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Effects of pH on the biology and distribution of Ephemerella funeralis (Ephemeroptera)

Sandy B. Fiance

Department of Entomology, Cornell University

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The life history, emergence, sex ratio, fecundity, food habits, microhabitat, distributional pattern, and effects of experimental acidification on the emergence, growth, and recruitment of *Ephemerella (Eurylophella) funeralis* McD. are reported. *E. funeralis* is the only member of the Ephemerellidae yet known to have a two-year life cycle. Emergence of adults was recorded from 1 June to 11 July in the Hubbard Brook watershed. Larval sex ratio from all study sites was approximately one male for every eight females. Sex ratio of adults was found to be site dependent, with males increasing in representation as stream size decreased. The average number of eggs per female was 1853 ± S.E. 87. Facultative parthenogenesis is indicated by the successful development of 53% of 381 eggs taken from an unmated female subimago. Larvae were found in accumulations of organic matter in slower flowing portions of streams and also in permanent woodland pools. Gut contents of larvae were predominantly composed of detritus and decomposing higher plant matter, especially leaves, and fungal growth of submerged wood. *E. funeralis* appears to decrease in abundance with decreasing stream pH and decreasing organic matter. The experimental acidification of Norris Brook had no effect on the emergence of adults but caused a decrease in growth and nearly eliminated recruitment of the new cohort. A direct relationship between pH and the abundance of *E. funeralis* in the Hubbard Brook watershed is indicated by the results.

S. B. Fiance, Dept of Entomology, Cornell University, Ithaca, NY 14853, USA.

Описаны жизненный цикл, вылупление, соотношение полов, плодовитость, биология питания, микростации, характер распределения и влияние опытной ацидификации на вылупление, рост и восстановление популяции *Ephemerella (Eurylophella) funeralis* McD. *E. funeralis* – единственный неарктический представитель *Ephemerellidae* с 2-летним жизненным циклом. Вылет имаго наблюдался с 1 июня по 11 июля в бассейне Хаббард Брук. Соотношение полов по определению на личинках во всех исследованных биотопах составляло примерно 1:8 (самцы : самки). Соотношение полов у имаго зависело от биотопа, причем, доля самцов возрастала с уменьшением величины русла. Среднее количество яиц на самку составляло 1853 ± 87. Наблюдался факультативный партеногенез, при котором развивалось 53% из 381 яица, взятых от неоплодотворенной самки субимаго. Личинки находились в скоплениях органического материала в участках рек с медленным течением, а также в постоянных лужах. В содержимом кишечника личинок преобладали детрит и остатки высших растений, преимущественно листвы, а также грибы, развивающиеся на затопленной древесине. У *E. funeralis* установлено снижение численности по мере снижения pH воды и содержания органики. Экспериментальная ацидификация Хаббард Брук не повлияла на вылет имаго, но вызвала снижение темпа роста и почти затормозила появление новой генерации. Результаты показали наличие прямой зависимости между pH и численностью *E. funeralis* в бассейне Хаббард Брук.

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1. Introduction

Ephemerella (Eurylophella) funeralis McD. is one of the most abundant and most widely distributed mayfly species in the Hubbard Brook watershed. Approximately 21% of all mayfly specimens taken during the two-year inventory phase of this study belonged to this species (Fiance unpubl). *E. funeralis* is the only mayfly species abundant in smaller tributaries to Hubbard Brook. Allen and Edmunds (1963) reviewed the biological observations on dates of emergence, geographical distributional limits, and notes on collection sites. My investigation focused on the life history, emergence, sex ratio, food habits, microhabitat, fecundity, and distributional patterns of *E. funeralis* in the Hubbard Brook watershed.

Several authors have commented on the potential importance of pH in determining stream community structure (Morgan and Egglishaw 1965, Minshall and Kuehne 1969, Sutcliffe and Carrick 1973, Ziemann 1975). These authors report that mayflies are the aquatic insect group most susceptible to low pH. This observation is supported by laboratory experiments which determined short-term lethal pH levels in a number of insect species (Bell and Nebeker 1969, Bell 1971). This paper helps fill the gap between descriptive studies correlating pH with benthic community structure and short-term laboratory experiments examining the lethal effects of low pH. The paper is divided into two major sections dealing with (1) the description of the biology and distributional pattern of *E. funeralis* in a watershed with streams of varying acidity, and (2) the response of *E. funeralis* to the experimental acidification of a small stream.

2. Description of study area

The Hubbard Brook watershed (Fig. 1) in the White Mountains of New Hampshire (43°57′N, 71°42′W) is a rugged terrain ranging from 200 to 1015 m a.s.l. and more than 3100 ha in extent. Two types of granitic bedrocks are equally represented in the watershed and are described in greater detail in Johnson et al. (1969). The climate is humid continental with short, cool summers and long, cold winters. Precipitation is evenly distributed throughout the year, averaging 1300 mm annually; about one-quarter to one-third is snow. Most water courses have a layer of ice covered by a thick mantle of snow from mid-December through March. Stream flow is unevenly distributed, with 58% occurring during the snow-melt period of March–May (Likens and Bormann 1975, Likens et al. 1977). Stream and air temperatures have been discussed by Federer (1973). A comparison of stream and precipitation pH can be found in Hornbeck et al. (1976). Streams are fed by ground-water seeps, many of which become dry in July and August of every year.

The Hubbard Brook watershed is nearly completely covered by a second growth forest of variable age composition that dates from extensive logging operations early in this century. Only the lower portion of the watershed around Mirror Lake is presently inhabited. A northern hardwood forest (described by Whittaker et al. 1974) is the dominant forest cover throughout the watershed but *Picea rubens* and *Abies balsamea* are dominant species at higher elevations, and *Tsuga canadensis* is the dominant tree species along Hubbard Brook below Bear Brook. Brook trout *Salvelinus fontinalis* are the dominant fish in Hubbard Brook and its larger tributaries. More detailed descriptions of parts of the watershed can be found in Likens et al. (1977) and Whittaker et al. (1974).

3. Methods

3.1. Descriptive period

Benthic samples were taken from 1974 through 1976 as part of an ecological survey of the mayflies and stoneflies of the watershed. Twenty-two sampling sites (Fig. 1) in the watershed were visited in May or June and again in August, 1975. Intensive handnet (mesh-size 471 μm) sampling and substrate inspection lasted about five hours at each visit at all sites. Additional samples were taken at these and other sampling sites during April, June, July, and August 1974; January and March

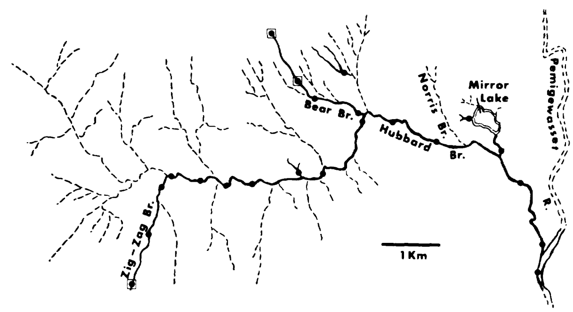

Fig. 1. Distributional pattern of *E. funeralis* in the Hubbard Brook watershed. Presence (darkened circles) or absence (darkened circles in squares) at study sites is indicated only for intensively studied streams (solid lines).

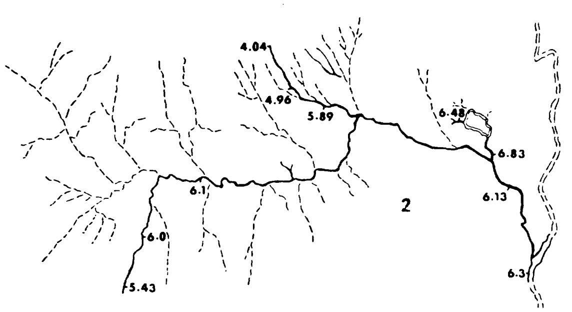

Fig. 2. Stream pH at selected study sites in the Hubbard Brook watershed on 1 July, 1975.

1975; January, March, May, and June 1976; and March 1977. Stream orders (as described by Leopold et al. 1964) were determined by field inspection during spring runoff. Organic cover of the stream bed was estimated by measurement of total aerial extent of organic dams in stream sections of known stream order, and did not include measurement of unconsolidated leaf or twig deposits. Thus, percent organic cover of the stream bed is an underestimate of the total organic cover. Water pH samples taken in polyethylene plastic 500 ml bottles were determined with an Orion Model 401 pH meter within 24 h of collection. Water temperature, conductivity, most anions and cations other than hydrogen, canopy cover, and chlorophyll a standing crop also were studied at 1975 sampling sites but did not significantly correlate with the abundance of *E. funeralis* (Fiance unpubl.). Potential fecundity was measured using methods detailed in Clifford and Boerger (1974).

3.2. Experimental period

Norris Brook (Fig. 1) was selected for experimental acidification. It is a southerly flowing tributary to Hubbard Brook immediately west of the Mirror Lake basin with a total mainstream length of 1700 m from source to mouth. It is unusually rich in mayfly and stonefly taxa for a stream of its size in the watershed; this may be a response to its relatively higher pH (Tab. 1).

Investigation of Norris Brook began in 1976 when study sites at 944, 1006, 1463, and 1676 m from the source were selected. In 1977, study of all these sites continued except at 1676 m and was begun at 0, 76, 274, 853 and 883 m. The 1976 field season began on 17 May and extended through 8 November. The 1977 field season began on 18 April and extended through 7 October. A cooperative investigation intended to study the effects of stream acidification on water chemistry, aquatic insects, periphyton, and microbes was initiated at this time and results will be reported in later papers. The experimental acidification point was located 855 m from the source. At this point, dilute sulfuric acid (0.5–1.0 N) was added continuously to yield a pH of 4.0

Tab. 1. Mean pH (and range) of Norris Brook study sites before and after acid addition. 1976 values are for 23 May through 8 Nov. 1977 values are for 18 Apr through 18 Sep.

Site (distance from source, m)	1976	1977
0	–	5.04 (4.89–5.26)
76	–	6.04 (5.62–6.27)
274	–	6.21 (5.79–6.48)
853	–	6.23 (5.85–6.45)
		Acid added
883	–	4.03 (3.89–4.29)
944	6.37 (6.29–6.50)	4.14 (3.91–4.30)
1006	6.37 (6.28–6.49)	4.19 (3.98–4.58)
1463	6.50 (6.37–6.59)	5.42 (4.41–6.19)
1676	6.58 (6.51–6.62)	–

Tab. 2. Number of emergence traps per site on Norris Brook during the 1976–77 field seasons.

Site (distance from source, m)	No. of traps per site 1976	1977	Stream order
0	0	3	1
76	0	3	1
274	0	3	1
853	0	10	3
884	0	9	3
944	3	10	3
1006	3	10	3
1463	3	12	3
1676	3	0	3

at the 883 m station. The acid drip rate was altered, depending on discharge, to maintain a pH of 4.0. Acidification began on 18 April, 1977 and continued through 22 September, 1977. The addition of sulfuric acid created a gradient of stream pH in which pH gradually increased with increasing distance from the point of acid addition (Tab. 2). This plume of acidified water fluctuated in length depending on the discharge. All sites between 855 and 1006 m had a pH fluctuating around 4.0, but the 1463 m site had only intermittent lowering of water pH during periods of high discharge when the plume lengthened (Tab. 1). Water samples taken at weekly intervals at all sites on Norris Brook were analyzed for pH with a Sargent-Welsh model PBL pH meter. The behavior of discharge, conductivity, anion and cation concentration, temperature, heavy metals, and chlorophyll a standing crop will be reported elsewhere (Hall et al. unpubl.).

Emergence of aquatic insects was measured with aluminum or fiberglass window-screen conical traps covering 0.138 m^2. Emerging insects were funneled through the trap into a plastic cup at the apex of the cone. The cups contained several ml of ethylene-glycol as a preservative. During both field seasons, traps were in place continuously and were cleared of specimens at weekly intervals, except after 18 July, 1977 when traps were cleared every other week. A summary of the number of traps per site during the study is found in Tab. 2.

Two types of benthic samples were employed during this study. Dip net samples described previously were employed in life history analysis. A simple cylindrical box sampler enclosing 0.034 m^2 of stream bottom was used to determine quantitative differences between the benthos of reference and acidified portions of Norris Brook. Nine random samples divided equally among fast-flow cobble and pebble, slow-flow cobble and pebble, and fast-flow organic debris substrates were taken from 25 m long sections of Norris Brook in both reference (820 m) and acidified (950 m) zones. Organic debris is defined as accumulations of leaves, twigs, wood pieces, and fine organic particles. The cylinder was placed over the desired substrate and twisted into the stream bottom. All large inorganic and organic sub-

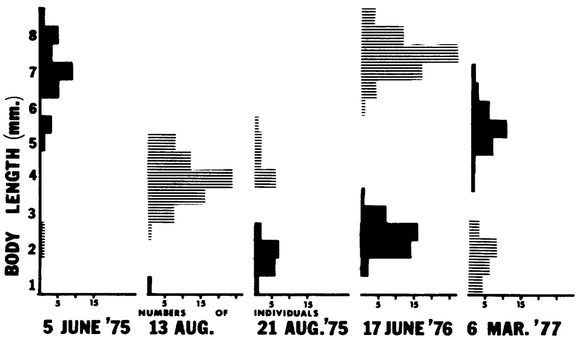

Fig. 3. Body length of *E. funeralis* larvae in relation to date of sample. 5 June 1975 sample from Hubbard Brook. All other samples from Zig-Zag Brook.

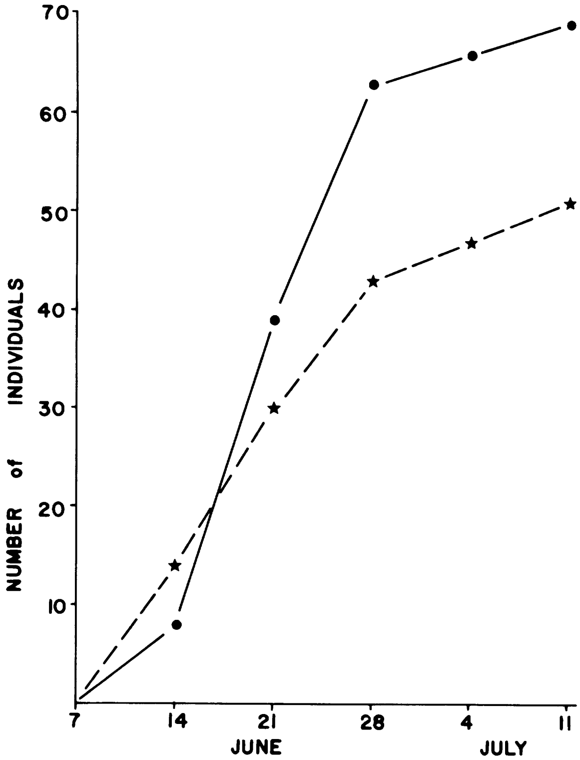

Fig. 4. Emergence phenology of *E. funeralis* in Norris Brook, 1977. Solid line represents cumulative emergence from three reference sites and the dashed line represents cumulative emergence from four acidified sites.

strates in the cylinder were removed to a water-filled enamel pan. Substrates remaining in the cylinder were stirred repeatedly down to a depth of approximately 15 cm and strained through a small 253 μm mesh handnet until no more organisms were collected. Large stones previously removed to the enamel pan were then scrubbed clean and all adhering organisms removed. The sample was then taken to the laboratory, refrigerated, inspected under 10 X magnification in small portions, organisms removed and preserved in 70% ethanol. The Student's t test for unpaired samples was used to test for significant differences between population means of both emergence trap and cylinder samples.

4. Results

4.1. Life history, emergence, sex ratio, and fecundity

Two separate larval cohorts of *E. funeralis* were present in the streams of the Hubbard Brook watershed (Fig. 3) except immediately following emergence. At this time, the smaller cohort was approximately one-third to one-half of mature size. Emergence of adults at any particular site was synchronous (Fig. 4). Adult flight period for all sites in the watershed extended from 1 June through 11 July. Emerging adults were observed at mid-day; mating was not observed. Oviposition occurred in mid-to late afternoon with females extruding yellow-orange egg masses while in near-stationary flight above the stream. Females dropped to the stream's surface for several moments at slower flowing sites to

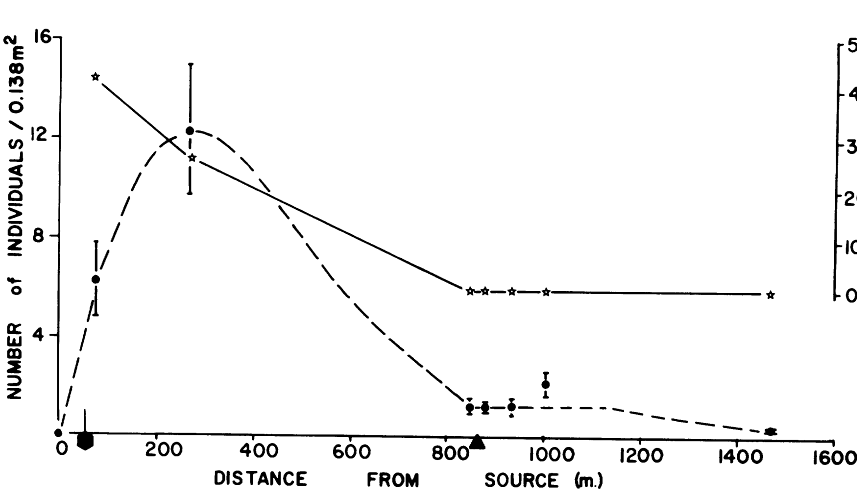

Fig. 5. Mean number of *E. funeralis* collected from emergence traps (dashed line) and percent males in that population (solid line) in relation to distance from the stream source, 1977. Hexagonal lollipop is at the upper limit of permanent streamflow, the solid triangle indicates the point of acid addition, and vertical lines represent the standard error of the mean.

deposit eggs and then resumed flight over the stream bed. Recruitment of larvae began in August. The presence of two distinct larval cohorts and one short, synchronous emergence each year denotes a two-year life cycle for *E. funeralis*.

A sex ratio of about one male to every eight females was found upon examination of 190 mature larval cimens collected from scattered localities in the watershed during April and May. The sex ratio obtained from emergence traps appears to be site-dependent in Norris Brook, as the percentage of males represented in the population increased with decreasing stream size (Fig. 5). Similar results have been observed in other streams under study.

Seven subimaginal females ranging in total body length from 6.5 to 7.5 mm (7 mm average) were examined for total numbers of eggs. Eggs were found in all thoracic and abdominal segments. A mean of 1852 eggs (S.E. ± 87) per female was recorded.

Of 381 eggs taken from a newly emerged, unmated female subimago, 203, or 53% hatched after 32 d of incubation at room temperature.

4.2. Microhabitat and food

Most larvae of *E. funeralis* are found in accumulations of leaves, and on submerged rotted wood in the slower flowing portions of streams (Fig. 6). Some smaller instar specimens can be found in cobbles and pebbles. Larvae can also be found in permanent standing-water pools created by subsurface seepage. However, this species was absent from Mirror Lake. Larvae in laboratory rearings were observed to strip softer portions of leaves and to graze on fungus found on the surface of submerged wood. Gut contents from 15 field-collected specimens were composed primarily of fine detritus and smaller proportions of decomposed higher plant material, fungal mycelia, and diatoms.

4.3. Distribution pattern

E. funeralis appears to reach its highest population densities in stream sections having the largest amount of organic cover and a high pH (Figs 1, 5, 7). Organic cover of leaves, twigs, and wood in the stream bed is greatest in the smallest streams and declines as the stream becomes larger and flushes out the organic material. A pH gradient exists in the streams of Hubbard Brook (Fig. 2). Smaller streams are the most acidic; pH increased downstream as the increasing concentrations of solutes neutralized free hydrogen ions. The pH remains relatively constant at the various sites along the stream (Hornbeck et al. 1976). Acidified precipitation may be the cause of low pH in the small streams (Hornbeck et al. 1976, Likens and Bormann 1974).

Emergence trap samples from Norris Brook have the greatest numbers of *E. funeralis* in first order stream sites (Fig. 5). Presumably, the population density in-

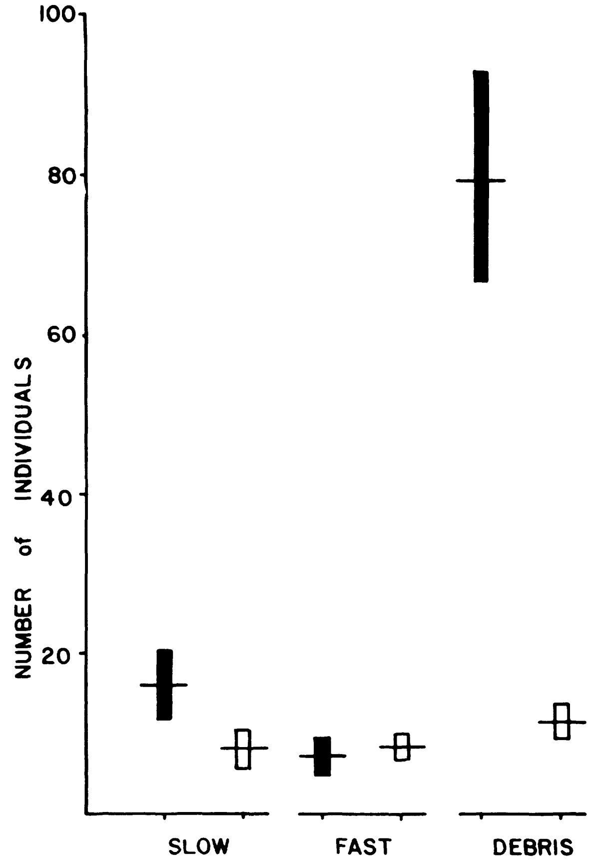

Fig. 6. Effects of acidification on benthic abundance and microhabitat specificity of *E. funeralis*. Vertical bars are equal to one standard error of the mean (solid = reference site, clear = acidified site; substrates are indicated as slow = slow velocity inorganic, fast = fast velocity inorganic, and debris = fast flow organic debris).

creases with increasing organic cover in the stream bed until the minimum pH approaches 5.5.

The relationship of total numbers of *E. funeralis* collected in the benthos per site to stream pH, stream order and organic cover can be found in Fig. 7. Three observations can be drawn from the data presented in this figure. First, sites in third order streams have the largest number of individuals per site. Second, in each stream order, larger populations were found at higher pH levels. Third, *E. funeralis* was absent from two permanent water sites which have a pH lower than 5.5.

4.4. Experimental effects of pH

Mean pH for all sites on Norris Brook are given in Tab. 1. Emergence phenology of *E. funeralis* was not altered by acidification (Fig. 4). The numbers of *E. funeralis* emerging from acidified sites were not significantly different from the reference site at 853 m, or from 1976 pre-acidification baseline values, although the larvae had been exposed to increased hydrogen ion concentra-

Tab. 3. A summary of life histories of members of the genus *Ephemerella* occurring in the Holarctic region.

Subgenus	Species	Years to complete life cycle	Source[1]
Eurylophella	*bicolor*	1	9
	funeralis	2	*this paper*
	lutulenta	1	9
	minimella	1	3
	temporalis	1	9
	trilineata	1	1
Ephemerella	*aurivilli*	1	7, 15
	excrucians	1	3
	ignita	1	4, 6, 8, 10, 11 & others
	inermis	1	2, 5
	krieghoffi	1	6, 12, 15
	mesoleuca	1	6, 12, 15
	mucronata	1	15
	notata	1	6
	subvaria	1	16
Seratella	*deficiens*	1	3
	tibialis	1	2, 5
Torleya	*major (= belgica)*	1	6, 12, 15
Drunella	*basalis*	1	14
	coloradensis	1	2, 13
	doddsi	1	2
	flavilinea (as *lapidula*?)	1	2, 5
	grandis ingens	1	2, 13
	trispina	1	14

1. References for life histories were drawn from the following citations: (1) Berner 1950, (2) Clifford et al. 1973, (3) Coleman and Hynes 1970, (4) Elliott 1967, (5) Hartland-Rowe 1964, (6) Landa 1968, (7) Larsen 1968, (8) Lavandier and Dumas 1971, (9) Lyman 1955, (10) Macan 1957, (11) Maitland 1965, (12) Petr 1961, (13) Radford and Hartland-Rowe 1971, (14) Tanaka 1966, (15) Ulfstrand 1968, (16) Waters and Crawford 1973.

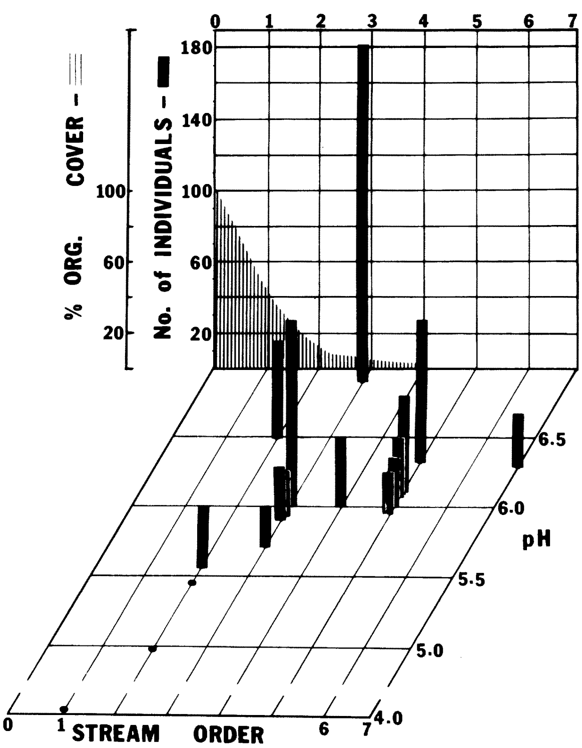

Fig. 7. The relationship of the total number of *E. funeralis* collected per site to stream order, pH (Fig. 2), and organic streambed cover in the Hubbard Brook watershed. Darkened ovals in first and second order streams indicate absence of larvae. Vertical black bars show number of individuals.

tions for approximately two months (Fig. 5). All other mayfly species were eliminated or severely reduced in the acidified zone at this time. After three months of exposure to pH 4, however, larvae of *E. funeralis* collected from the acidified stream section were significantly smaller (approximately 29%) in total body length than their counterparts in the reference zone (Fig. 8). Additional benthic dip-net samples taken in September show that larvae from the acidified stream section remained about 20% smaller than those from the reference site. These samples also show that recruitment of the new cohort in the acidified zone was severely reduced (Fig. 8). The new cohort represented 77 and 94% of the total numbers of *E. funeralis* present in dip net and cylinder samples, respectively, from the reference section of Norris Brook. Individuals of the same size-range from the acidified section of Norris Brook represented only 11% of the total population present in dip net samples. Further, some of these individuals are certainly members of the older cohort that have not

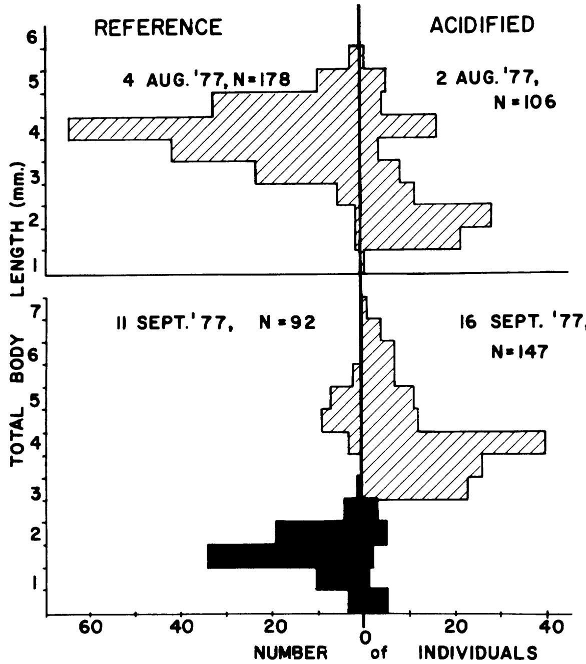

Fig. 8. Effects of acidification on growth and recruitment of *E. funeralis* larvae. Black histograms indicate the newly recruited cohort; striped histograms indicate the cohort recruited in 1976.

grown beyond the arbitrary cut off point between cohorts at 2.5 mm. Thus, there was at least a 66% reduction in the proportion of the population represented by the new cohort in the acidified zone. Cylinder samples employed to estimate relative larval densities between stream sections show no significant difference in total number of larvae from acidified and reference fast- and slow-water inorganic substrates (Fig. 6). However, there may be significantly fewer larvae in debris samples from the acidified zone ($P = 0.15$). This difference is due entirely to the recruitment failure of *E. funeralis* in the acidified zone. Larger larvae were approximately equally abundant in acidified and reference debris samples. All of the larvae from the reference zone inorganic substrates belonged to the newly hatched cohort and members of the older cohort were found only in the debris samples. In contrast, members of the older cohort were found in all substrates sampled in the acidified zone.

5. Discussion

E. funeralis is an unusual mayfly in many respects. It appears to be the first member of the Ephemerellidae reported to have a two-year life cycle (Tab. 3). All other members of this family in which the life histories are known have a one-year life cycle. Since *E. funeralis* is not larger than many of the species requiring only one year to complete development, some other reason for the reduced growth rate in this species must exist. In an oligotrophic stream system such as the Hubbard Brook watershed, a species able to pace its growth with the low food production rate may be at a competitive advantage.

McDunnough (1931) found no males among a large series of specimens from Ontario and suggested that the species was represented only by females. Males have since been associated with this species (Allen and Edmunds 1963). The skewed sex ratio for the watershed as a whole and the peculiar relationship of sex ratio and stream size are therefore compatible with previous observations but present interesting problems of interpretation. Potential reasons for the variable sex ratio include differential mortality of the sexes induced by environmental factors, and stress induction of males. The successful hatching of over 50% of eggs from an unmated subimago indicates facultative parthenogenesis in *E. funeralis*. Populations which are entirely female and that reproduce parthenogenetically possess a potentially greater rate of increase than those that produce males (Cuellar 1977).

The tolerance of later instars of *E. funeralis* to occasional low pH episodes has been demonstrated in the experimentally acidified stream. Presumably, the dominance of *E. funeralis* in smaller streams is the result of this ability as *E. funeralis* was the only mayfly that survived and emerged in approximately normal numbers following the experimental acidification. However, continued presence of low pH may have decreased its growth rate and severely reduced the recruitment of the new cohort. Alternate explanations of the decreased size of the larger cohort are (1) a size-dependent mortality of larger larvae, and (2) a decrease in the amount or quality of food due to acidification. The first has been discounted because of slightly greater abundance of the larger cohort in the acidified vs. the reference zones, lack of response of larger larvae for the first two months of acidification, and also because preliminary laboratory experiments showed no mortality of larger larvae after two weeks exposure to pH of 5.0, 4.0, and 3.5 (Fiance unpubl.). The second has been discounted because of the observed increase in algal standing crop following acidification (Hall et al. unpubl.). Lowered pH has been shown to inhibit gill ion-exchange mechanisms and to alter acid-base balance in fish (Packer and Dunson 1970, Schofield 1976). Since ion-exchange structures have also been found in mayflies (Komnick et al. 1972), it is possible that lowered pH would affect the osmoregulatory function of mayflies as well, although experimental evidence is lacking. The inferred decrease in growth rate observed in the acidified zone may be the result of greater allocation of energy resources to the maintenance of internal ionic balance and concomitant increases in metabolic rates to supply the energy for active transport. This may help to explain the movement of the older cohort away from low velocity organic accumulations into inorganic substrates where greater velocities would allow more oxygen exchange. Populations of *E. funeralis* in permanently acidified streams would most likely be eliminated through recruitment failure. These experimental results agree well with the observed pattern of abundance found in the watershed.

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J. Peters and Dr W. L. Peters at Florida A & M University, Tallahassee, verified the species indentification and also crically read an early draft of the manuscript, suggesting many improvements. The aid of all these people is very much appreciated. This is a contribution to the Hubbard Brook Ecosystem Study. The investigation was conducted with partial assistance from the Dept of Entomology, Cornell University and National Science Foundation grants to G. E. Likens and R. H. Borman. Field work was made possible through to cooperation of the Northeastern Forest Service Experimental Station, Forest Service USDA, Upper Darby, Pennsylvania.

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