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Ecology, Vol. 62, No. 5 (Oct., 1981), 1353-1369.

Stable URL:
http://links.jstor.org/sici?sici=0012-9658%28198110%2962%3A5%3C1353%3AEMOWCC%3E2.0.CO%3B2-Y

Ecology

Ecology, 62(5), 1981, pp. 1353–1369

EPHEMERELLA MAYFLIES OF WHITE CLAY CREEK: BIOENERGETIC AND ECOLOGICAL RELATIONSHIPS AMONG SIX COEXISTING SPECIES[1]

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Abstract. Comparative data are presented on feeding, growth, respiration, mortality, adult emergence and fecundity, egg development, and calorimetry for *Ephemerella subvaria, E. dorothea, E. verisimilis, E. funeralis, E. serrata,* and *E. deficiens* in White Clay Creek (WCC), Pennsylvania, USA.

All species are univoltine in WCC. The larvae of most species hatch from eggs in late summer–early fall and complete their growth by the following spring or early summer. The magnitude and rate of larval growth for each species appears to be affected largely by seasonal variation in both stream temperature and food quality. Net growth efficiencies (NGE) tend to be positively correlated with larval size for most species. NGE ranged from 3.5 to 81.6% for *Ephemerella* species.

The timing of adult emergence and the size and fecundity of adults appears to be determined largely by the magnitude and pattern of temperatures that are experienced during the larval growth period. An hypothesis is presented to explain the interaction of temperature, insect developmental processes, and physiology in determining both the timing of adult emergence and the resultant size and fecundity of individuals. Empirical data are presented to support the hypothesis.

Quantitative data concerning seasonal variation of larval densities are given for all species except *E. funeralis*. Results indicate that the remaining five species all coexist in riffle habitat in WCC (as opposed to pool habitat). There is also minimal overlap in the relative abundance of consubgeneric species. It is suggested that competition between pairs of consubgeneric species may be reduced somewhat by having the relative abundance and body size, as well as resource requirements (i.e., biomass production), of each pair segregated temporally.

Key words: bioenergetics; calorimetry; Ephemerella; *Ephemeroptera; fecundity; growth; larval density; mayfly; metamorphosis; net growth efficiency.*

White Clay Creek, a piedmont stream in rural southeastern Pennsylvania, supports a high diversity of mayfly species, particularly in the headwaters. Over 50 species have been collected from first- through fourth-order tributaries draining a single 725-ha subbasin. In some habitats, two or more congeneric species are often collected simultaneously. The co-occurrence of systematically related aquatic species often raises questions concerning interspecific competition for environmental resources as well as the relationship between species during the structuring of ecological communities (Cummins 1964, Grant and Mackay 1969, Mackay 1972, Resh 1976).

In this paper we assess and compare several ecologically important parameters (e.g., larval growth, feeding, metabolism, reproduction, etc.) for six species of *Ephemerella* mayflies. This species complex in White Clay Creek (WCC) is composed of three subgenera, each represented by two species. (Allen [1980] has just reclassified the subfamily Ephemerellinae and now treats each of these subgenera as distinct genera. In this paper all the species are referred to as *Ephemerella*.) The subgenera and species are: (1) *Ephemerella* (*Eurylophella*) *verisimilis* and *funeralis*; (2) *Ephemerella* (*Ephemerella*) *dorothea* and *subvaria*; and (3) *Ephemerella* (*Serratella*) *serrata* and *deficiens*. Five of the species (all except *E. funeralis*) can be collected in significant numbers from the same riffle during any given year.

[1] Manuscript received 20 September 1979; revised 28 December 1980; accepted 27 January 1981.

METHODS AND MATERIALS

Adult emergence, fecundity, and egg development

Adult emergence for all species except *E. funeralis* was studied by collecting several hundred larvae from WCC about 1 wk before the usual emergence date of each species and rearing them in an indoor laboratory stream supplied continuously with fresh stream water. Newly emerged adults were collected daily from vegetation, lights, and the inside environs of the wet laboratory. The timing of adult emergence in the indoor stream is similar to WCC because photoperiod, temperature, and water chemistry do not differ significantly in the laboratory stream.

E. funeralis adults were obtained by collecting larvae from a first-order tributary of WCC just before normal emergence and placing the larvae into polypropylene trays (18 × 9 cm, 8 cm deep) which were submerged halfway in the same tributary. Each tray had part of its sides removed and replaced with 1-mm Nitex netting to permit stream water to circulate through the tray. Trays were provided with detritus and algae for food. A fine-mesh net fastened over the top of the tray prevented adults from leaving. Adults were removed daily from each tray.

Ephemerella adults collected as described above for characterizing the diel frequency of emergence were

also used: (1) for dry mass determination to estimate mean adult mass for a given date; (2) to establish the statistical relationship between adult dry mass and fecundity; or (3) as a source of eggs for studies on embryonic development. In this study, all larval and adult masses were determined with an electrobalance (±1 μg) after drying at 60°C for 48 h. Dry mass and fecundity were measured on individual adult females by dissecting the eggs onto a preweighed (nearest 0.01 mg) glass coverslip (30 × 15 mm), drying the coverslip containing eggs and remaining tissue for 48 h at 60°, reweighing the coverslip, placing the coverslip on a triplex microprojector (Bausch and Lomb Company), and projecting the image of the dried eggs onto a grid for counting. Eggs used in embryonic development studies were dissected from live adults (or subimagoes), fertilized with sperm stripped from males, and incubated in jars (5.5 cm outside diameter, 6.5 cm deep) with 100 mL filtered (0.45 μm) stream water. Jars were then partially submerged in water baths kept at a specific constant temperature or at ambient WCC temperatures. Jars were examined daily for embryonic development and hatching.

Larval growth

The rate and magnitude of larval growth in WCC was estimated from qualitative samples collected at random from the creek at ≈1-mo intervals. These samples were taken with a 0.25-mm mesh hand net, returned to the laboratory, and all specimens of a given *Ephemerella* species were removed, killed in hot water, and dried at 60°. Estimates of mean masses from these samples are probably biased somewhat towards the larger larvae, even though samples were examined under a dissecting microscope when larvae were small and all specimens were removed from each sample for mass determinations.

Larval growth experiments for *E. subvaria* at 9.5°, 15°, and ambient WCC temperature were initiated by collecting larvae from the creek at random and sorting the larvae visually into three or four size classes. Ten to 20 larvae were taken at random from each size class to estimate mean individual mass for the group. The remaining larvae of each size class were placed in separate flow-through troughs (concave bottom; 16 cm × 1 m, 7 cm deep). The initial number of larvae placed in each trough varied between 40 and 133 because intermediate-size larvae were very abundant while smaller and larger animals were not as readily available.

The effects of abnormally high winter temperatures on larval growth of *E. funeralis* and *E. subvaria* was tested by collecting small larvae of each species in midwinter and rearing them under nonlimiting food conditions at each of four thermal regimes. The coldest regime was ambient WCC temperatures (WC) and warmer regimes were provided by adding 2° and 5° to ambient WCC temperatures (WC + 2° and WC + 5°, respectively). The warmest regime was kept at a constant 15.5 ± 1° (15.5). Larvae were kept in flow-through polypropylene trays (18 × 9 cm, 8 cm deep); about 50 larvae were placed in each tray.

For the above described growth experiments, creek water kept at either a specific constant or fluctuating temperature regime was pumped continuously from a reservoir into each trough or tray. Reservoir water was changed twice a week. Nitex screens (0.250 mm) prevented drift loss of larvae; nylon netting fitted tightly over each rearing container kept adults confined until they could be collected. Each trough or tray contained substrate (rocks, gravel, sand, etc.) which varied according to the needs of each species. Algae (mainly diatoms) and/or detritus were added to each container at frequent intervals in order to assure adequate nutrition for larvae. Laboratory lighting corresponded to the existing daily photoperiod.

Larval density

Larval densities (number per unit area) of all species except *E. funeralis* were estimated from 0.09-m^2 bottom samples (mesh size = 0.45 mm) taken at random from the creek each month using a slightly modified Coffman (1967) sampler. For this sampling, a continuous riffle-pool segment was mapped and divided into 0.09-m^2 quadrats. Twenty such quadrats were chosen at random for collecting each month. Thus, the number of monthly samples per habitat type (e.g., riffle) varied. Additional field sampling in riffles for two species (*E. subvaria* and *E. dorothea*) using fine-mesh nets (0.1 mm), microscopic sorting, and a stratified sampling technique was undertaken periodically in addition to the normal random sampling regime. This additional sampling was used to estimate the amount of error due to low sample number, mesh size of the samples, macroscopic sorting, and changes in the microdistribution of the species toward the end of larval development (e.g., tendency for larvae to migrate toward the stream margin prior to metamorphosis). Quantitative samples were not used for growth calculations; specimens were preserved in alcohol, which precludes obtaining accurate dry mass estimates due to extraction of body constituents by the preservative.

Larval feeding

Gut contents of live larvae were removed and prepared according to Coffman (1967). For most species, the gut contents of 5–30 animals were removed and combined on a depression slide, washed through a millipore filter, cleared with oil, mounted, and examined as a single sample. Prepared slides were analyzed for three food categories: algae (mainly diatoms), detritus, and animals. Fifteen separate fields (Wipple eyepiece) were counted per slide at 450×. Raw data such as the number of diatoms counted and square millimetres of detritus on the slides were converted to dry mass (milligrams) using Coffman's (1967) values.

TABLE 1. Duration of embryonic development as a function of temperature for *Ephemerella* mayflies in White Clay Creek. For each species, the reported data are days to first hatch for each replicate clutch of eggs. n = the number of replicates in which at least one hatch was observed.

Temp. (°C)	*E. subvaria**			*E. dorothea*†			*E. funeralis*†			*E. deficiens*§		
	n	Avg. (SD)	Range	n	Avg. (SD)	Range	n	Avg. (SD)	Range	n	Avg. (SD)	Range
5	0	...	...	0	...	...	0	...	...	0	...	...
10	2	88 (—)	88	0	...	...	7	64 (8.3)	59–82	0	...	...
15	5	53 (19.3)	39–84	3	110 (0.57)	110–111	7	33 (4.9)	22–36	1	40 (—)	40
20	5	32 (9.7)	23–48	1	122 (—)	122	7	20 (1.2)	18–21	1	36 (—)	36
25	1	20 (—)	20	0	...	...	7	19 (2.8)	17–24	0	...	...
30	0	...	...	0	...	...	7	...	...	0	...	...
WCC‖	7	44 (15.1)	39–48	8	111 (6.6)	109–122	17	44 (13.3)	29–72	1	41 (—)	41

* Each constant temperature regime had six replicates; WCC had seven.
† Each temperature regime had 10 replicates.
‡ Each constant temperature regime had seven replicates; WCC had seventeen.
§ Each temperature regime had one clutch of eggs (no replicates).
‖ WCC = ambient stream temperatures of a fourth-order tributary of White Clay Creek. Maximum, minimum, and average temperatures, respectively, for the developmental period of each species are as follows: *E. subvaria*: 21.3°, 8.2°, 14.7°C; *E. dorothea*: 21.6°, 10.4°, 17.9°C; *E. funeralis*: 21.6°, 10.4°, 16.2°C; *E. deficiens*: 21.3°, 11.6°, 16.2°C.

Our data are reported as percent composition by mass of diatoms vs. detritus since animal parts were not observed in *Ephemerella* mayflies.

Larval respiration

Oxygen uptake was measured with a differential respirometer (Gilson 1963). Larvae were collected from the creek, placed immediately in test vessels with 7 mL of filtered (0.45 μm) stream water, and acclimated to test conditions for 1 h. A cheesecloth strip (2 × 2 cm) was put in each vessel for larvae to grasp. Oxygen use was measured at 30-min intervals for 3 h. Carbon dioxide evolved during respiration was absorbed by 0.5 mL KOH kept in a side arm flask of the reaction vessel. The number of animals per vessel varied from 1 to 30 depending on species, body size, and temperature so that measurable quantities of oxygen were consumed in each vessel. Density effects were not significant when vessels with one and more than one animal of similar size were compared at the same temperature.

Calorimetry and energy budget

Energy content (J/mg) of larvae was estimated using a microbomb calorimeter (Phillipson 1964). Energy budgets were constructed according to the following equation:

$$A = G + R,$$

where

A = assimilation = energy consumed in food less the energy lost by egestion, excretion, and secretion;
G = growth = total change in energy value of body materials;
R = respiration = energy metabolically used or released in all ways for all purposes.

Growth was measured as changes in biomass (milligrams) between sample intervals and converted to joules using energy equivalents obtained experimentally for each species. Relative growth rates (milligrams per milligram per day) for each sampling period and season were calculated using the following growth model.

$$\text{growth rate} = (W_f - W_i) \cdot 2\,(W_f + W_i)^{-1} \cdot t^{-1},$$

where

W_f = mean individual mass (milligrams) at the end of the time period,
W_i = mean individual mass (milligrams) at the beginning of the time period, and
t = duration of time period in days.

Growth rates in milligrams per milligram per day were converted to joules per milligram per day for use in the energy budget by multiplying by the appropriate energy conversion factor for each species.

RESULTS AND DISCUSSION

Egg development

Egg development at ambient creek temperatures requires about 41, 44, 44, and 111 d for *E. deficiens*, *E. subvaria*, *E. funeralis*, and *E. dorothea*, respectively (Table 1). The precise developmental time for the remaining species is unknown but the occurrence of small (<0.01 mg dry mass) *E. verisimilis* larvae in the stream by early July suggests that the embryonic period is probably ≈40–60 d for this species. Small larvae of *E. serrata* do not appear in collections until late May, suggesting a possible embryonic or early larval diapause. The long developmental time for *E. dorothea* strongly suggests a possible embryonic diapause. If a diapause is present, however, it must occur early in development, because once we observed embryonic differentiation within the eggs, development proceeded to eclosion without interruption.

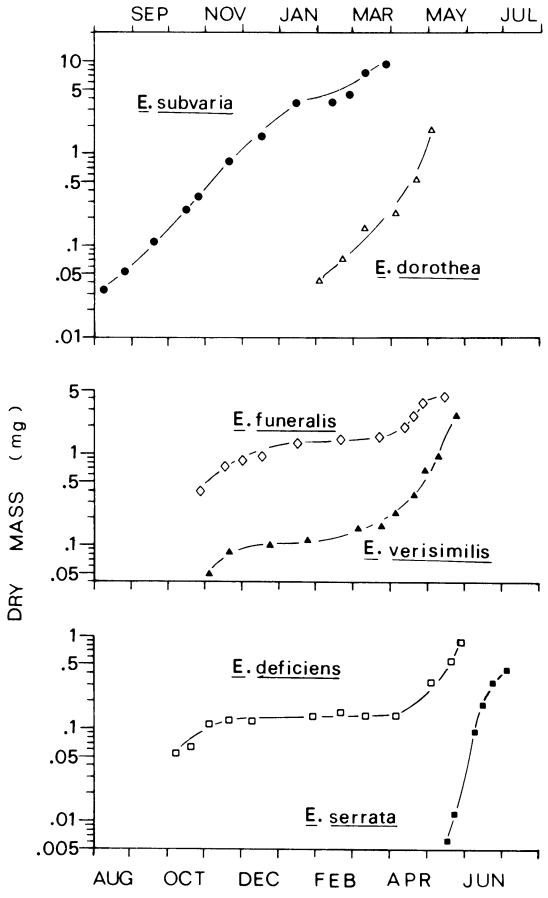

FIG. 1. Average larval growth of six species of *Ephemerella* mayflies in White Clay Creek. Each data point is the average mass of at least 100 larvae. Lines were drawn by eye; actual means and standard deviations are given in Tables 7, 8, and 9.

For each species studied, temperature appears to have a large effect on the rate of embryonic development. For example, *E. funeralis* and *E. subvaria* eggs can complete development in ≈20 d at 25° but require more than three times as long at 10°. Degree-day accumulation for egg development is not constant and appears to be inversely related to incubation temperature for *E. funeralis* and *E. subvaria*. This results from the nonlinear relationship between developmental rate and temperature. The failure of *E. dorothea* and *E. deficiens* to develop successfully at 10° and 20° may be misleading because for all species except *E. funeralis*, eggs were removed from females and artificially inseminated with sperm stripped from males. In many instances, therefore, few (or no) replicates hatching at a given temperature could have resulted from technical failure during the fertilization process, although we generally had good success in the controls (i.e., WCC regime) which were treated similarly. Since fertilization was not a factor for the parthenogenetic *E. funeralis*, it appears that temperatures of 5° and 30° are not conducive to successful embryonic development of this species.

Larval growth

Although first instar larvae of all species weigh about 1 μg, the smallest identifiable larva collected in qualitative field samples was about 0.01 mg. Fig. 1 contains the average growth curves of all six species in White Clay Creek. Each data point is the mean dry mass of ≥100 larvae collected at random from the creek. These samples are undoubtedly biased toward the larger individuals during the early larval growth period of each species (e.g., data points <0.1 mg) due to small larvae passing through our sampling net. We consider other qualitative samples to be relatively unbiased because each sample was carefully sorted to obtain all larvae. *E. funeralis* data are from a headwater spring seep (first order); all other data are from a fourth-order tributary.

For all species, larval growth was continuous during the fall and spring, but slowed for all or part of the winter. Reduced growth during winter was most pronounced in *E. deficiens* and *E. verisimilis*. Population growth curves also show that, for a given date, the mean larval size for each coexisting species within a subgenus does not overlap significantly, especially during periods of maximum resource use (e.g., fall and spring). Although it is unknown whether trophic and physical habitat requirements change as a function of larval size, ecological segregation among *Ephemerella* species may result, in part, from having no two closely related species at the same size during periods of rapid growth. This assumes, although not always true, that a species' closest competitor would most likely be another species in the same genus or subgenus. At this time we do not have enough data to assess whether other species belonging to the same functional group or guild as *Ephemerella* would be closer competitors.

Adult emergence

Although the general shape of the frequency distributions varied among species, it seems noteworthy that for all species, most individuals emerged synchronously (i.e., >80% emerge during a 10–20 d interval, Fig. 2). It has been suggested that a synchronous adult emergence may be adaptive largely because it increases the probability of successful mating (Macan 1958). This untested hypothesis implies that early- and late-emerging individuals rarely, if ever, mate successfully and thus are strongly selected out of the population.

We suggest an alternate hypothesis that predator satiation may be an important factor underlying the evolution of synchronous emergence patterns. Adult emergence of aquatic species, particularly mayflies, attracts large numbers of birds, bats, fish, and other predators which vigorously feed on them (Needham et al. 1935). When predators are territorial or few in

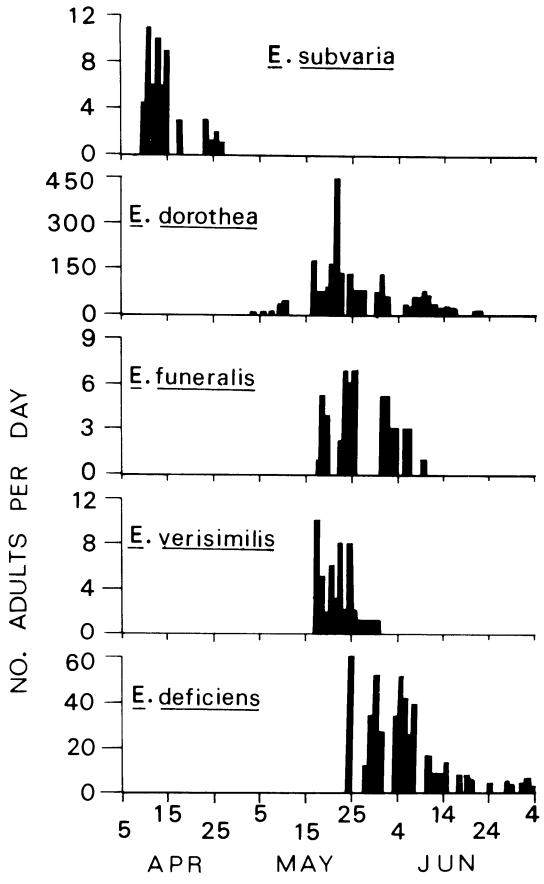

FIG. 2. Frequency distribution for daily observations of adult emergence of six species of *Ephemerella* mayflies from White Clay Creek.

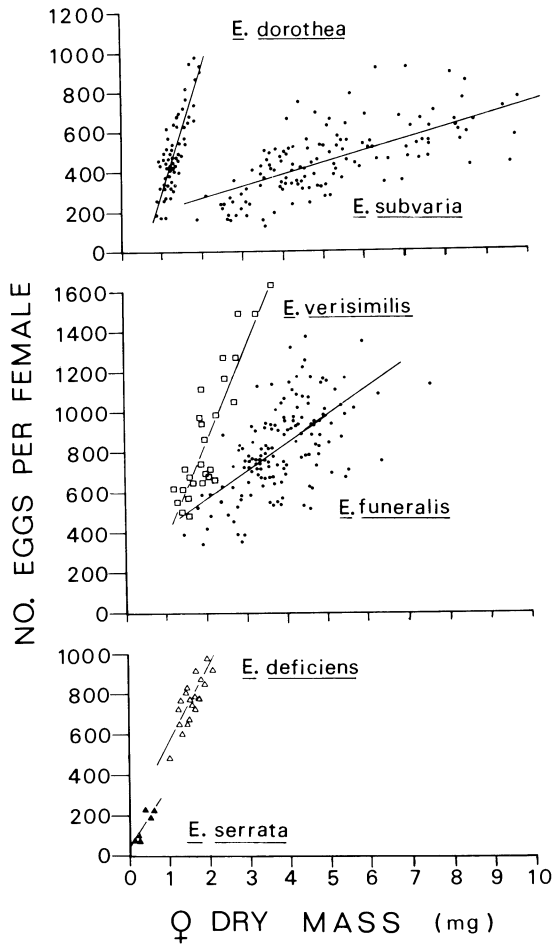

FIG. 3. Fecundity per unit mass for adult females of six *Ephemerella* species from White Clay Creek. Regression equations for each species are: *E. dorothea*: $Y = -300 + 614X$, $r^2 = .74$; *E. subvaria*: $Y = 143 + 59X$, $r^2 = .44$; *E. verisimilis*: $Y = -112 + 488X$, $r^2 = .81$; *E. funeralis*: $Y = 299 + 135X$, $r^2 = .33$; *E. deficiens*: $Y = 157 + 389X$, $r^2 = .66$; *E. serrata*: $Y = 24 + 336X$, $r^2 = .72$.

numbers relative to the prey, mass emergence tends to reduce the percentage of prey mortality due to predator satiation. Although we have no quantitative data for *Ephemerella* species, recent evidence for the stream mayfly *Dolania americana* suggests that predator satiation does occur during adult emergence (i.e., predators take a smaller percentage of adults somewhere near the mode of the emergence frequency distribution and a higher percentage of adults on either tail of the distribution—B. W. Sweeney and R. L. Vannote, *personal observation*). We suspect that further studies will show that predator satiation is an important adaptive function of population synchrony for mayflies, just as it has been suggested for other insects (Corbet 1964, Lloyd and Dybas 1966) as well as plants (Jansen 1976).

Adult body size, fecundity, and emergence time

Laboratory experiments.—Although the number of eggs produced per female was correlated positively with adult mass for each species, there was no clear relationship between a species mass and its fecundity (Fig. 3). For example, *E. subvaria* was the heaviest species but most other species had more eggs. Also, the slopes of fitted regression equations describing mass-specific egg production vary considerably from species to species, even within a given subgenus. These differences suggest that egg quality (e.g., size, shape, nutrient content, etc.) and the placement of eggs should not be considered insignificant parameters relative to egg quantity when evaluating the overall reproductive effort of *Ephemerella* mayflies.

Several past studies of mayfly fecundity have used wing and body length as predictors of individual fecundity (see Clifford and Boerger 1974 for review). We have found dry mass to be the most accurate predictor of adult fecundity. Most of the scatter associated with *E. subvaria* and *E. funeralis* in Fig. 3 was due to technique problems associated with obtaining

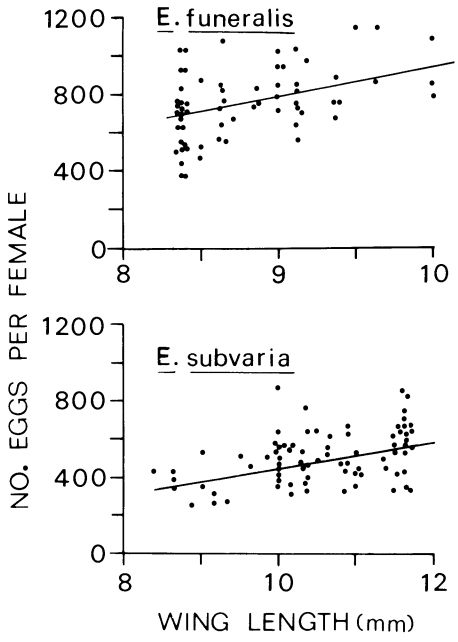

FIG. 4. Fecundity per female as a function of adult wing length for two *Ephemerella* species in White Clay Creek. Regression equations are as follows: *E. funeralis*: $Y = -550 + 149X$, $r^2 = .15$; *E. subvaria*: $Y = -142 + 6X$, $r^2 = .15$.

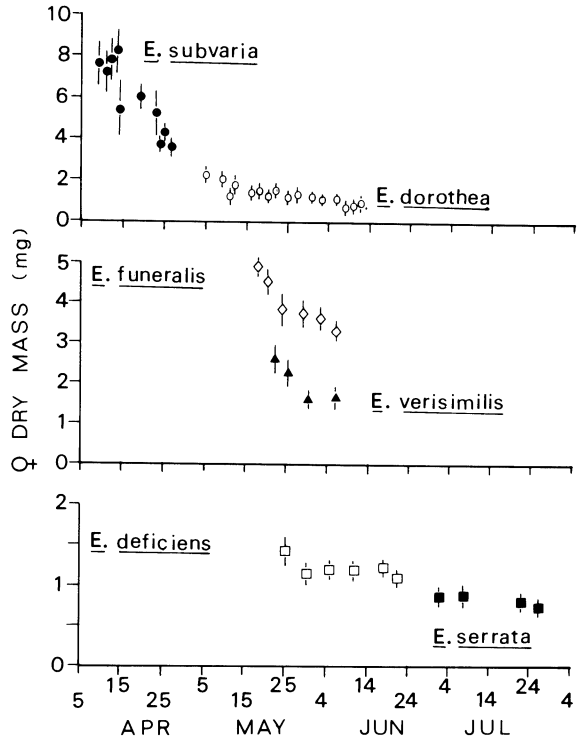

FIG. 5. Adult female size as a function of emergence date for six species of *Ephemerella* mayflies from White Clay Creek. Each data point depicts the mean and standard deviation of adults collected on a single day.

both an egg count and dry mass of dissected specimens. Our current procedure described in the methods section has yielded regressions with high correlation coefficients (e.g., *E. dorothea, E. verisimilis, E. deficiens* in Fig. 3).

Since our adult masses include the mass of eggs, one might expect a priori that individual fecundity and mass should be correlated positively. Thus, the regressions may leave some doubt as to whether heavy individuals are in fact larger. To strengthen this point somewhat, we have replotted individual fecundity data for *E. subvaria* and *E. funeralis* as a function of wing length, which is taken as a measure of insect body size (Fig. 4). Total adult body length would probably have been a better independent measure of largeness and yield a more convincing relationship between fecundity and size, but unfortunately it was not measured. However, despite the high variance in wing length data, the positive slopes of the resulting regressions are significant ($P < .001$), indicating that heavy adult females are indeed larger and produce more eggs than light adult females. Clifford and Boerger (1974) have shown a similar positive correlation between fecundity and adult length for several other mayfly species. This correlation between female size and egg production is important because *Ephemerella* females get progressively lighter and smaller during the emergence period (Fig. 5). This size decrease during emergence is not unique to *Ephemerella* mayflies and has been shown for other mayfly species (Harker 1952, Macan 1957, Langford 1975). The ecological significance of decreasing size is untested. It would appear a priori that the relative contribution of late-emerging adults to overall population recruitment of each species may be small relative to large, early adults because individual egg production is lower and the susceptibility to predators and probability of not finding a mate may be higher. Since a size decrease is observed year after year in most species studied, either the intensity of natural selection is not more severe on late-emerging adults within a given species, or late-emerging adults are not always offspring of late-emerging adults of the previous generation. Larvae that metamorphose late may arise as offspring of early-emerging adults of the previous generation because either the larvae hatched late (e.g., if eggs were buried in silt and oxygen levels reduced—see Hunt 1951) or the larvae were on suboptimal habitat initially and growth was slowed. Delayed egg hatching of a low percentage of eggs, which has been observed in natural populations of several mayfly species (Gledhill 1960, Clifford et al. 1979), may be a very important factor causing high variance in sizes of larvae during the growth cycle as well as adults during emergence. To our knowledge, there are no data available for any aquatic species to prove or disprove genetic similarity between the earliest and latest adults to emerge during a given generation.

TABLE 2. Male and female adult mass, date of first adult emergence, and adult sex ratio for four size classes of *E. subvaria* larvae reared at ambient White Clay Creek temperatures. Note that all adults in the "extra large" size class were females.

Size class	Starting date	Initial larval mass (mg)			Final adult mass (mg)								Adult sex ratio	Date of first adult
					Female			Male			Female and male combined			
		Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Female: male	Female (male)
Extra large	24 Dec	4.6	0.5	3.9–5.5	10.8	0.6	7.1–12.5				10.8	0.6	100:0	25 Mar
Large	24 Dec	3.0	0.3	3.0–4.4	8.0	0.3	4.0–13.1	4.9	0.1	4.9–5.1	7.8	0.4	91:9	26 Mar (30 Mar)
Medium	24 Dec	3.1	0.2	2.1–3.9	5.5	0.4	2.5–8.1	3.0	0.3	2.8–6.5	5.0	0.3	42:58	4 Apr (26 Mar)
Small	24 Dec	2.5	0.2	1.7–3.7	6.6	0.5	1.8–8.8	4.3	0.3	2.4–6.1	5.5	0.3	35:65	2 Apr (26 Mar)

For our *Ephemerella* species, magnitude of size reduction during the emergence period (Fig. 5) does not seem to be correlated with initial size of the species. For example, *E. subvaria* is four times larger than *E. dorothea*, but *E. dorothea* exhibits a larger size decrease (70% vs. 52%). It appears that the degree of size reduction may be related to the timing of emergence. *E. subvaria* and *E. dorothea* emerge earliest in the spring and show the largest relative size decrease (52% and 70%, respectively). *E. funeralis*, *E. verisimilis*, and *E. deficiens* emerge later in the spring and exhibit an intermediate decrease (32%, 40%, and 33%, respectively). The smallest size decrease (16%) occurs in *E. serrata* which emerges in early summer.

A temperature hypothesis.—We believe one of the important underlying factors causing the above correlation between emergence time and relative degree of size reduction is water temperature. More specifically, we hypothesize that the thermal regime experienced by larvae during their final growth phase is largely responsible for both the occurrence and magnitude of decrease in adult size during emergence. To develop this hypothesis we present below experimental results for *E. subvaria* and *E. funeralis*. Similar supporting data for other mayflies have been published previously (Clifford 1970, Sweeney 1978, Sweeney and Vannote 1978, Clifford et al. 1979, Vannote and Sweeney 1980).

We initially thought that the large range of larval masses observed throughout the larval growth period for each species was one of the key components underlying the size decrease during adult emergence. To test this possibility for *E. subvaria*, we collected larvae at random from the creek in December, visually sorted the larvae into four size categories (small, medium, large, extra-large). These nonterminal instar larvae were then reared to the adult stage, keeping each size class separate under nonlimiting trophic conditions and at ambient WCC temperatures (Table 2). We found that: (1) the larvae largest in December were all females, emerged first, and were the largest adults; (2) the smallest larvae in December contained a low percentage of females and emerged toward the end of the emergence period at a small size. These data suggest strongly that the number of instars varies among individuals of a given species. Although all larvae within a size group did not metamorphose on the same date, most adults of a given size group did appear on the first few days. The largest adults in each size group were females.

The decrease in adult female size for *E. subvaria*, therefore, seems to result from the sequential metamorphosis of progressively smaller larvae. However, since larvae in the smaller size classes were actively molting and growing at the time of emergence, it was unclear why these small larvae did not postpone emergence, complete growth, and emerge later at a larger size. We hypothesize that they cannot postpone emergence because they are physiologically regulated to complete metamorphosis.

Our interpretation of Table 2 is based on the idea that *E. subvaria* larvae contain two sets of tissues, larval and adult, whose development is under endocrine control (see Novak 1965 for review). During the early stages of development (e.g., from August to late winter) only the larval tissues exhibit significant differentiation and growth. Adult tissues (i.e., those associated with adult structures such as wing buds, genitalia, and gonads) apparently develop very slowly, if at all, during this period. As the water begins to warm in the spring, a threshold temperature is exceeded which results in a change in the metabolism or production of one or more endocrine compounds and subsequent initiation of adult tissue synthesis. Once adult tissues begin differentiating, each larva proceeds uninterrupted towards metamorphosis and has only a finite amount of time remaining for additional larval tissue growth and storage of nutrients. The ultimate size of each larva at metamorphosis, and hence the adult size, depends largely on the size of the larva at the onset of adult tissue synthesis and how efficiently the larva can subsequently grow before metamorphosis. We suspect that for each species there is a lower size limit for larvae below which adult tissues will not dif-

TABLE 3. Number of days to metamorphosis for four size classes of *Ephemerella subvaria* larvae collected in late December and reared at 9.5° and 15°C. The mean and standard error of larval and adult masses are for dried specimens. The number of larvae (n_i) at the start of the experiment is given for each size class.

Temp. (°C)	n_i	Initial larval mass (unsexed)	Final adult mass		No. days to first adult	No. days to last adult	No. days to median adult
			Males	Females			
9.5	45	1.627 (0.308)	3.060 (0.321)	3.412 (0.960)	91	107	91
	133	2.544 (0.180)	2.791 (0.256)	3.981 (0.296)	90	107	96
	121	2.912 (0.232)	3.854 (0.364)	4.083 (0.370)	83	101	88
	44	3.578 (0.326)	3.743 (0.379)	4.743 (0.500)	85	95	91
15.0	52	2.442 (0.222)	3.169 (0.139)	3.576 (0.287)	54	60	58
	123	3.037 (0.182)	3.605 (0.201)	3.910 (0.610)	53	60	54
	113	3.913 (0.351)	3.773 (0.253)	5.367 (0.277)	53	58	54
	40	4.612 (0.428)	...	5.775 (0.244)	53	53	53

ferentiate regardless of temperature. Starved or semistarved animals (e.g., those on nonoptimum diets) may not respond to threshold temperatures as readily as unstarved animals because certain materials necessary for hormone production may be lacking in their diet. These materials, such as certain sterols, cannot be synthesized by the individual animals.

The above interpretation was synthesized from an array of known physiological information concerning developmental dynamics of both hemi- and holometabolous insects (see Slama et al. 1974, Riddiford and Truman 1978, Gilbert et al. 1980 for some reviews) and experimental observations on *E. subvaria* and several other species of mayflies that seem to be closely aligned with many past studies on other insect orders. Our data are consistent with past studies showing that while the larva itself greatly increases in size during larval life, adult tissues do not differentiate significantly until stimulated by a change in hormone titers during the later stages of development.

Our principal criterion for assessing the onset of adult tissue synthesis has been the first appearance of wing buds and forceps (male abdominal appendage) on larvae. Using these criteria we noted that larvae of *E. subvaria* (and other winter-spring mayfly species) collected throughout the winter did not exhibit signs of adult tissue synthesis until the stream began warming in late winter or early spring. Given our "thermal threshold" hypothesis, we predicted that increasing winter temperatures above the threshold would stimulate a premature adult emergence of all size larvae on or about the same date.

Table 3 shows the results of an experiment where four size classes of *E. subvaria* larvae were subjected to unseasonably warm (9.5° and 15.0°) temperatures in December and reared to metamorphosis. Adult emergence began only a few days pre-mature at 9.5° but almost 6 wk pre-mature at 15°. All size classes began metamorphosing on about the same date at 15°; smaller size classes were somewhat delayed relative to large ones at 9.5°. The duration of the emergence period was reduced at 15° relative to 9.5°; higher emergence synchrony was also noted for large size classes relative to small ones at both temperatures. These data seem consistent with the hypothesis that temperatures above a given threshold stimulate adult tissues to differentiate at about the same time in all size larvae. However, the prolonged emergence of small size classes suggests that the rate and degree of adult tissue synthesis for larvae whose size exceeds the lower limit may be somewhat size dependent (i.e., large larvae reach metamorphosis sooner than small larvae, even though both are stimulated initially at the same time).

Our hypothesis also suggests that the ultimate size of each adult will depend largely on how fast and efficiently each larva grows in the time interval between the onset of adult tissue synthesis and metamorphosis. We studied this for *E. subvaria* and *E. funeralis* by collecting small larvae of each species in midwinter and rearing them in each of four thermal regimes (Fig. 6). The coldest regime was ambient WCC temperatures (WC), and warmer regimes consisted of adding 2° and 5° to ambient WCC temperatures (WC + 2 and WC + 5, respectively). The warmest regime was kept at a constant 15.5° ± 1° (15.5). Results for both species show that the warmer the thermal regime, the earlier the emergence. Adult size of *E. subvaria,* however, was inversely correlated with temperature while adult size of *E. funeralis* showed a positive correlation. These data indicate that, although temperature may be the critical factor determining the timing of adult metamorphosis, temperature is probably interrelated with nutrition and the intrinsic growth capacity of each species in determining adult size.

As we suggested above, the effect of temperature is probably mediated through the insect's endocrine system. Specifically, changes in the rate of production and metabolism of juvenile hormone (JH) and ecdysone are undoubtedly the most critical factors underlying the patterns of growth and development observed for *Ephemerella* mayflies (and other aquatic insects in general). In hemimetabolous species, JH titer declines to undetectable levels around the time of the last larval instar (Riddiford and Truman 1978). The timing of this decrease in *Ephemerella* mayflies, as judged by subsequent development of adult structures,

seems to be correlated largely with temperature or with unknown factors which parallel temperature.

Little is known regarding the direct influence of environmental factors on JH and ecdysone titers. Wigglesworth's (1952) study on fourth instar larvae of *Rhodnius prolixus*, a hemimetabolous terrestrial insect, showed that high temperatures (≈35°) caused the development of adultoid fifth instars, whereas relatively cold temperatures (≈20°) resulted in somewhat juvenile fifth instars. These results appear consistent with our data on mayflies (i.e., warm water stimulates a reduction in JH titer causing premature adult tissue differentiation, and cool water favors high titers of JH with no noticeable progression towards metamorphosis). The titer of JH in the hemolymph depends on several parameters: the rate of secretion by the corpora allata, hormone catabolism in the blood by JH-specific esterases, uptake and metabolism by various tissues, and the presence or absence of carrier proteins which protect JH from catabolism by nonspecific general esterases (Riddiford and Truman 1978). For our study species it is difficult to relate premature metamorphosis at high temperatures to a temperature-induced suppression of secretory activity by the corpora allata because most other aspects of larval activity (e.g., locomotion, feeding activity, etc.) seem stimulated by the higher temperatures. A more viable hypothesis would involve the increased efficiency of JH catabolism at high temperatures due to either: (1) increased concentration of catabolic enzymes, or (2) increased enzymatic efficiency due to the thermal kinetics of esterase enzymes.

Interaction of temperature and other factors.—Our hypothesis and experimental data suggest that temperature is an important factor in determining when *Ephemerella* mayflies emerge. Numerous other laboratory studies have shown that subjecting larvae to unseasonably warm or cool water consistently causes premature or delayed emergence, respectively, for mayflies (Nebeker and Lemke 1968, Nebeker 1971*a*, *b*, Sweeney 1978, Sweeney and Vannote 1978, Rogers 1980) and other aquatic orders (Nebeker 1971*a*, Branham et al. 1975, Rupprecht 1975). These laboratory results seem consistent with field observations on natural aquatic populations where: (1) adult emergence of a species occurs on different dates within a given drainage depending on the thermal regime of the various tributaries (Ide 1935, Sprules 1947, Clifford 1969); (2) adult emergence on a given tributary occurs earlier or later during years that are either abnormally warm or cool relative to average conditions (Gledhill 1960, Illies 1971, Langford 1975); (3) adult emergence of a given species occurs at an earlier date at more southern latitudes (Thibault 1971, Lehmkuhl 1974) or at lower altitudes (Nebeker 1971*c*) in streams notably warmer than more northern or high altitude streams respectively; (4) adult emergence occurs earlier on sections of a river receiving warm effluent of power plants (Coutant 1967, cited by Langford 1975; Mattice and Dye 1978) but later in the year if receiving cold hypolimnetic water from a large reservoir (Pearson et al. 1968).

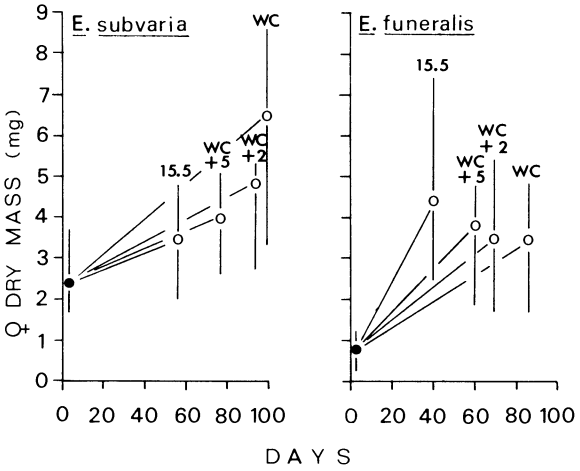

FIG. 6. Larval growth and adult female mass for two *Ephemerella* mayfly species reared in various thermal regimes. The thermal regimes were (1) WC = ambient White Clay Creek (WCC) temperatures; (2) WC + 2 = 2°C added to ambient WCC temperatures; (3) WC + 5 = 5°C added to ambient WCC temperatures; (4) 15.5 = 15.5°C + 1°C constant temperature. More detailed thermal data are given in Sweeney and Vannote (1978). Closed and open circles depict average larval and adult masses, respectively; vertical bars delimit the range for each mean.

Our results also suggest that adult size of *Ephemerella* mayflies at emergence may depend partially on thermal conditions during the larval growth period. Variation in adult size has also been shown to be correlated with rearing temperature for several other species of mayflies (Sweeney 1978, Sweeney and Vannote 1978, Vannote and Sweeney 1980) as well as other aquatic orders (Konstantinov 1958, Heuvel 1963, Brust 1967, Sweeney and Schnack 1977). Similar data have been reported for several terrestrial insect species (see David and Clavel 1967 for review). Adult size variation of natural populations has also been correlated with temperature. For example, studies on polyvoltine mayfly species consistently show that winter-spring cohorts are significantly larger than summer cohorts (Thibault 1971, Benech 1972, Fahy 1973, Clifford and Boerger 1974, Sweeney 1978, Illies 1979, Vannote and Sweeney 1980). Also, studies of natural populations of mayflies in rivers warmed by power plant effluent have shown either slight (Langford 1975) or extensive (Obrdlik et al. 1979) modification of adult size depending on the species and degree of warming. It remains to be demonstrated clearly, however, to what extent temperature affects natural populations directly through physiological and developmental processes or indirectly through seasonal variation in the quantity or quality of food and habitat.

TABLE 4. Percent occurrence of five species of *Ephemerella* mayflies in pool and riffle habitat of White Clay Creek. For each species the total number of specimens collected in each habitat type during the quantitative sampling program was used to calculate percent occurrence.

	Riffle (%)	Pool (%)
E. deficiens	95.6	4.4
E. subvaria	90.7	9.3
E. dorothea	90.3	9.6
E. serrata	89.6	10.4
E. verisimilis	56.9	43.1

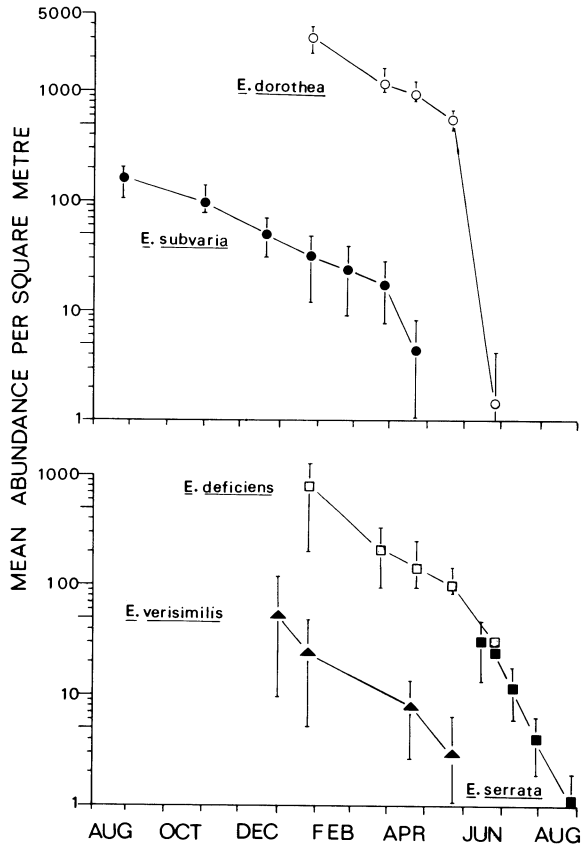

FIG. 7. Mean larval abundance per square metre for five species of *Ephemerella* mayflies in a riffle area of White Clay Creek. Vertical bars denote standard deviation of means.

Photoperiod is another factor that must be considered with respect to the growth, development, and emergence of aquatic species. It has been clearly shown that terrestrial insects have exploited extensively the geophysical patterns of photoperiod during the evolution of ecological, physiological, and behavioral adaptations (Beck 1968 for review). Photoperiod has not been studied extensively for aquatic species, especially mayflies. All of our experiments on *Ephemerella* species have taken place under the daily photoperiod that existed at the time of the experiment (e.g., premature and midwinter emergence of *E. subvaria* occurred in a midwinter photoperiod). Nebeker (1971) concluded from his study of 10 species of aquatic insects (including Ephemeroptera, Trichoptera, Plecoptera, and Diptera) that altering photoperiod had little or no measurable effect on emergence time, while elevating winter temperatures induced premature emergence. The relative effects of temperature and photoperiod on larval growth and adult emergence have been partially studied for the aquatic insect order Odonata (dragonflies and damselflies). Most of these studies have involved collecting final or penultimate instar larvae at different times from August through the winter and exposing them to various combinations of temperature and photoperiod. Results of these studies are highly variable. For some species it can be reasonably inferred that photoperiod either induces a larval diapause (Corbet 1956) or affects the amount of time needed to complete larval development and emerge as an adult (Lutz 1968, Lutz 1974*a*, *b*, Ingram 1975, Ingram and Jenner 1976). For other species, completed experiments (i.e., those where data were not extrapolated for the sake of analysis) indicate little or no photoperiodic effect (Proctor 1973). Interpretation of many experiments is difficult, however, because the experimental design either lacked controls entirely (i.e., animals were not kept at either natural temperature or photoperiod for comparison—Corbet 1956, Ingram 1975, Ingram and Jenner 1976) or only partial controls were maintained (i.e., animals kept at natural temperatures but not at natural photoperiods—Lutz 1968, 1974*a*, *b*). This seems relatively important because experiments which demonstrated a marked photoperiod effect at certain high constant temperatures (Lutz 1968, 1974*a*, *b*) always failed to show any photoperiodic effect in the limited control groups (e.g., animals kept at natural temperatures failed to respond to photoperiodic manipulation).

Although the temperature–photoperiod interaction for Odonata needs further study, we cannot ignore the fact that, for most species, larval development was correlated positively with temperature and, within a temperature, development was fastest at long-day photoperiods. One reasonable but untested hypothesis is that increased day-length at a given temperature increases the overall feeding period of these species, especially since most are visual predators and feeding activity may be reduced or less efficient in the darkness. For example, increasing daylength from 11 to 14 h not only increases the feeding period for a daytime predator by 27% but also reduces the amount of time each day that the animal relies on reserve energy for maintenance metabolism. It is interesting to note that significant photoperiodic effects at a given constant temperature were only observed for Odonata that were provided with food ad libitum and permitted to feed according to their own schedule (Corbet 1956, Lutz 1968, 1974*a*, *b*, Ingram 1975, Ingram and Jenner

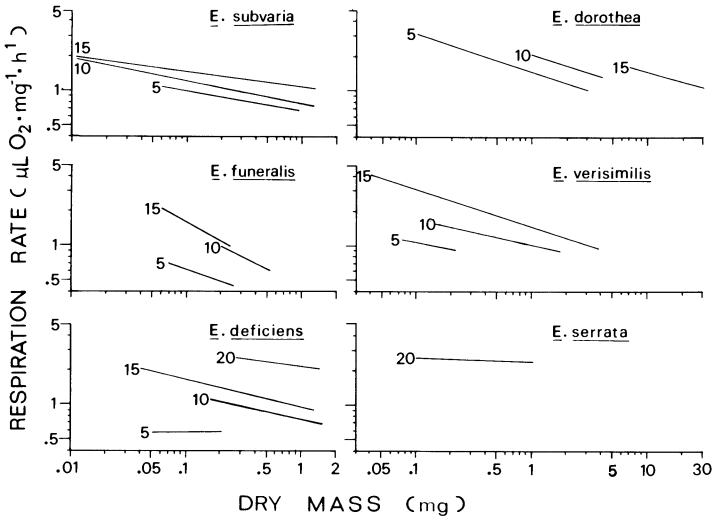

FIG. 8. Respiration rates per unit mass for larvae of six species of *Ephemerella* mayflies from White Clay Creek. Each fitted regression describes respiration rates for a specific constant temperature (e.g., 5°, 10°, 15°, 20°C). Regression equations are given in Table 4.

1976). Proctor (1973), however, fed his animals by hand each day until each larva was satiated and failed to show significant photoperiodic effects for completed experiments on three species of Odonata.

Our growth studies on *Ephemerella* mayflies in White Clay Creek indicate that several species exhibit a marked increase in growth during the spring. During this period both water temperature, photoperiod, and algal food supply are increasing. Anderson and Cummins (1979) have reviewed the importance of diet to the life histories of aquatic insects. We have shown an effect of temperature but the relative importance of photoperiod and nutrition are clearly areas for further study.

TABLE 5. Regression equations (log $Y = b \log X + \log a$) for mass-specific metabolism (μL $O_2 \cdot mg^{-1} \cdot h^{-1}$) of six species of *Ephemerella* mayflies at various constant temperatures (range 1°–20°C).

Species	Temp. (°C)	$b_{Y \cdot X}$	log a	df	F	r^2
E. subvaria	1	−0.018	−0.175	1, 13	0.04	0.003
	5	−0.168	0.0002	1, 83	13.7	0.14
	7	−0.306	−0.0012	1, 10	4.2	0.29
	9	−0.135	0.049	1, 13	3.2	0.20
	10	−0.171	0.112	1, 37	29.4	0.44
	11	−0.080	0.131	1, 9	0.63	0.06
	12	−0.236	0.154	1, 13	5.70	0.30
	13	−0.122	0.129	1, 6	0.59	0.09
	15	−0.123	0.160	1, 20	27.0	0.57
E. dorothea	5	−0.337	−0.162	1, 13	51.3	0.79
	10	−0.306	0.010	1, 12	34.3	0.74
	13	−0.336	0.100	1, 7	329.1	0.97
	14	−0.326	0.123	1, 9	159.8	0.94
	15	−0.286	0.162	1, 21	21.9	0.51
	16	−0.327	0.246	1, 5	6.4	0.56
E. funeralis	5	−0.330	−0.219	1, 19	5.3	0.21
	10	−0.490	0.154	1, 12	30.5	0.71
	15	−0.594	0.212	1, 10	10.6	0.51
E. verisimilis	5	−0.188	−0.159	1, 9	0.2	0.01
	10	−0.186	0.027	1, 8	2.3	0.22
	15	−0.310	0.176	1, 12	35.6	0.74
E. deficiens	5	0.077	−0.132	1, 15	0.2	0.01
	10	−0.213	−0.116	1, 10	9.1	0.47
	15	−0.242	−0.014	1, 14	13.2	0.48
	20	−0.147	0.330	1, 21	0.5	0.02
E. serrata	20	−0.024	0.390	1, 21	0.003	0.01

TABLE 6. Energy content of various developmental stages of six species of *Ephemerella* mayflies in White Clay Creek.

	Joules/mg ash-free dry mass														
	Larvae			Exuvia			Adult males			Adult females			Eggs		
Species	*n*	Avg.	(SD)	*n*	Avg.	(SD)	*n*	Avg.	(SD)	*n*	Avg.	(SD)	*n*	Avg.	(SD)
E. subvaria	5	20.204	(0.556)	1	20.740	(—)	2	23.577	(0.347)	2	23.192	(1.201)	3	27.342	(1.544)
E. dorothea	2	22.041	(0.385)				2	23.832	(2.188)	2	20.958	(0.653)	2	25.062	(1.381)
E. verisimilis				2	20.338	(0.121)	3	25.049	(2.063)	2	24.631	(1.179)	2	23.660	(0.690)
E. funeralis	4	21.652	(0.975)	2	21.924	(0.962)				3	24.903	(0.544)	5	24.698	(0.460)
E. deficiens	2	22.782	(0.766)				2	26.656	(1.858)	2	24.819	(0.046)	3	24.811	(0.724)
E. serrata	2	22.464	(0.644)												

Larval distribution and mean abundance

E. funeralis is the only *Ephemerella* mayfly whose main population occurs in spring seeps and first-order spring brooks of White Clay Creek. Larvae are collected occasionally in higher order tributaries along the pool margins or in loose leaf deposits. We have not sampled quantitatively for this species.

The other *Ephemerella* species occur generally in higher order tributaries (≥ third order); we have quantitatively sampled for these species in a pool and riffle on a fourth-order tributary. Results show that four of the five species have ≥90% of their larvae in riffle habitat (Table 4). *E. verisimilis* had more larvae in riffle (56.9%) than pool (43.1%) habitat when averaged for the entire year, but on many sample dates larvae were absent completely from riffle samples or were found mainly in the pool. This suggests that the distribution of larvae may be somewhat restricted to pools and slow flow areas of riffles.

Data on mean abundance per square metre of riffle show that *E. dorothea* and *E. deficiens* had the highest densities (Fig. 7). Also, there appears to be a relatively constant percent larval mortality for all species which does not seem to increase or decrease significantly with changes in the rate of larval growth (see Fig. 1 for comparison with seasonal patterns of growth).

Although all five species occur in the same riffle, the question arises as to whether each species occupies a unique microhabitat. We statistically analyzed the presence-absence component of our quantitative riffle samples to determine whether the patterns of occurrence of the five *Ephemerella* species were indicative of differences in microhabitat preference. The analysis was done by date, with each species scored as being either present or absent from each 0.09-m² sample. Two test statistics were used: (1) the Cochran Q-statistic (Cochran 1950); and (2) the M-statistic (Hendrickson 1978). The underlying distribution of both statistics appears to be approximated adequately by the chi-square distribution. The two statistics are related such that if Q is significant, then M is significant, but when the Q statistic is not significant, then the M statistic is, in fact, an independent test of the same hypothesis (J. A. Hendrickson, *personal communication*). A significant result for either statistic indicates unusual heterogeneity in the number of *Ephemerella* species per 0.09-m² sample when tested over all samples (i.e., that a given 0.09 m² of riffle

TABLE 7. Partial energy budgets for *E. subvaria* and *E. dorothea* larvae in White Clay Creek. G = growth, R = respiration, A = assimilation, NGE = net growth efficiency = (G ÷ A) 100.

Ephemerella (Ephemerella) subvaria							*Ephemerella (Ephemerella) dorothea*						
	mg		Joules·mg⁻¹·d⁻¹			%		mg		Joules·mg⁻¹·d⁻¹			%
Date	Avg. mass	(SD)	G	R	A	NGE	Date	Avg. mass	(SD)	G	R	A	NGE
7 Aug	0.034	(0.008)	.494	1.268	1.761	28.0	1 Feb	0.041	(0.033)	.598	.619	1.218	49.1
25 Aug	0.052	(0.008)	.477	1.138	1.741	34.6	21 Feb	0.073	(0.073)	.925	.661	1.586	58.3
18 Sep	0.106	(0.072)	1.029	1.590	2.619	39.2	10 Mar	0.158	(0.150)	.184	.427	.611	30.1
15 Oct	0.254	(0.097)	.623	.908	1.531	40.7	5 Apr	0.198	(0.125)	1.121	.690	1.812	61.8
25 Oct	0.341	(0.184)	.741	.711	1.452	51.0	22 Apr	0.518	(0.411)	2.389	.536	2.925	81.6
18 Nov	0.833	(0.289)	.502	.490	.992	50.6	2 May	1.837	(0.767)				
15 Dec	1.613	(0.566)	.523	.356	.879	59.5							
14 Jan	3.495	(1.544)	0	.343	.343	...							
12 Feb	3.474	(1.338)	.364	.372	.720	49.4							
26 Feb	4.313	(1.622)	.916	.360	1.276	71.8							
10 Mar	7.320	(2.804)	.297	.393	.690	43.0							
28 Mar	9.440	(3.340)											

TABLE 8. Partial energy budgets for *E. funeralis* and *E. verisimilis* larvae in White Clay Creek. G = growth, R = respiration, A = assimilation, NGE = net growth efficiency = (G ÷ A) 100.

Ephemerella (Eurylophella) funeralis							*Ephemerella (Eurylophella) verisimilis*						
	mg		Joules·mg^{-1}·d^{-1}			%		mg		Joules·mg^{-1}·d^{-1}			%
Date	Avg. mass	(SD)	G	R	A	NGE	Date	Avg. mass	(SD)	G	R	A	NGE
26 Oct	0.398	(0.212)	.615	.967	1.582	38.8	1 Nov	0.050	(0.033)	.623	.987	1.611	38.6
15 Nov	0.729	(0.310)	.209	.527	.736	28.3	18 Nov	0.084	(0.025)	.100	.569	.669	14.9
30 Nov	0.851	(0.486)	.117	.364	.481	24.3	22 Dec	0.099	(0.032)	.067	.343	.410	16.2
15 Dec	0.927	(0.346)	.192	.146	.339	56.3	23 Jan	0.110	(0.041)	.121	.381	.502	24.2
14 Jan	1.223	(0.905)	.054	.063	.117	45.9	4 Mar	0.139	(0.051)	.163	.556	.720	22.6
18 Feb	1.343	(0.653)	0	.339	.339	. . ..	22 Mar	0.160	(0.050)	.636	.519	1.138	54.9
21 Mar	1.341	(0.543)	.381	.410	.791	48.1	1 Apr	0.217	(0.059)	.669	.619	1.289	51.8
11 Apr	1.941	(0.806)	.808	.456	1.268	63.8	18 Apr	0.379	(0.115)	1.377	.640	2.017	61.1
18 Apr	2.546	(0.766)	1.017	.393	1.414	71.9	27 Apr	0.697	(0.385)	.912	.623	1.536	59.3
25 Apr	3.587	(1.120)	.134	.377	.510	26.2	8 May	0.908	(0.610)	1.452	.586	2.038	71.1
13 May	4.026	(1.110)					22 May	2.615	(1.121)				

probably will not contain all species). Conversely, nonsignificant Q and M statistics would suggest that most 0.09-m^2 samples probably contain all the possible *Ephemerella* species found at that time in the riffle as a whole.

Out of 25 sampling dates over a 2.5-yr span, a significant Q statistic occurred twice and M statistic eight times. Most significant values occurred during fall or in early winter samples when most species are small and difficult to collect. Thus, from the analysis it appears that each species generally occupies the same areas within the riffle. There may be more subtle microhabitat preferences (e.g., rock top vs. rock bottom, etc.) but our data were not detailed enough to test for these. Interspecific competition for available resources, however, may not be as severe as the above analysis suggests for *Ephemerella* larvae because: (1) the mortality curves (Fig. 7) show that no two species in the same subgenus are equally abundant at any time of the year; and (2) the population growth curves (Fig. 1) indicate that maximum larval growth (and hence resource use) of each species is temporally out of phase with the others and that there is usually at least a 10-fold difference in size on any given date between two species in the same subgenus.

The shape and magnitude of the mortality curves in Fig. 7 are useful for making comparisons of relative abundance among the five species. They cannot, however, be used to calculate the absolute production of each species because additional field sampling using more refined techniques revealed that our collection and processing procedures for the quantitative samples were missing a percentage of the specimens, especially when the larvae were small, and that an "edge effect" (i.e., nonrandom distribution within the habitat) tends to develop in certain species. The additional

TABLE 9. Partial energy budgets for *E. deficiens* and *E. serrata* larvae in White Clay Creek. G = growth, R = respiration, A = assimilation, NGE = net growth efficiency = (G ÷ A) 100. Asterisk (*) indicates calculation for time period between 19 Nov and 5 Apr.

Ephemerella (Serratella) deficiens							*Ephemerella (Serratella) serrata*						
	mg		Joules·mg^{-1}·d^{-1}			%		mg		Joules·mg^{-1}·d^{-1}			%
Date	Avg. mass	(SD)	G	R	A	NGE	Date	Avg. mass	(SD)	G	R	A	NGE
6 Oct	0.054	(0.029)	.272	.916	1.188	22.8	16 May	0.006	(0.004)	2.326	.849	3.217	73.6
19 Oct	0.064	(0.034)	.786	.686	1.472	53.2	22 May	0.012	(0.005)	2.088	.648	2.736	76.2
3 Nov	0.114	(0.100)	.054	.598	.652	8.3	7 Jun	0.095	(0.051)	1.958	1.163	3.121	62.7
19 Nov	0.119	(0.052)					14 Jun	0.185	(0.129)	1.594	1.176	2.769	57.5
8 Dec	0.115	(0.024)					21 Jun	0.316	(0.177)	.523	1.192	1.715	30.4
28 Jan	0.134	(0.062)	.017*	.456	.473	3.5	5 Jul	0.447	(0.225)				
19 Feb	0.151	(0.101)											
11 Mar	0.139	(0.043)											
5 Apr	0.134	(0.068)											
3 May	0.333	(0.240)	.640	.502	1.142	56.0							
19 May	0.642	(0.379)	.833	.506	1.339	62.0							
26 May	0.900	(0.421)	1.004	.631	1.636	61.2							

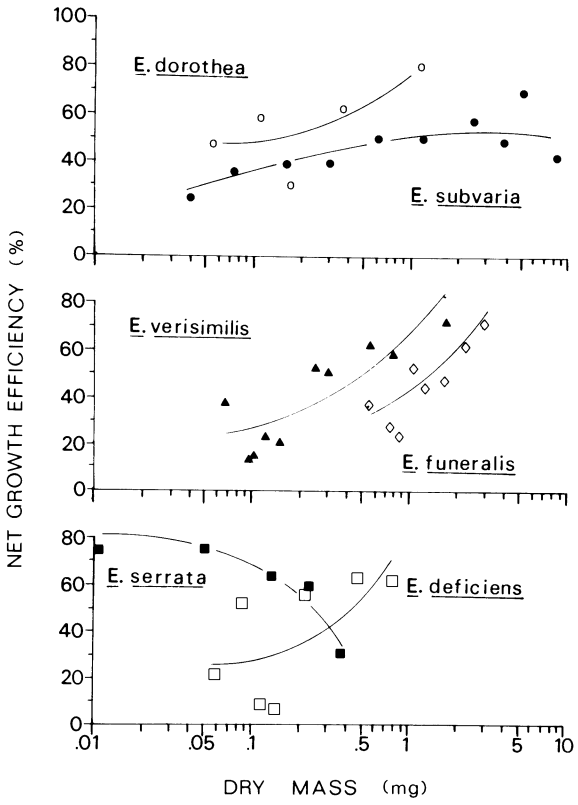

FIG. 9. Net growth efficiency as a function of mean larval mass for six *Ephemerella* species in White Clay Creek. Lines through data points were fitted using the following equations which yielded the best fit of six equations tried: *E. dorothea*: $Y = 44.1 + 32.9^X$, $r^2 = .61$; *E. subvaria*: $Y = X \div (0.0006 + 0.0192^X)$, $r^2 = .80$; *E. verisimilis*: $Y = 66.8X^{0.43}$, $r^2 = .62$; *E. funeralis*: $Y = 23.1 + 16.9X$, $r^2 = .71$; *E. serrata*: $Y = 79.1 - 118.1X$, $r^2 = .92$; *E. deficiens*: $Y = 21.9 + 59.9X$, $r^2 = .39$.

samples suggest that, although the magnitude of all curves should be elevated, the shape of the mortality curves and interspecific differences in relative abundance remain essentially unchanged. This might enable us to compare seasonal "patterns" of production for *Ephemerella* species by combining field estimates of growth (Fig. 1) and density (Fig. 7) for each species. We hesitate to tabulate these data in this paper because, without careful reading of the text, they might falsely be construed as absolute estimates of production.

Energetics

In order to provide comparative information on seasonal flux of energy per species and interspecific differences in growth efficiency of larvae, we present data in subsequent sections concerning larval respiration and calorimetry of life history stages. These data are combined with the growth data of Fig. 1 to develop partial energy budgets for each species during the year.

Larval respiration.—Respiration rates were inversely related to body mass for a given temperature (Fig. 8, Table 5). Rates for most species ranged between 1.0 and 2.5 μL $O_2 \cdot mg^{-1} \cdot h^{-1}$ during periods of rapid larval growth. At 5°, rates were ≤ 1.0 μL $O_2 \cdot mg^{-1} \cdot h^{-1}$ for all species except *E. dorothea*, which exhibited rates between 1.0 and 3.0 μL $O_2 \cdot mg^{-1} \cdot h^{-1}$.

A comparison between consubgeneric species indicates that respiration rates were significantly different at all temperatures for the *Ephemerella* (*E. subvaria* vs. *E. dorothea*) and *Eurylophella* (*E. funeralis* vs. *E. verisimilis*) subgenera (analysis of covariance; $P < .05$). Respiration rates of *E. deficiens* and *E. serrata* (subgenus *Serratella*) did not differ significantly at 20° ($P > .05$); a comparison for temperatures <20° was impossible because experimental data were not available for *E. serrata*.

Calorimetry of life history stages.—The energy content of *Ephemerella* mayflies depends largely on the developmental stage (Table 6). By averaging values for all *Ephemerella* species, it appears that the lowest energy content occurs in larval tissue (21.81 J/mg) and the highest in eggs (25.11 J/mg). Adult females and males average about 23.70 and 25.07 J/mg, respectively. The increase in energy content from larvae to adults to eggs probably represents a disproportionate increase in lipids per unit mass relative to other materials (e.g., carbohydrates and proteins). The tissue of lowest energy content in *Ephemerella* mayflies is the larval molt skin (average for three species = 21.00 J/mg). Since larval molt skins generally weigh between 5 and 12% of the mass of the molting larvae, the molting process for these mayflies appears to be costly in terms of the overall energy flux of an individual.

Larval energy budgets.—The flux of energy through each *Ephemerella* species was estimated by: (1) converting growth data presented in Fig. 1 to joules and calculating a mean individual growth rate (joules per milligram per day) for each sample interval; (2) using the fitted regression equations (see Table 5) for respiration experiments to estimate daily respiration costs (microlitres of oxygen per milligram per day) using a respiratory quotient of 0.87 and an oxyenergetic equivalent of 2.019×10^{-2} J/μL O_2. Respiration rates at temperatures intermediate between actual experimental temperatures were estimated by averaging between the slopes and intercepts of known equations. Mean daily assimilation rate for each interval was estimated by summing values for growth and respiration. This is a partial and somewhat conservative estimate because larval molt skin production and excretory products are not included. The estimates, however, are sufficient to provide comparative information on seasonal flux of energy per species and interspecific differences in net growth efficiency (i.e., growth ÷ assimilation) of larvae.

Partial energy budgets for species in each subgenus (*Ephemerella, Eurylophella,* and *Serratella*) are pre-

TABLE 10. Percent composition of larval gut contents for *Ephemerella* mayflies from White Clay Creek.

Species	Nov–Feb				Mar–May			
	% diatoms		% detritus		% diatoms		% detritus	
	n	Avg.	(SD)	Avg.	(SD)	*n*	Avg.	(SD)	Avg.	(SD)
E. subvaria	4	41.7	(26.6)	58.2	(26.6)	17	75.7	(13.8)	24.3	(13.8)
E. dorothea	3	6.2	(4.1)	93.8	(4.1)	12	71.9	(10.4)	28.1	(10.4)
E. funeralis	3	4.8	(2.4)	95.2	(2.4)	2	21.0	(10.3)	79.0	(10.3)
E. deficiens	3	30.1	(24.2)	69.9	(24.2)	4	44.5	(7.5)	55.4	(7.5)

sented separately in Tables 7, 8, and 9, respectively. For most species (except *E. serrata*) there was a general tendency for net growth efficiency to be correlated positively with body size (Fig. 9). Respiration energy usually exceeded the amount put into growth during the early larval instars of most species. In general, each species exhibited a net growth efficiency exceeding 70% for at least one sample interval during larval growth. This sample interval occurred usually during the last 4–5 wk of larval growth, except for *E. serrata* where it occurred during the early larval instars.

Although net growth efficiencies generally tended to change in a consistent direction during the larval period of each species, our estimates are often irregular and seem to shift abruptly from one sample interval to the next. This is probably due to errors inherent in estimating mean larval mass at the beginning and end of each sample date. For example, an underestimate followed by an overestimate of mean mass would result in an artificially high net growth efficiency for the sample interval.

For all species, net growth efficiencies above 50% occur only during late spring and early summer. This may be related to many factors, both physiological and environmental. Three possibly important environmental parameters are temperature, food quantity, and food quality. The period between late winter and early summer is characterized by large temperature fluctuations. The importance of these fluctuations to energy processing by stream insects in WCC has been reviewed elsewhere (Sweeney 1978, Vannote 1978, Vannote and Sweeney 1980). Although standing crop of detritus is decreasing during this period (R. L. Vannote, *personal observation*) primary productivity is increasing (T. L. Bott, *personal communication*). Thus the quality of available food is probably changing more significantly than the quantity. A comparison of the quality of gut contents for *Ephemerella* larvae seems to reflect this seasonal change (Table 10). These data show that the diet of *E. dorothea, E. funeralis,* and *E. deficiens* shifted from largely detritus in the winter to diatoms in the spring. *E. funeralis,* which inhabits heavily wooded, spring seep areas and eats mainly particulate detritus also shows a slight increase in the percentage of diatoms during the spring period. This shift from a detritus- to a diatom-based diet may be partially responsible for the observed changes in net growth efficiency. This interpretation would at least be consistent with past studies that have shown a strong correlation between low net growth efficiencies and a detritus diet (see Sweeney and Schnack 1977 for review) as well as recent studies that demonstrate the importance of food quality in insect bioenergetics (Anderson and Cummins 1979, Ward and Cummins 1979).

We computed from our energy budgets a conservative estimate of total energy that must be assimilated by an average larva of each species to complete larval growth (Table 11). The estimate is conservative because only growth and respiration energy are considered. Growth values were calculated for each species by taking the average amount of biomass (in milligrams) gained between the first and the last sampling date (from Tables 7, 8, 9) and converting to joules with a species-specific conversion factor (see Table 6). Respiration values were calculated on a daily basis (microlitres of oxygen per animal per day) and converted to joules for the budget (conversion factor reported in energy budget section of methods). Respiration rates in a given day were predicted by equations (Table 5) from the estimated mean larval mass for the day which was either read directly or estimated by interpolation from Tables 7, 8, or 9. The equation used each day depended on the mean daily water temperature (read at 0.5° intervals). Known equations (Table 5) at specific temperatures were used to estimate slopes and intercepts for equations predicting respiration rates at intermediate temperatures.

TABLE 11. Minimum number of joules required by six *Ephemerella* species during the larval growth period. G = growth, R = respiration, A = assimilation. Note that A = G + R. Asterisk (*) indicates mean larval mass just prior to metamorphosis.

	Mean larval* mass (mg)	Joules		
		G	R	A
E. subvaria	9.44	196.6	204.1	400.8
E. dorothea	1.83	39.3	14.2	53.5
E. funeralis	4.02	85.7	98.7	184.1
E. verisimilis	2.61	55.6	34.3	89.9
E. deficiens	0.90	19.2	20.0	39.3
E. serrata	0.49	9.6	10.0	19.6

The total energy requirement was correlated positively with size of the mature larva (e.g., the requirement ranged from 19.7 J for the smallest species, *E. serrata*, to 401 J for the largest, *E. subvaria*). Four of the six species (*E. subvaria*, *E. funeralis*, *E. deficiens*, and *E. serrata*) exhibited nearly equal partitioning of energy between growth and respiration. For two species (*E. dorothea* and *E. verisimilis*), a disproportionately large amount of energy went into growth relative to respiration. It is interesting to note that an *E. subvaria* larva has about a 14-fold greater respiration requirement to complete growth than *E. dorothea*. This large discrepancy might mean that although *E. dorothea* are far more abundant than *E. subvaria*, the overall flux of energy through each population on an annual basis may be nearly equal.

ACKNOWLEDGMENTS

This work was supported by grants from the Rockefeller Foundation, the Frances Boyer Research Endowment, the Stroud Foundation, and the Shell Company Fund. We thank B. Anderson, S. Duczkowski, D. Funk, S. Hyatt, K. McGrath, J. Peirson, C. Staub, P. Thorn, and B. Vannote for technical assistance. We also thank C. Hawkins and an anonymous reviewer for helpful comments, and J. Hendrickson for valuable statistical advice in several aspects of this study. This paper was communicated at the Third International Conference on Ephemeroptera, Winnipeg, Manitoba, 1979.

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