nat. **91**: 90–102.

HILSENHOFF, W. L., J. L. LONGRIDGE, R. P. NARF, K. J. TENNESSEN, and C. P. WALTON. 1972. Aquatic insects of the Pine-Popple River, Wisconsin. Tech. Bull. 54, Dep. Nat. Res., Madison, Wisc.

IDE, F. P. 1935. Life history notes on *Ephoron*, *Potamanthus*, *Leptophlebia* and *Blasturus* with descriptions (Ephemeroptera). Can. Entomol. **67**: 113–125.

——— 1940. Quantitative determination of the insect fauna of rapid water. Publ. Ont. Fish. Res. Lab. **50**: 1–20.

KELSO, J. R. M. 1973. Seasonal energy changes in walleye and their diet in West Blue Lake, Manitoba. Trans. Am. Fish Soc. **102**: 363–368.

KOSS, R. W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. Ann. Entomol. Soc. Am. **61**: 696–721.

LEONARD, J. W., and F. A. LEONARD. 1962. Mayflies of Michigan trout streams. Cranbrook Institute of Science, Bloomfield Hills.

LYMAN, F. E. 1955. Seasonal distribution and life cycles of Ephemeroptera. Ann. Entomol. Soc. Am. **48**: 380–391.

——— 1956. Environmental factors affecting distribution of mayfly nymphs in Douglas Lake, Michigan. Ecology, **37**: 568–576.

MCCLURE, R. G., and K. W. STEWART. 1976. Life cycle and production of the mayfly *Choroterpes* (*Neochoroterpes*) *mexicanus* Allen (Ephemeroptera: Leptophlebiidae). Ann. Entomol. Soc. Am. **69**: 134–144.

MOORE, J. W. 1977. Some factors affecting algal consumption in subarctic Ephemeroptera, Plecoptera and Simuliidae. Oecologica, **27**: 261–273.

MORGAN, A. H. 1911. May-flies of Fall Creek. Ann. Entomol. Soc. Am. **4**: 93–118.

——— 1913. A contribution to the biology of mayflies. Ann. Entomol. Soc. Am. **6**: 371–413.

NEAVE, F. 1930. Migratory habits of the mayfly *Blasturus cupidus* Say. Ecology, **11**: 568–576.

NEBECKER, A. V. 1972. Effect of low oxygen concentration on survival and emergence of aquatic insects. Trans. Am. Fish. Soc. **101**: 675–679.

PETERS, W. L., and L. O. WARREN. 1966. Seasonal distribution of adult Ephemeroptera in northwestern Arkansas. J. Kans. Entomol. Soc. **39**: 390–401.

PETERSEN, R. C., and K. W. CUMMINS. 1974. Leaf processing in a woodland stream. Freshwater Biol. **4**: 343–368.

PRATHER, B. L. 1969. A quantitative analysis of the mayfly naiad *Leptophlebia cupida* in a temporary woods pool near Defiance, Ohio. Bios (Madison, N.J.), **40**: 122–127.

RAWLINSON, R. 1939. Studies on the life history and breeding of *Ecdyonurus venosus*. Proc. Zool. Soc. London, Ser. B, **109**: 377–450.

SCHWIEBERT, E. 1973. Nymphs. A complete guide to naturals and their imitations. Winchester Press, New York.

SCUDDER, G. G. E. 1975. An annotated checklist of the Ephemeroptera (Insecta) of British Columbia. Syesis, **8**: 311–315.

SHAPAS, T. J., and W. L. HILSENHOFF. 1976. Feeding habits of Wisconsin's predominant lotic Plecoptera, Ephemeroptera, and Trichoptera. Great Lakes Entomol. **9**: 175–188.

SIMPSON, G. G., A. ROE, and R. C. LEWONTIN. 1960. Quantitative zoology. Harcourt, Brace and World, Inc.

SMITH, O. R. 1935. The eggs and egg-laying habits of North American mayflies. *In* The biology of mayflies with a systematic account of North American species. *Edited by* J. G. Needham, J. R. Traver, and Y.-C. Hsu. Comstock. pp. 67–89.

SPIETH, H. T. 1938. Studies on the biology of the Ephemeroptera. I. Coloration and its relation to seasonal emergence. Can. Entomol. **70**: 210–218.

SVENSSON, B. 1977. Life cycle, energy fluctuations and sexual differentiation in *Ephemera danica* (Ephemeroptera), a stream-living mayfly. Oikos, **29**: 78–86.

SWEENEY, B. W. 1978. Bioenergetic and developmental response of a mayfly to thermal variation. Limnol. Oceanogr. **23**: 461–477.

SWEENEY, B. W., and R. L. VANNOTE. 1978. Size variation and the distribution of hemimetabolous aquatic insects: two thermal equilibrium hypotheses. Science, **200**: 444–446.

SWISHER, D., and C. RICHARDS. 1971. Selective trout. Crown Publ. Inc., New York.

TRAVER, J. R. 1925. Observations on the ecology of the mayfly, *Blasturus cupidus*. Can. Entomol. **57**: 211–218.

TROST, L. M., and L. BERNER. 1963. The biology of *Callibaetis floridanus* Banks (Ephemeroptera, Baetidae). Fla. Entomol. **46**: 285–299.

WATERS, T. F., and G. W. CRAWFORD. 1973. Annual production of a stream mayfly population: a comparison of methods. Limnol. Oceanogr. **18**: 286–296.

WIENS, A. P., D. M. ROSENBERG, and N. B. SNOW. 1975. Species list of aquatic plants and animals collected from the Mackenzie and Porcupine River watersheds from 1971–1973. Dep. Environ. (Canada), Fish Mar. Serv., Tech. Rep. 557. Winnipeg.