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## APPLIED ISSUES

# Effects of single and repeated experimental acid pulses on invertebrates in a high altitude Sierra Nevada stream

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### SUMMARY

1. We examined responses of aquatic macroinvertebrates to pulsed acidification experiments in twelve streamside channels located in the Sierra Nevada, California. Experiment 1 consisted of a single 8 h acid addition, and Experiment 2 consisted of two 8 h acid additions administered 2 weeks apart. Replicated treatments (four reps/treatment) consisted of a control (pH 6.5–6.7) and pH levels of 5.1–5.2 and 4.4–4.6. Invertebrate drift was monitored continuously and benthic densities were determined before and after acid addition.
2. Drift responses to pH reduction were: (i) increased drift during acidification in pH 5.2 and pH 4.6 treatment channels, often with depressed post-acidification drift in treatment channels relative to controls (exhibited by *Baetis* only). Depressed post-acidification drift in treatment channels appeared to be due to low benthic densities because a positive relationship between benthic and drift densities was noted for most common taxa; (ii) increased drift rates during acidification only at pH 4.6 (*Epeorus*, *Drunella*, *Paraleptophlebia*, *Zapada*, and *Simulium*); (iii) decreased drift at pH 5.2 and/or pH 4.6 relative to control channels (*Rhyacophila* and chironomid larvae); (iv) no significant response to acidification (*Ameletus*, *Amiocentrus*, *Dixa* and *Hydroporus*).
3. A high proportion (45–100%) of acid-induced drift in *Baetis*, *Epeorus*, and chironomid larvae could be attributed to dead, drifting individuals.
4. Except for chironomids, most common invertebrates (i.e. *Baetis* and *Paraleptophlebia*) showed reduced benthic densities in treatment relative to control channels after acidification.
5. For sensitive taxa, drift was enhanced and benthic densities reduced by single (Experiment 1) and initial [Experiment 2(a)] acid pulses. Drift responses to a second acid pulse [Experiment 2(b)] were not as pronounced as those to the single or initial acid pulses [Experiments 1 and 2(a)], and the second acid pulse had no additional effect on benthic density.

### Introduction

The depression of pH in lakes and streams by anthropogenic acidic deposition can have substantial effects on the aquatic biota (Schindler, 1988; Schindler, Kasian & Hesslein, 1989). Decline in stream pH has been shown to decrease the diversity of benthic

invertebrate and algal communities, increase periphyton abundance, alter invertebrate life histories and may decrease benthic invertebrate biomass (Bell, 1970; Almer *et al.*, 1978; Fiance, 1978; Kimmel *et al.*, 1985; Mackay & Kersey, 1985; Okland & Okland, 1986; Feldman & Connor, 1992). Chronic long-term acidification can shift community composition

through the loss of sensitive taxa and replacement by more tolerant forms (Hall & Ide, 1987; Sutcliffe & Hildrew, 1989).

Increased drift of benthic invertebrates often occurs during acidification of streams (Hall, Driscoll & Likens, 1987; Ormerod *et al.*, 1987; Merrett *et al.*, 1991) and may be enhanced by associated increases in aluminium concentration (Hall *et al.*, 1987). Mayflies (Order Ephemeroptera), except *Ameletus* (Rosemond *et al.*, 1992), are especially sensitive to low pH waters (Bell, 1971; Sutcliffe & Carrick, 1973; Fiance, 1978; MacKay & Kersey, 1985), often having higher acid-induced drift rates than other benthic invertebrates (Overrein, Seip & Tollan, 1980). Species-specific susceptibility to acidification has been considered a function of differing physiology (Bell, 1971), habitat preferences (Allard & Moreau, 1987), and behaviour (Bernard, Neill & Rowe, 1990). Taxa that drift in response to short-term inputs are sensitive species lost from chronically acidified streams (Hall & Ide, 1987). Drift induced by perturbations may be responsible for observed reductions in benthic invertebrate density (cf. Hall, 1990), and may be a primary mechanism of species loss in low-order headwater streams where recolonization from upstream is precluded.

Acidic inputs can indirectly affect organisms through their influence on other organisms. Because many stream fish, such as trout, feed primarily on drifting invertebrates (Jenkins, Feldmeth & Elliot, 1970), changes in stream acidity can affect fish food resources. Further, the elimination of fish in chronically acidified habitats alters species assemblages, resulting in a community with invertebrates as top predators (Henrikson & Oscarson, 1978; Eriksson *et al.*, 1980).

Most previous stream or stream channel experiments have employed sustained acid additions to stream sections or channels (Hall *et al.*, 1980; Zischke *et al.*, 1983; Allard & Moreau, 1984; Burton, Stanford & Allan, 1985; Griffiths, 1987). Although these experiments demonstrate biotic responses to severe, continuous acid exposure, the results are less applicable to episodic, surface-water acidification associated with rain and snowmelt events. Moreover, increasing evidence suggests that the rapid decline of pH and concomitant increase in metal ions (particularly aluminium) associated with episodic events may be more deleterious than chronic exposure (Reader &

Dempsey, 1989). Episodic pH depressions may have substantial impacts on the aquatic biota before long-term decline in pH is evident (Servos & Mackie, 1986). Indeed, recent studies have shown significant responses of invertebrate communities to episodic pH depressions lasting from 6 to 24 h (Ormerod *et al.*, 1987; Hopkins, Kratz & Cooper, 1989; Bernard *et al.*, 1990; Merrett *et al.*, 1991).

Previous pulse studies have simulated single, localized pH depressions, while allowing immigration by drifting invertebrates from untreated areas above the experimental reach. Episodic acid events may have a proportionately large influence on streams draining small, weakly buffered alpine catchments, by affecting entire headwater areas thereby precluding subsequent recolonization via drift. In experiments reported here, we prevented immigration of invertebrates into stream channels during our experiments, to assess impacts associated with entire first-order streams rather than reaches within higher order systems. In contrast to previous studies, these experiments also address the cumulative effect of multiple acid events on the benthic community by preventing nymphal recolonization between acidification events.

Aquatic systems in the Sierra Nevada have the lowest ionic concentrations in the United States (Landers *et al.*, 1987) and their associated drainage basins have a limited capacity to neutralize acids (Melack, Stoddard & Ochs, 1985; Sickman & Melack, 1989). Streams and lakes in the Sierra Nevada of California generally range in pH from 6.2 to 6.5 (Melack *et al.*, 1985). Acidic precipitation (pH 4–5) sometimes falls during the summer and autumn (Melack, Stoddard & Dawson, 1982; Williams & Melack, 1991a), and pH as low as 5.5 has been recorded during snowmelt (Melack *et al.*, 1989; Williams & Melack, 1991b). During periods of heavy run-off, these catchments may not be able to neutralize acidic inputs (Sickman & Melack, 1989; Melack & Stoddard, 1991) and short-term pH depression of surface waters may occur (Dillon, Yan & Harvey, 1984; Melack *et al.*, 1989). Although the effect of pH depression on the invertebrate assemblage of a Sierran lake has been documented (Barmuta *et al.*, 1990), only limited information is available concerning the responses of Sierran stream invertebrates to increased acid deposition (Hopkins *et al.*, 1989).

In order to assess the sensitivity of a Sierran stream

community we simulated episodic acidification in twelve experimental channels and measured invertebrate benthic densities and drift rates.

### Materials and Methods

Simulations of low pH episodes were performed in stream channels fed by the Marble Fork of the Kaweah River, a second-order stream with an aquatic invertebrate fauna that is representative of Sierra Nevada streams and a fish community that is restricted to introduced brook trout (*Salvelinus fontinalis* Mitchell) (Cooper *et al.*, 1988). The simulated acid pulses contained sulphuric and nitric acids in equal proportions by equivalents, and were similar in acidity to naturally occurring summer storms (Williams & Melack, 1991a). Acidic rain and spring snowmelt comprise the primary acidic inputs in the area (Williams & Melack, 1991b). Twelve experimental stream channels were constructed next to the Marble Fork of the Kaweah River, in Sequoia National Park on the western side of the Sierra Nevada, California, U.S.A. (elevation 2780 m, latitude 36°35'N, longitude 118°40'W). At the experimental site the Marble Fork was approximately 1 m wide and 0.1 m deep. The substratum was granitic boulders and cobble interspersed with sand and gravel. The surrounding catchment is granitic with thin soils and sparse vegetation comprised of willows (*Salix* spp.) and conifers (*Pinus* spp.). Experiments were conducted in August and September 1986.

Channels were constructed from two 1.2 × 2.4 m plywood sheets. These plywood bases were each divided into six, 0.2 m × 2.4 m, channels with 0.2 m high Plexiglas plates as partitions between channels. A 6 cm high Plexiglas plate was affixed to the downstream end of each channel to ensure adequate water depth throughout the channel. Immediately in front of each of the downstream plates, retainers on the Plexiglas walls held a drift net (mesh size 250 µm) with the same cross-sectional area as the channel. Each net captured all invertebrates drifting from each channel. The gradient in each set of stream channels was adjusted to provide a drop of 5 cm from the upstream to the downstream end.

Water was diverted from a pool in the Marble Fork approximately 40 m upstream and with a 2 m head above the channels. Plastic pipes transported the unfiltered water by gravity to a 0.5 m<sup>3</sup> central reservoir.

Three 12.7 cm diameter, 5 m PVC delivery pipes, positioned perpendicular to the channels, carried water from the bottom front panel of the reservoir past the head of the channels. Four PVC 'T's were placed at approximate 0.6 m intervals along each PVC delivery pipe, and flexible plastic pipes (internal diameter 12 cm) carried water from each T to the head of a stream channel. Flow into individual channels was regulated by 12.7 cm diameter butterfly valves that connected the delivery pipes to each of the pipes delivering water from the Ts to experimental channels. During acidifications two 30 l plastic containers discharged acid solution through Tygon tubing to the appropriate delivery pipe. Precise control of acid flow was accomplished with the use of stopcocks mounted in the Tygon tubing. Effluent waters from the channels flowed through open plastic pipes back into the Marble Fork. A more detailed description and pictures of the channels can be found in Hopkins *et al.* (1989) and Cooper & Barmuta (1993).

The stream channels were leached with stream water for 2 weeks and then stocked with cleaned substratum (all invertebrates had been elutriated off) from the Marble Fork. Six large rocks (approximately 9 cm diameter) and 2 cm of gravel (0.5–2.0 cm diameter) and sand were placed in each channel. Invertebrates were allowed to colonize the channels naturally (via drift) for a 2-week period before the first and second experiments. In addition, invertebrates were collected from the Marble Fork through gentle kick sampling into drift nets (mesh size 250 µm), placed into buckets of water, and equally apportioned among the channels at least 3 days before the acid pulse. In Experiment 1, drift colonization was excluded from 24 h before until 40 h after the acid pulse to prevent potential 'swamping' effects by recolonizing invertebrates from the river (Hopkins *et al.*, 1989). Colonization via drift was additionally prevented in Experiment 2 for the 2 week period between the two acid events [Experiment 2(a) and 2(b)] to determine stream community responses to repeated acid episodes. Invertebrate exclusion was effected by directing diverted water through a 250 µm mesh net (1 m × 1.5 m length) located in the reservoir. This net was emptied daily during daylight hours, when natural drift was minimal. Between Experiments 2(a) and (b) a small tear (c. 2 cm) formed in the block net; however, the net was repaired within 24 h. The passage of invertebrate drift through this small

tear may have accounted for some of the increase in benthic densities of some taxa between the two acidifications in Experiment 2.

Each experiment consisted of a single 8 h pulse of three treatment levels of acid: pH 6.5 (control, no acid), pH 5.2 and pH 4.6. These treatments were chosen on the basis of earlier experiments (Hopkins *et al.*, 1989) and a search of the literature (cf. Baker *et al.*, 1990). Acid additions were made on 5 August (Experiment 1), 4 September [Experiment 2(a)], and 17 September 1986 [Experiment 2(b)]. Experimental channels were acidified with solutions of nitric and sulphuric acids (1:1 by equivalents). Acid additions were conducted in late morning and afternoon (10.00–18.00 h), to simulate rains which typically occur in the afternoon in the summer. In two of the treatment channels (one each at pH 4.6 and 5.2), pH was monitored continuously. pH in the remaining channels was measured at approximately 60 min intervals. Continuous pH measurements were made using portable pH meters and gel-filled electrodes. We measured the pH of water samples from other channels in the field with a Ross combination electrode (8104) and an Orion SH250 pH meter. Additional measurements were made downstream of the channels to insure that Marble Fork pH level was not affected by our experiments.

Water samples for chemical analyses were collected before, during and after acidification. The accuracy of field pH measurements was verified by laboratory measurements of pH of unfiltered samples with a combination electrode suitable for use in dilute waters (Sargent-Welch S-30072-15) and a Fisher Acumet 805 pH meter. Acid neutralizing capacity (ANC) was determined by incremental titration of the same sample with 0.1 N HCl. Laboratory analyses of pH and ANC were performed within 36 h of collection. Filtered samples (Gelman AE glass-fibre filtered) were analysed by ion chromatography for nitrate and sulphate. Unfiltered subsamples which had been acidified in the field were analysed for total iron and total aluminium by atomic absorption with the graphite furnace technique. Ion chromatograph and atomic absorption samples were stored and analysed within 6 months.

Invertebrate drift was monitored for 24 h before, 8 h during and 40 h after each acid pulse. Nets fixed to the end of each channel were emptied every 4 h during the time of acid addition (c. 10.00–18.00 h),

and every 8 h during each 24 h pre-acidification and 40 h post-acidification period. Discharge was measured in each channel before and after each drift sample was taken, by collecting the discharge in a calibrated bucket set at the end of each channel. Using these data, flow into channels was regulated to ensure similar discharge among channels. Drift rates were calculated as number per unit volume ( $m^{-3}$ ) to correct for slight variation in flow among channels (Allan, 1987). Live *v* dead counts for common taxa in the drift were obtained by immediately washing the contents of the drift nets into buckets and counting the numbers of live and dead individuals of selected taxa. An individual was considered dead if it did not move when prodded. Owing to the time required to determine numbers of live and dead individuals from all channels this assessment was restricted to the most common drift taxa (*Baetis*, *Epeorus* and chironomid larvae).

The proportion of drift that was caused by acid additions in treatment channels was determined for each time period and was partitioned into two components, death and escape. The number of individuals drifting as the result of escape was determined by subtracting the mean number of live individuals ( $m^{-3}$ ) of a taxon drifting from the control channels from the mean number of live drifting individuals ( $m^{-3}$ ) of that taxon in the treatment channels. Similarly, enhanced dead drift owing to acidification was determined for each acid treatment by subtracting the mean number of dead individuals ( $m^{-3}$ ) in a taxon drifting from the control channels from the mean number of dead drifting individuals ( $m^{-3}$ ) of that taxon in the treatment channels. The percentage of dead acid-induced drift was then determined by dividing the enhanced dead drift by the total number of live and dead acid-enhanced drifting individuals ( $\times 100$ ). This fraction of drifting individuals that was dead that was caused by acid inputs was termed 'killed' drift. Dead drift and killed drift were determined for *Baetis* and chironomid larvae in all experiments and for *Epeorus* in Experiments 2(a) and 2(b).

Benthic invertebrate densities were determined 24 h before, and 40 h and 2 weeks after each acid experiment. Stream benthos was sampled by cleaning all substrata in a randomly selected  $10 \times 20$  cm area on the bottom of each channel into a drift net (250  $\mu m$  mesh) placed just downstream. Two such samples (a total of 400  $cm^2$ ) were collected from each channel at

each sampling time and combined for analyses. In order to compare river fauna with the fauna of the experimental channels, three samples were taken using the same procedures from the Marble Fork adjacent to the stream channels on both 6 August and 19 September 1986 and compared with benthic samples collected on similar dates in the control stream channels. Invertebrate samples were preserved in 70% ethanol and were later identified to family or genus and counted under a dissecting microscope at  $\times 12$ .

In our analyses a taxon was defined as 'common' if present at a mean density of at least five individuals per sample in at least one treatment. Over the course of the two experimental periods a total of sixteen taxa in the channels were sufficiently 'common' in drift or benthic samples (Table 1) to be subjected to statistical analyses (Cooper & Barmuta, 1993).

Drift and benthic densities were log transformed if necessary following analyses of the residuals. Similarly, percentage of drift that was dead was arcsine square root transformed prior to analyses to insure homogeneity of variances (Allan & Russek, 1985). The relationship between benthic density and drift was assessed using cumulative drift densities from 24 h following the initial, and 24 h before the final, channel density estimates. Treatment effects on benthic densities and drift densities were determined differently. Treatment effects on benthic densities in Experiment 1 and pre-acidification Experiment 2(a) were analysed with one-way ANOVAs, by taxon, with pH treatment as the independent variable. Significant ANOVAs were followed with Tukey's HSD test. Treatment effects on live drift and percentage dead drift from both experiments, and the remaining benthic densities from Experiment 2, were analysed by taxon with repeated measures ANOVAs. In each experiment, the time series data for each common taxon were divided into three discrete blocks, namely the four net sets occurring before acidification (pre-acid), the two net sets collected during acidification (acid), and the six net sets collected following acidification (post-acid). In analysing live drift we assumed these three temporal blocks to be independent since the onset and the cessation of acid in these experiments was immediate. Continued increased active drift following the removal of the acid stimulus was not expected. If a significant time by treatment interaction was observed in repeated measures

ANOVAs, one-way ANOVAs were then performed on each time within a period. Significant ANOVAs were followed by Tukey's HSD test. The effects of acid treatment on aquatic invertebrates are therefore presented in two ways: (i) only significant main (treatment) effects on responses are presented by block (pre-acid, acid or post-acid) in tables; (ii) results for taxa showing significant interaction (time  $\times$  treatment) effects are presented by time period within blocks in figures. All analyses of variance and regression were performed using the general linear models procedure of SYSTAT (Wilkinson, 1989). Treatment means and standard errors are included for all one-way ANOVAs performed (Yoccoz, 1991).

## Results

### *Physical and chemical factors*

The mean discharge ( $\pm 1$  SE) of individual stream channels was  $3.4 \pm 0.02$ ,  $3.3 \pm 0.05$  and  $2.0 \pm 0.031 \text{ s}^{-1}$  during Experiments 1, 2(a) and 2(b), respectively. Discharge remained constant for the duration of Experiment 1, declined over Experiment 2(a), and increased over the course of Experiment 2(b) (Fig. 1). To ensure adequate discharge in Experiment 2(b), three channels were eliminated (one from each treatment). Although discharge differed among treatments in Experiment 2(b) before acid addition, there were no significant differences in discharge among treatments, during or after acidification in any experiment. Mean channel water depth showed a pattern similar to discharge. Mean depths over the substratum ( $\pm 1$  SD) were  $10.7 \pm 0.6$ ,  $7.0 \pm 0.5$  and  $6.0 \pm 0.3$  cm, during Experiments 1, 2(a) and 2(b), respectively. Water temperatures in the experimental channels were similar to those in the Marble Fork, ranging from 10 to 19°C during Experiment 1, 11 to 19°C during Experiment 2(a) and 4.5 to 8°C in Experiment 2(b).

Control channel pH means ranged from approximately 6.41 to 6.78 across experiments and fluctuated no more than 0.20 pH units within an experiment. During experimental acidifications, the pH of channels to which acid was added were within 0.2 pH units of target pH (5.2 and 4.6), except in the case of the pH 4.6 treatment in Experiment 1 (Table 2). ANC, which was low over the course of the summer, was exhausted by acid addition in the pH 5.2 treatment of

Order Ephemeroptera	
Siphonuridae	Rhyacophilidae
<i>Ameletus</i> sp.*	<i>Rhyacophila angelita</i> (Banks)*
Baetidae	<i>R. oreta</i> (Ross)
<i>Baetis</i> sp.*	<i>R. rotunda</i> (Banks)
<i>B. bicaudatus</i> (Dodds)	<i>R. alberti</i> grp.
Heptageniidae	<i>R. brunnea</i> grp.
<i>Cinygma</i> sp.	Order Coleoptera
<i>Cinygmula</i> sp.	Dytiscidae
<i>Epeorus</i> ( <i>Ironopsis</i> ) <i>grandis</i> (McDunnough)*	<i>Oreodytes</i> sp.
<i>Heptagenia</i> sp.	<i>Uvarus</i> sp.
Ephemerellidae	<i>Agabus</i> sp.†
<i>Attenella</i> sp.	<i>Coptotomus</i> sp.
<i>Caudatella hystrix</i> (Traver)	<i>Hydrovatus</i> sp.
<i>Drunella grandis</i> (Eaton)*	<i>Hydroporus</i> sp.*
<i>D. doddsi</i> (Needham)	Hydrophilidae
<i>D. spinifera</i> (Needham)	<i>Tropisternus</i> sp.
<i>Ephemerella</i> sp.	Elmidae
<i>Serratella</i> sp.	<i>Zaitzevia</i> sp.
Leptophlebiidae	
<i>Paraleptophlebia</i> sp.*	Order Diptera
Order Plecoptera	Deuterophlebiidae
Nemouridae	<i>Deuterophlebia</i> sp.
<i>Malenka californica</i> (Claassen)	Tipulidae
<i>Zapada cinctipes</i> (Banks)*	<i>Dicranota</i> sp.
<i>Z. oregonensis</i> (Claassen)	Simuliidae
Leuctridae	<i>Prosimulium</i> sp.*
<i>Perlomyia</i> sp.	<i>Simulium</i> sp.*
Perlodidae	Chironomidae
<i>Megarocys</i> sp.	Larvae*
<i>Isoperla quinquipunctata</i> (Banks)*	Pupae*
Chloroperlidae	Adults*
<i>Suwallia</i> sp.	Dixidae
Order Megaloptera	<i>Dixa</i> sp.*
Sialidae	<i>Meringodixa</i> sp.
<i>Sialis</i> sp.	Empididae
Order Trichoptera	<i>Clinocera</i> sp.
Brachycentridae	Muscidae
<i>Amiocentrus</i> sp.*	<i>Limnophora</i> sp.
<i>Micrasema</i> sp.	Class Arachnida
Hydropsychidae	Hydracarina*
<i>Hydropsyche</i> sp.	Phylum Nematoda
Limnephilidae	Phylum Annelida
<i>Ecclisomyia</i> sp.	Class Oligochaeta*
<i>Dicosmoecus</i> sp.†	
<i>Neophylax</i> sp.	
<i>Desmona</i> sp.	

**Table 1** Invertebrate taxa found in drift and benthic samples collected from the Marble Fork of the Kaweah River and the experimental stream channels, August–September 1986. \*Indicates that that genus was considered 'common' over the course of at least one experiment; see text for details. †Denotes river taxa not found in the stream channels

Experiment 1. Because ANC was relatively constant in control channels throughout experiments and because ANC was easily exhausted by acid additions in Experiment 1, we assumed that ANC was exhausted during acid additions to treatment channels in subsequent experiments and only monitored ANC in

control channels. Concentrations of nitrate and sulphate directly reflected additions of nitric and sulphuric acids (Table 3). Concentrations of total iron and total aluminium were low and variable, and did not show consistent responses to acidification (Table 4).

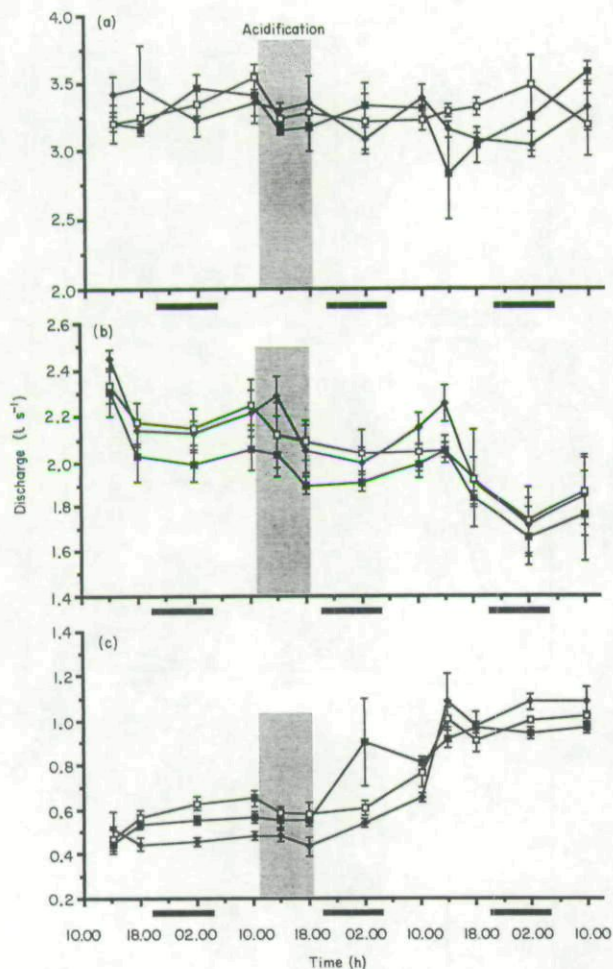


Fig. 1 Mean discharge ( $l s^{-1}$ ) from channels in each treatment. (a) Experiment 1: 5–8 August; (b) Experiment 2(a): 3–6 September; (c) Experiment 2(b): 17–19 September 1986. The shaded areas indicate the time of acid additions, the dark horizontal bars under the time line represent the periods of darkness (night). Vertical lines represent  $\pm 1$  SE. ■, pH 4.6; ◆, pH 5.2; □, control.

### Invertebrates

A total of forty-five taxa (generic level or higher) were found both in benthic samples taken from the channels and from the Marble Fork of the Kaweah River (Table 1). Two additional rare taxa were found only in the Marble Fork. Absolute abundances of taxa in channels were within the range of density values recorded from the Marble Fork, and the rank order of abundances of the twenty most common taxa was similar in the channels and the Marble Fork (Spearman's  $r = +0.53$  and  $+0.69$ ) in August and September, respectively ( $P < 0.05$ ).

### Drift

Drift rates for all taxa were similar in control and treatment channels prior to acidification in all experiments, with the exception of chironomid larvae in Experiment 1. In this experiment chironomid larvae showed significantly lower drift rates in treatment channels relative to controls the night before acidification (means =  $1.3$  and  $2.8 m^{-3}$ , pH 4.6 v control, respectively;  $F = 6.5$ ,  $P = 0.02$ , treatment effect, Tukey's HSD).

Diel drift patterns before acidification differed among taxa within experiments and within taxa across experiments. Nocturnal drift peaks in the mayfly nymphs *Epeorus*, *Paraleptophlebia* and *Drunella* ranged from pronounced to undetectable in Experiment 2(a) (Fig. 2). Diel drift patterns of *Baetis* varied among experiments, with a pronounced pretreatment nocturnal peak only in Experiment 2(a) (Fig. 3). *Baetis* had the most consistent and pronounced drift responses to acid inputs. There was significantly higher baetid drift in acidified than control channels during acidification in two of the three experiments (Table 5) with drift rates in acid treatments eleven to twenty-six times greater than those in controls during the first 4 h of acidification (Fig. 3). Following acidification *Baetis* drift was significantly greater in control channels than acid treatment channels ( $F = 14.0$  to  $46.4$ ;  $P = 0.002$  to  $0.0001$ ).

Prior to acidification the percentage of drifting *Baetis* that were dead (*c.* 15%) was similar across treatments and experiments (Table 6). This proportion increased significantly with decreasing pH during acidification and declined following acidification. The effects of treatment on the proportion of drift that was dead were significant after acidification only in Experiment 1 (Fig. 4). In response to acid addition the percentages of drifting *Baetis* that were dead in the pH 4.6 channels increased to 72%, 77% and 55%, in Experiments 1, 2(a) and 2(b), respectively. The percentage of enhanced drift that could be attributed to individuals killed outright by acid ('killed drift') increased with declining pH. In Experiments 1 and 2(a), 71% and 73% of the enhanced drift at treatment pH 5.2, and 76% and 100% at treatment pH 4.6 were the result of killed drift. During the acid pulse in Experiment 2(b), 31% of the increased drift at pH 5.2 and 82% of the increased drift at pH 4.6 could be attributed to killed drift.



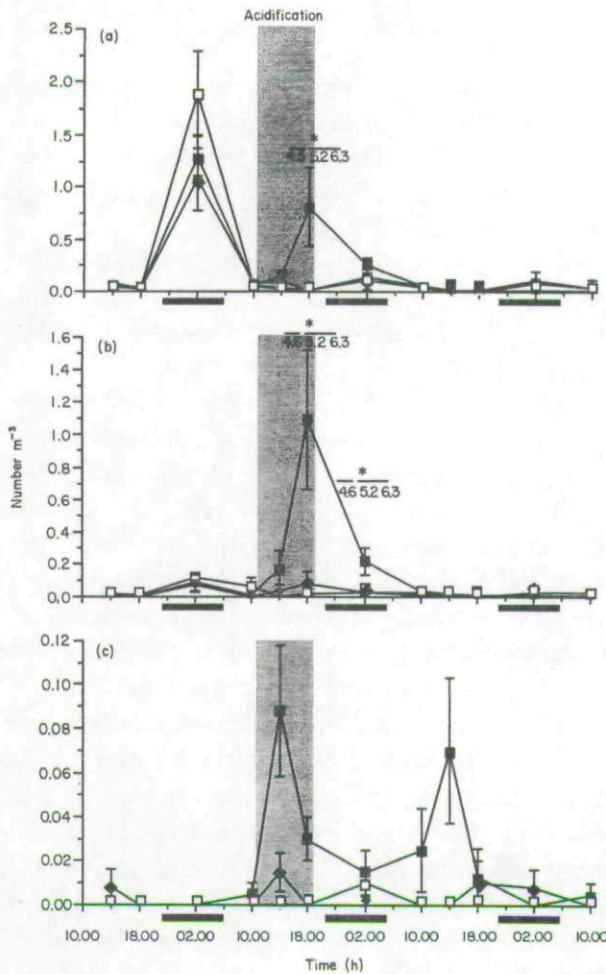


Fig. 2 Drift rates ( $\text{no. m}^{-3} \pm 1$  SE) of (a) *Epeorus*, (b) *Drunella* and (c) *Paraleptophlebia* in Experiment 2(a). Shaded areas indicate times of acid additions. Lower dark bars indicate periods of darkness. The time(s) below an asterisk were significantly different (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , Tukey's HSD test). Asterisks indicate significant interaction (time  $\times$  treatment) effects were observed within experimental blocks (pre-acid, acid and post-acid). If main (treatment) effects only were observed these results are presented in Table 5. See text for details. ■, pH 4.6; ◆, pH 5.2; □, control.

Drift rates of the mayfly nymph *Epeorus* were significantly affected by acidification in Experiments 1 and 2(a) (Table 5). Although *Epeorus* drift density varied among experiments, it was higher in acidified channels than controls throughout the acid pulse in Experiment 1 ( $F = 8.8$ ,  $P = 0.008$ ; Fig. 5) and in the latter half of the pulse in Experiment 2(a) ( $F = 6.4$ ,  $P = 0.023$ ). Conversely, nocturnal drift following acidification was two times higher in control relative to acidified channels in Experiment 1 ( $F = 9.9$ ,  $P < 0.005$ ; Fig. 5). Live to dead ratios of drifting

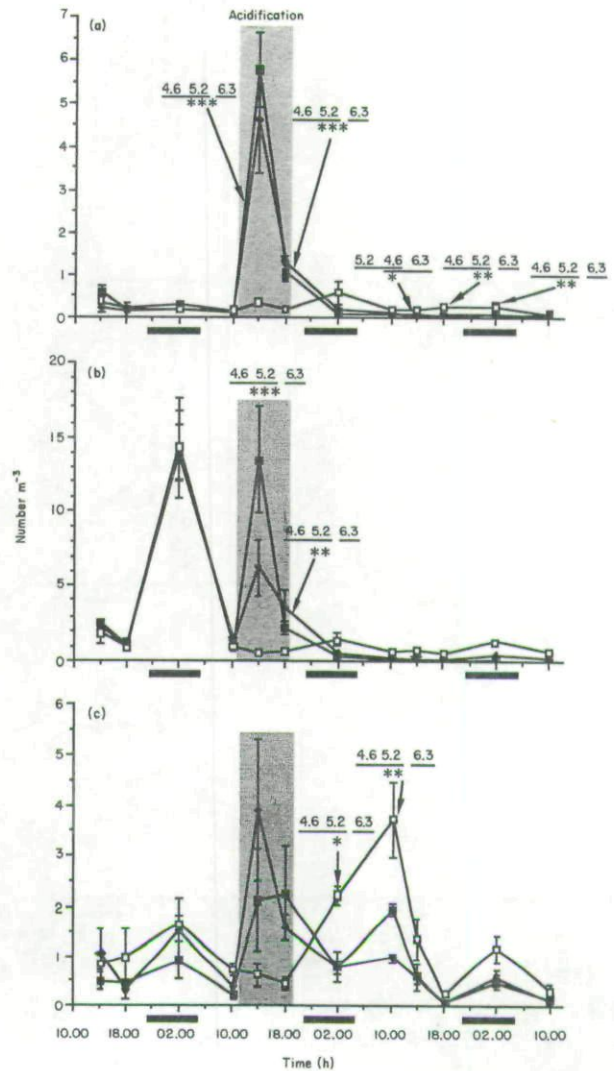


Fig. 3 *Baetis* drift rates ( $\text{no. m}^{-3} \pm 1$  SE) in each treatment throughout the course of all experiments. Other designations as in Fig. 2.

*Epeorus* were not monitored in Experiment 1. The percentage of drifting *Epeorus* that were dead was significantly higher in the pH 4.6 channels than in the pH 5.2 and control channels ( $F = 13.5$ ,  $P = 0.002$ ), and rose to 90% during the last half of the acid pulse in Experiment 2(a) (Fig. 6, Table 6). Approximately 45% of the increased drift in the pH 4.6 channels could be attributed to killed drift in these experiments. Where *Paraleptophlebia* and *Drunella* drift densities were sufficient for analysis, drift rates were enhanced by acidification during and immediately following the acid pulse (Figs 2b, and 2c; Table 5).

Drift responses of chironomids were variable (Table 5). Drift rates were depressed by acidic inputs

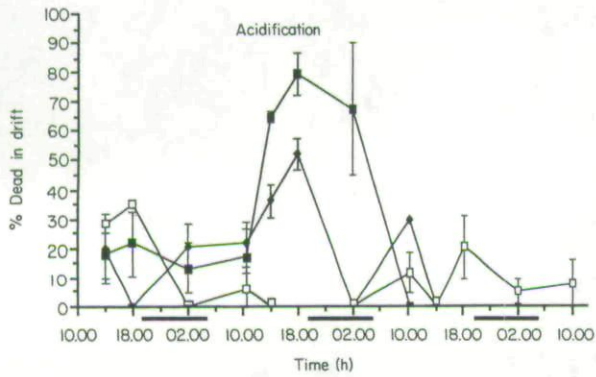


Fig. 4 The percentages of *Baetis* that were dead in each drift set over the course of Experiment 1 ( $\pm 1$  SE). Other designations as in Fig. 2. Other experiments gave similar results. Main effects are presented in Table 6.

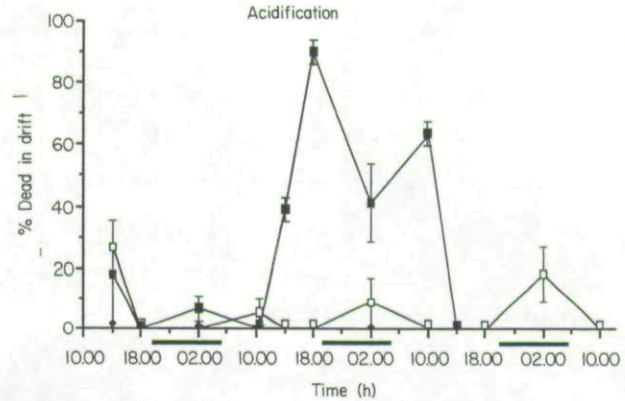


Fig. 6 The percentages of *Epeorus* that were dead in each drift set over the course of Experiment 2(a) ( $\pm 1$  SE). Other designations as in Fig. 2. Other experiments gave similar results. Main effects are presented in Table 6.

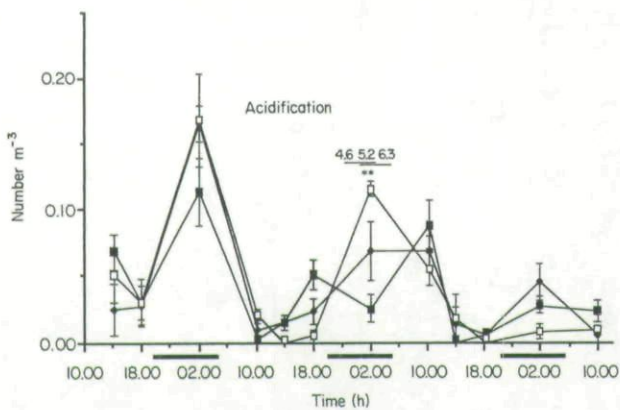


Fig. 5 *Epeorus* drift rates ( $\text{no. m}^{-3} \pm 1$  SE) in each treatment throughout the course of Experiment 1. Other designations as in Fig. 2.

during the acid pulse in Experiment 1, and were lowest in the pH 5.2 treatment in Experiment 2(a). Like *Baetis*, treatments with the greatest drift rates during acidification usually had significantly lower drift rates relative to other treatments in the post-acid period (Fig. 7). Drift rates of chironomid pupae were not affected by acidification except for a single post-acid net set in Experiment 1 (Fig. 8a).

The percentage of drifting chironomid larvae that were dead was 1.5–2.0 times higher at pH 5.2 and 4.6, respectively, than in control channels during both the acid and post-acid periods in Experiment 2(a) ( $F = 21.1, P = 0.0001$ ; Table 6). All increased drift in the acidified channels during acidification was owing to the drift of dead individuals. Increased

Table 2 Representative pHs in experimental channels across experiments. Times shown are as follows: TBA, 16.00 h (day before acidification); T1, 10.20 h (20 min after acidification); T2, 17.40 h (20 min before end of acidification); TAA, 14.00 h (day after acidification). Data are means for all four channels (pH was converted to  $[H^+]$  to calculate means)

		Experiment								
		1			2(a)			2(b)		
Variable	Time	Control	pH 5.2	pH 4.6	Control	pH 5.2	pH 4.6	Control	pH 5.2	pH 4.6
pH	TBA	6.48	6.48	6.45	6.67	6.76	6.71	6.77	6.68	6.66
	T1	6.41	5.35	4.89	6.32	5.17	4.57	6.78	5.25	4.68
	T2	6.51	5.15	5.25	6.69	5.29	4.62	6.52	5.32	4.69
	TAA	6.56	6.50	6.55	6.62	6.64	6.63	6.37	6.50	6.51
Range during acidification										
Upper		6.78	5.36	5.25	6.72	5.35	4.66	6.78	5.32	4.75
Mean		6.64	5.23	4.62	6.52	5.06	4.43	6.58	5.10	4.46
Lower		6.40	5.08	4.46	6.32	5.00	4.37	6.45	4.95	4.03

**Table 3** Chemical conditions in the experimental channel for all experiments. Times are as follows: TBA, 16.00 h (day before acidification); T1, 10.20 h (20 min after acidification); T2, 17.40 h (20 min before end of acidification); TAA, 14.00 h (day after acidification). Data are means (standard deviations in parentheses) for all four channels; values without standard deviations were measured in only one channel from each treatment. NA, not analysed

Variable	Experiment																	
	1						2(a)						2(b)					
	Time	Control	pH 5.2	pH 4.6	Control	pH 5.2	pH 4.6	Control	pH 5.2	pH 4.6	Control	pH 5.2	pH 4.6					
$H^+$ ( $\mu eq l^{-1}$ )	TBA	0.33 (0.02)	0.33 (0.02)	0.35 (0.04)	0.21 (0.06)	0.17 (0.03)	0.20 (0.05)	0.21 (0.06)	0.17 (0.03)	0.17 (0.03)	0.17 (0.03)	0.21 (0.05)	0.22 (0.02)					
	T1	0.39 (0.03)	4.45 (0.25)	13.0 (0.90)	0.48 (0.17)	6.72 (0.70)	26.9 (14.70)	0.48 (0.17)	6.72 (0.70)	26.9 (14.70)	0.17 (0.03)	5.60 (1.04)	21.0 (1.80)					
	T2	0.31 (0.03)	7.02 (0.53)	5.62 (1.50)	0.20 (0.02)	5.11 (1.43)	23.9 (4.70)	0.20 (0.02)	5.11 (1.43)	23.9 (4.70)	0.34 (0.06)	4.83 (0.61)	20.5 (1.30)					
ANC ( $\mu eq l^{-1}$ )	TAA	0.27 (0.03)	0.32 (0.02)	0.28 (0.06)	0.24 (0.01)	0.23 (0.04)	0.23 (0.04)	0.24 (0.01)	0.23 (0.04)	0.23 (0.04)	0.43 (0.13)	0.31 (0.02)	0.31 (0.01)					
	TBA	23.8 (1.0)	25.1 (1.0)	24.4 (3.0)	53.0 (2.2)	47.4 (1.1)	49.8 (1.8)	53.0 (2.2)	47.4 (1.1)	49.8 (1.8)	50.9 (1.7)	47.4 (1.1)	46.6 (0.2)					
	T1	21.1 (1.2)	0.5 (0.9)	NA	44.6 (3.4)	NA	NA	44.6 (3.4)	NA	NA	52.6 (0.5)	NA	NA					
$NO_3^-$ ( $\mu eq l^{-1}$ )	T2	25.9 (1.0)	-1.7 (1.5)	NA	49.1 (1.6)	NA	49.1 (1.6)	49.1 (1.6)	NA	NA	51.4 (1.7)	NA	NA					
	TAA	24.3 (0.1)	22.6 (2.1)	26.6 (1.2)	47.2 (1.1)	45.1 (1.5)	47.6 (1.6)	47.2 (1.1)	45.1 (1.5)	47.6 (1.6)	50.8 (1.3)	50.9 (0.8)	52.6 (1.5)					
	TBA	0.1	0.0	0.3	0.0 (0.0)	0.2 (0.2)	0.1 (0.1)	0.0 (0.0)	0.2 (0.2)	0.1 (0.1)	0.0	0.0	0.1					
$SO_4^{2-}$ ( $\mu eq l^{-1}$ )	T1	0.6	13.3	26.4	0.0 (0.0)	22.9 (0.4)	53.9 (1.4)	0.0 (0.0)	22.9 (0.4)	53.9 (1.4)	0.1	19.7	34.1					
	T2	0.1	12.3	11.6	0.1 (0.1)	23.1 (4.2)	32.9 (11.0)	0.1 (0.1)	23.1 (4.2)	32.9 (11.0)	0.0	18.1	28.7					
	TAA	0.1	0.1	0.4	0.2 (0.3)	0.1 (0.1)	0.1 (0.1)	0.2 (0.3)	0.1 (0.1)	0.1 (0.1)	5.5	8.3	8.3					
TBA	T1	3.1	3.5	3.8	4.6 (0.1)	4.8 (0.4)	4.6 (0.3)	4.6 (0.1)	4.8 (0.4)	4.6 (0.3)	7.1	4.3	4.3					
	T2	1.9	16.1	22.5	5.0 (0.2)	29.0 (0.6)	58.8 (1.7)	5.0 (0.2)	29.0 (0.6)	58.8 (1.7)	4.3	27.8	41.5					
	TAA	3.5	18.2	19.6	5.3 (0.1)	30.5 (3.1)	39.2 (10.3)	5.3 (0.1)	30.5 (3.1)	39.2 (10.3)	4.1	26.0	36.7					
TAA	3.3	3.4	3.2	4.2 (0.3)	4.3 (0.3)	4.1 (0.4)	4.2 (0.3)	4.3 (0.3)	4.1 (0.4)	7.2	6.9	8.2						

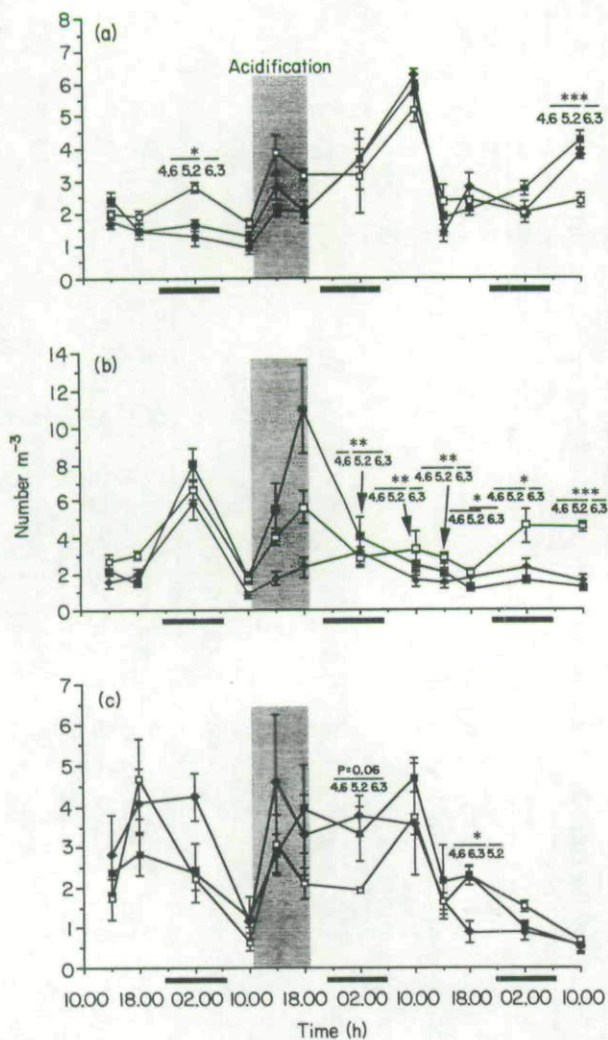


Fig. 7 Chironomid drift rates (no.  $m^{-3} \pm 1$  SE) in each treatment during (a) Experiment 1, (b) Experiment 2(a), and (c) Experiment 2(b). Other designations as in Fig. 2.

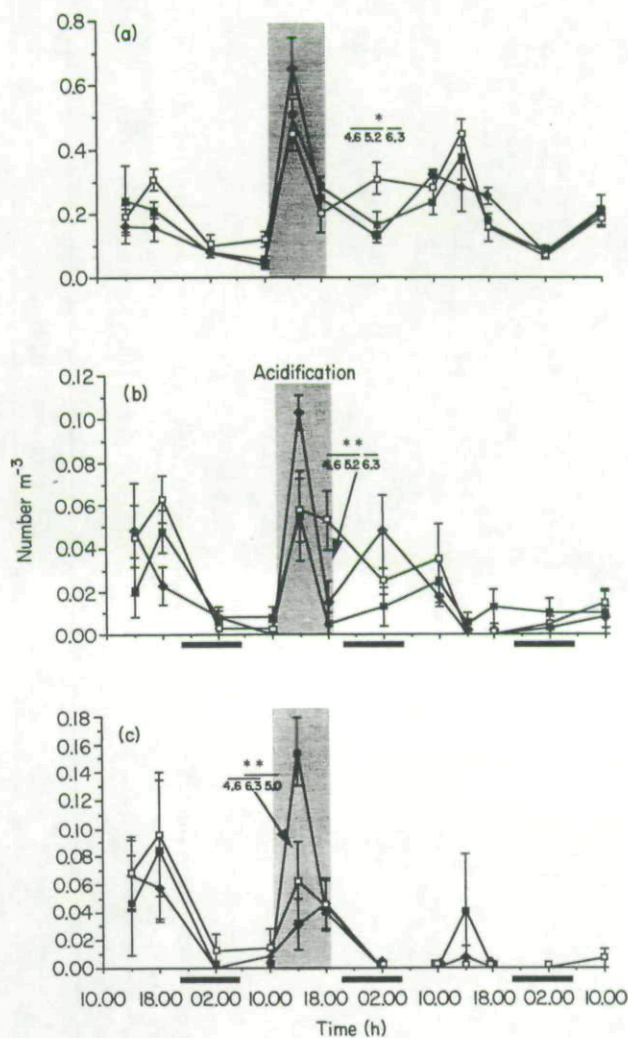


Fig. 8 Drift rates (no.  $m^{-3} \pm 1$  SE) of (a) chironomid pupae in Experiment 1, (b) mites in Experiment 1, and (c) mites in Experiment 2(a). Other designations as in Fig. 2.

**Table 4** Concentrations of iron and aluminium in the experimental channels. Data are means (standard deviations in parentheses) for two channels, except for the control in Experiment 2(b) (three channels). TBA, day before acidification; T2, 20–60 min before the end of acidification

Experiment	Treatment	Fe ( $\mu M$ )		Al ( $\mu M$ )	
		TBA	T2	TBA	T2
1 (5–6 August)	Control	0.37 (0.26)	0.50 (0.07)	0.26 (0.01)	0.79*
	pH 4.6	0.17 (0.06)	0.08 (0.00)	0.28* —	0.39 (0.02)
2(a) (2–3 September)	Control	0.32 (0.05)	0.25 (0.05)	0.35 (0.03)	0.48 (0.03)
	pH 4.6	0.33 (0.05)	0.86 (0.28)	0.33 (0.03)	0.87 (0.40)
2(b) (16–17 September)	Control	0.31 (0.09)	0.19 (0.06)	0.43 (0.02)	0.52 (0.02)
	pH 4.6	0.26 (0.03)	0.18 (0.07)	0.31 (0.02)	0.50 (0.20)

\* Data available for only one of the replicates.

**Table 5** The effect of pH on drift densities—mean (1 SE) per m<sup>3</sup> in taxa with a significant main effect in repeated measure ANOVAs. Values are the means (1 SE) of all channels within each treatment (pH 4.6, pH 5.2, pH 6.3—control) over all net sets in a time period, acidification = two sets, post-acidification = six sets. ID indicates times when taxa abundances were of insufficient density to allow statistical analyses. Values sharing the same line are not significantly different, Tukey's HSD. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Taxa	Experiment 1			Experiment 2(a)			Experiment 2(b)											
	Acidification	Post-acidification	Acidification	Acidification	Post-acidification	Acidification	Acidification	Post-acidification	Post-acidification									
	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3						
<i>Baetis</i>	3.4 (0.51)	2.9 (0.62)	0.27 (0.05)	0.07 (0.01)	0.08 (0.01)	0.26 (0.07)	7.9 (1.9)	4.9 (1.4)	0.6 (0.17)	0.13 (0.002)	0.27 (0.003)	0.92 (0.047)	2.2 (0.8)	2.6 (0.8)	0.5 (0.1)	0.66 (0.09)	0.50 (0.07)	1.5 (0.26)
<i>Ameletus</i>	0.03 (0.01)	0.03 (0.02)	0	ID	ID	ID	0.06 (0.01)	0.03 (0.02)	0	ID	ID	ID	ID	ID	ID	ID	ID	ID
<i>Epeorus</i>	0.03 (0.02)	0.02 (0.01)	0.003 (0.003)	0.03 (0.002)	0.03 (0.01)	0.04 (0.002)	0.48 (0.20)	0.01 (0.01)	0	0.06 (0.02)	0.06 (0.02)	0.05 (0.01)	ID	ID	ID	ID	ID	ID
<i>Drunella</i>	ID	ID	ID	ID	ID	ID	0.06 (0.02)	0.01 (0.01)	0	0.02 (0.01)	0.003 (0.003)	0.002 (0.001)	ID	ID	ID	ID	ID	ID
<i>Paraleptophlebia</i>	ID	ID	ID	ID	ID	ID	0.63 (0.25)	0.06 (0.03)	0.01 (0.005)	0.05 (0.02)	0.02 (0.001)	0.01 (0.003)	0.04 (0.04)	0.12 (0.09)	0.02 (0.02)	ID	ID	ID
<i>Zapada</i>	ID	ID	ID	ID	ID	ID	0.09 (0.03)	0.004 (0.004)	0	0.02 (0.01)	0.01 (0.004)	0	ID	ID	ID	ID	ID	ID



**Table 6** Mean (1 SE) percentages of drifting invertebrates that were dead for each taxon with a significant treatment effect in repeated measures ANOVAs (see text). Means per channel were determined across all net sets within the acid and post-acid time periods (pre = four, acid = two, post = six). Four channels per treatment were used in Experiments 1 and 2(a), three channels per treatment in Experiment 2(b). *Baetis* and chironomid larvae were assessed during pre-, acid and post-acid periods of all three acid additions. *Epeorus* was assessed only during Experiments 2(a) and 2(b), and there were insufficient numbers of *Epeorus* for analysis in Experiment 2(b). Treatments sharing the same bar are not significantly different as determined by Tukey's HSD. NS = not significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

	Pre-acidification			Acidification			Post-acidification		
	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3
<b>Experiment 1</b>									
<i>Baetis</i>		NS			***			*	
Mean (1 SE)	14.5 (0.9)	19.5 (1.6)	9.5 (4.4)	72.1 (3.8)	44.2 (2.8)	28.5 (9.3)	33.2 (5.6)	15.5 (4.2)	7.1 (4.0)
Chironomid larvae		NS			NS			NS	
Mean (1 SE)	21.7 (3.8)	17.9 (2.9)	17.1 (3.0)	35.5 (3.6)	30.7 (2.1)	28.2 (2.5)	27.8 (3.4)	24.2 (3.1)	22.4 (1.8)
<b>Experiment 2(a)</b>									
<i>Baetis</i>		NS			**			NS	
Mean (1 SE)	12.7 (1.9)	8.2 (1.5)	10.8 (3.1)	76.7 (3.5)	57.6 (5.0)	2.3 (2.3)	20.2 (4.9)	14.5 (5.2)	11.4 (1.2)
<i>Epeorus</i>		NS			***			**	
Mean (1 SE)	10.5 (1.7)	6.0 (3.7)	8.2 (1.3)	49.9 (4.9)	14.5 (5.2)	15.0 (5.9)	31.6 (5.4)	4.5 (4.5)	5.4 (1.8)
Chironomid larvae		NS			***			***	
Mean (1 SE)	23.2 (3.8)	20.2 (2.2)	15.5 (2.1)	46.4 (2.4)	36.9 (2.6)	23.5 (2.4)	36.1 (3.1)	21.5 (1.0)	17.2 (1.6)
<b>Experiment 2(b)</b>									
<i>Baetis</i>		NS			**			NS	
Mean (1 SE)	20.2 (9.3)	24.8 (9.1)	21.5 (2.9)	54.7 (4.0)	16.3 (8.5)	5.9 (5.9)	31.1 (9.3)	13.5 (5.1)	17.1 (2.1)
Chironomid larvae		NS			NS			NS	
Mean (1 SE)	28.8 (7.9)	28.5 (4.3)	29.0 (4.2)	50.2 (2.5)	33.7 (10.8)	40.2 (6.7)	32.2 (6.4)	29.1 (3.3)	30.5 (6.4)

percentages of dead drifting chironomids were maintained after acid additions stopped. No consistent patterns in the proportion of drifting chironomids that were dead were noted in other experiments.

Drift rates of mites were enhanced by acidification in two of the pulses (Table 5, Figs 8b and 8c). Responses appeared within the first 4 h of the pulses. Control channel drift was significantly greater than treatment channel drift during the second half of acidification in Experiment 1, although rates peaked at pH 5.2 during the first half. Drift rates in pH 4.6 channels were significantly greater than those of pH 5.2 and control channels in Experiment 2(a).

A number of rare taxa were only occasionally

abundant enough in the drift for statistical analyses. Most of these taxa increased in the drift during acidification in treatment channels (Table 5). The mayfly *Drunella* was abundant only during the acid pulse in Experiment 2(a), showing higher drift rates in acidified channels relative to controls (Fig. 2c). Similarly, the stonefly *Zapada* increased drift densities with acidification in Experiment 2(a). Drift rates of the caddisfly *Rhyacophila* were depressed in highly acidified channels during acidification in Experiment 1. *Simulium* increased drift rates at the lowest pH during acidification in Experiment 2(a). Adult winged insects were composed largely of chironomids and had higher drift rates in control

channels relative to highly acidified channels after acidification in Experiments 1 and 2(a). Drift patterns of a number of rarer taxa were not significantly associated with acidification, although the mayfly *Ameletus*, the larvae of the caddisfly *Amiocentrus* and the dipteran *Prosimulium* often reached highest drift densities during acidification in acidified channels.

Across these experiments drift rates were related to benthic densities. For example, low baetid drift rates were observed after acidification in treatment channels where benthic densities were reduced relative to pre-acidification and post-acidification control channels (Fig. 9). *Baetis* drift rate increased with increasing benthic density over all experiments with 47% of the variation in *Baetis* drift explained by variation in benthic density. Seven of the nine taxa examined, and total invertebrates, showed positive associations between drift and benthic densities (Table 7).

#### Benthic densities

There were no significant differences among treatments before acidification for any of the most common benthic taxa. Only *Baetis* was affected by pH

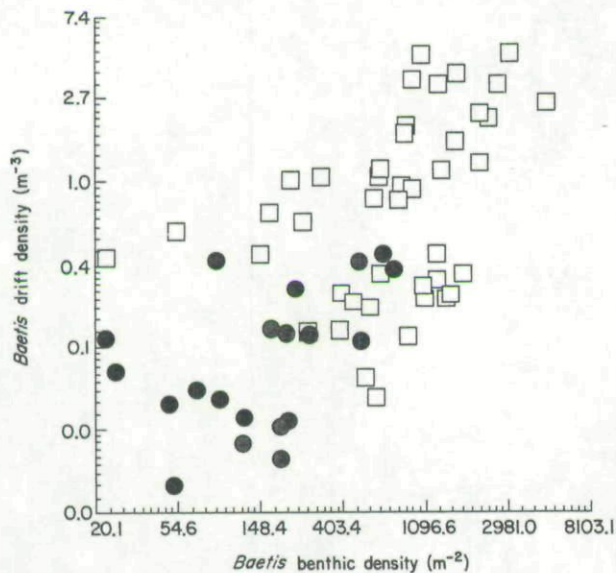


Fig. 9 The relationship between *Baetis* drift density and benthic abundance in control and pre-acid treatment channels, and in post-acidification treatment channels across all experiments. Regression equation and statistical significance can be found in Table 7. pH 5.2 and pH 4.6 are differentiated only for inspection. ●, Post-acid pH 5.2 channels; ●, post-acid pH 4.6 channels; □, control and pre-acid channels.

Table 7 Regression equations, coefficients of determination and the significance of the regression between invertebrate benthic density ( $x$ , number/400 cm<sup>2</sup>) and drift density ( $y$ , number m<sup>-3</sup>) in abundant benthic taxa in the experimental stream channels.  $n = 66$  for all data. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Taxa	Model	$r^2$	F
<i>Baetis</i>	$y = 0.001x + 0.011$	0.47	57.53***
<i>Epeorus</i>	$y = 0.017x + 0.044$	0.17	13.12***
<i>Paraleptophlebia</i>	$y = 0.003x + 0.006$	0.14	10.68**
<i>Zapada</i>	$y = 0.002x + 0.006$	0.08	5.16**
<i>Isoperla</i>	$y = 0.009x + 0.001$	0.25	21.63***
<i>Rhyacophila</i>	$y = 0.001x + 0.009$	0.01	0.56
<i>Simulium</i> larvae	$y = 0.0001x + 0.017$	0.09	6.93*
Chironomid larvae	$y = -0.017x + 1.28$	0.01	0.07
Chironomid pupae	$y = 0.136x - 0.47$	0.20	16.59***
Total invertebrates	$y = 0.179x + 0.02$	0.14	10.15**

in Experiment 1 with reduced treatment relative to control benthic densities following acidification ( $P < 0.001$ ,  $F = 31.72$ , ANOVA; Fig. 10). The benthic densities of the nymphs *Baetis* and *Paraleptophlebia* showed main effects of acid treatment and acid by time interactions in Experiment 2 ( $P = 0.04$  to  $0.009$ ,  $F = 2.24$  to  $11.65$ , repeated measures ANOVAs; Table 8). Although constant through Experiment 1, *Baetis* densities in control channels declined in Experiment 2(a) probably owing to drift losses (Table 6). Following acidification, *Baetis* benthic densities were reduced in pH 4.6 and 5.2 channels to 10% and 16%,

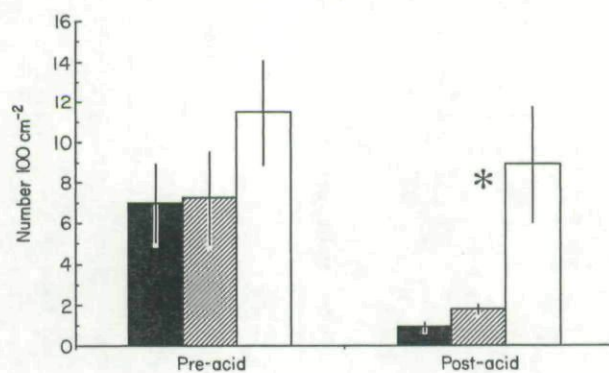


Fig. 10 Benthic densities (no. 100 cm<sup>-2</sup> ± 1 SE) of *Baetis* among treatments in Experiment 1. Pre-acid samples were taken 24 h prior to acid addition, post-acid samples were taken 40 h after the conclusion of acid additions. An asterisk over a histogram indicates that it, and any histograms to the left, are significantly lower than the next histogram to the right ( $P < 0.05$ , Tukey's HSD test). ■, pH 4.6; ▨, pH 5.2; □, control.



Table 8 Treatment means ( $0.01 \text{ m}^{-2}$ ) (1 SE) for all taxa in benthic samples with sufficient abundances for statistical analyses in Experiments 2(a) and 2(b). Treatments sharing the same bar are not significantly different as determined by Tukey's HSD. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Taxa	Experiment 2(a)						Experiment 2(b)								
	Pre-acid			Post-acid			Pre-acid			Post-acid (2 days)			Post-acid (14 days)		
	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3	pH 4.6	pH 5.2	pH 6.3
<i>Baetis</i>															
Mean (1 SE)	F = 0.53 22.8 (3.5)	P = 0.60 18.1 (7.4)	P = 0.004 18.8 (3.9)	** 0.2 (0.2)	P = 0.004 1.5 (0.5)	5.9 (1.2)	F = 17.57 3.9 (2.2)	** 5.1 (2.1)	P = 0.001 7.8 (1.6)	F = 0.66 4.0 (1.7)	P = 0.55 4.1 (0.9)	7.7 (6.1)	F = 9.85 1.8 (1.0)	* 2.9 (0.2)	P = 0.01 5.7 (2.9)
<i>Paraleptophlebia</i>															
Mean (1 SE)	F = 0.49 3.1 (1.3)	P = 0.63 1.2 (0.6)	2.8 (0.8)	F = 0.23 0.1 (0.1)	P = 0.80 0.2 (0.1)	1.1 (0.8)	F = 10.53 1.1 (0.7)	** 6.6 (1.8)	P = 0.004 8.2 (4.1)	F = 2.32 1.2 (1.1)	P = 0.15 4.1 (2.6)	10.3 (10.1)	F = 26.07 1.0 (0.5)	*** 5.3 (1.2)	P < 0.0001 11.2 (5.6)
Chironomid															
larvae	F = 2.92 120.3 (25.2)	P = 0.11 90.4 (14.8)	177.7 (31.2)	F = 1.17 85.6 (11.3)	P = 0.35 71.0 (10.8)	103.5 (17.9)	F = 0.38 146.9 (48.7)	P = 0.69 187.7 (50.6)	171.8 (34.5)	F = 0.54 185.2 (16.4)	P = 0.61 149.1 (39.7)	140.9 (62.8)	F = 1.52 175.7 (45.6)	P = 0.29 271.5 (57.3)	172.1 (33.1)
Chironomid pupae	F = 1.17 7.6 (1.0)	P = 0.35 6.2 (1.2)	10.7 (2.7)	F = 0.43 9.9 (3.1)	P = 0.66 6.5 (1.5)	9.4 (2.3)	F = 0.60 6.4 (3.2)	P = 0.57 11.3 (5.5)	12.9 (5.7)	F = 0.28 17.9 (1.0)	P = 0.76 17.8 (8.8)	13.3 (10.2)	F = 0.44 9.2 (3.9)	P = 0.66 12.9 (4.9)	8.3 (3.7)

and 3% and 25%, of control levels, respectively, in Experiments 1 and 2(a) ( $F = 31.05$ ,  $P = 0.001$  and  $F = 11.04$ ,  $P = 0.01$ ). The second acid pulse had no immediate additional effect on benthic density. Benthic densities in pH 4.6 and 5.2 channels were 50 and 65% of control channels before the second acid pulse in Experiment 2(b) and remained at these levels after the pulse (Table 9). Approximately 2 weeks after acid addition in Experiment 2(b) mean *Baetis* densities in pH 4.6 and 5.2 channels were 32 and 50% of those in control channels.

Densities of *Paraleptophlebia* appeared to be depressed by acid input in Experiment 2(a). Densities at pH 4.6 and 5.2 were approximately 9 and 18% of those in controls after the Experiment 2(a) acidification (Table 9), but densities were low and variable and treatment effects were not statistically significant (Table 8). Despite increasing densities of this mayfly in all channels, treatment differences were maintained following Experiment 2(a) and density differences were significant prior to acid input in Experiment 2(b). Densities of *Paraleptophlebia* remained relatively constant in pH 4.6 channels in pre- and post-acid periods of Experiment 2(b). Densities in pH 4.6 channels were c. 9–14% of those in control channels, whereas densities in pH 5.2 channels were approximately 80% of those in controls before acidification but declined to 40–50% of controls after the second set of acid inputs. No large additive effects of multiple acid pulses on *Paraleptophlebia* density were apparent immediately after the acid pulse (Table 9). Significant effects of acid inputs were apparent 2 weeks after the Experiment 2(b) acidification (Tables 8 and 9).

Benthic densities of chironomid larvae and pupae were not affected by acid additions in these experiments (Tables 8 and 9). Benthic densities of other

taxa (*Epeorus*, oligochaetes) were too low to assess statistically the effects of acid additions on abundances.

## Discussion

In contrast to most previous studies, we examined the effects of episodic pH depressions on stream communities rather than the effects of continuous acid additions. We found that stream invertebrates showed a variety of responses to acid inputs. Increased drift in response to acid inputs in Ephemeroptera and some chironomid stages is consistent with earlier studies (Hall *et al.*, 1980; Ormerod *et al.*, 1987; Hopkins *et al.*, 1989; Bernard *et al.*, 1990; Merrett *et al.*, 1991). *Baetis* was the taxon most sensitive to acid inputs, with large increases in drift causing decline in benthic density. Lower drift rates observed in acidified channels relative to controls after acid pulses were probably due to lower benthic densities in acidified channels (Fig. 9). Other mayfly genera, such as *Epeorus* and *Paraleptophlebia*, showed enhanced drift when pH was reduced to 4.6, and *Paraleptophlebia* showed reduced benthic densities owing to acid inputs. Ephemeroptera are especially sensitive to acid (Sutcliffe & Carrick, 1973; Fiance 1978; Howells, Howells & Alabaster, 1983; Mackay & Kersey, 1985; Allard & Moreau, 1984, 1987; Ormerod *et al.*, 1987; Hopkins *et al.*, 1989; Peterson & Van Eeckhaute, 1992) and often demonstrate high drift rates following stream acidification (Hall *et al.*, 1980; Overrein *et al.*, 1980; Zischke *et al.*, 1983; Merrett *et al.*, 1991).

We observed depressed numbers of chironomid adults in acidified *v* control channels. We cannot determine whether lower adult drift in acidified channels resulted from lower pupal densities, de-

**Table 9** Benthic densities in treatment channels as a proportion of benthic densities in control channels in Experiments 1, 2(a), and 2(b). \* Indicates an acid addition immediately following that benthic sampling date. ID, insufficient densities available for analysis

Experiment	Date	<i>Baetis</i>		<i>Paraleptophlebia</i>		Chironomids	
		pH 4.6	pH 5.2	pH 4.6	pH 5.2	pH 4.6	pH 5.2
1	5 August*	0.59	0.62	ID	ID	1.03	1.18
1	8 August	0.10	0.16	ID	ID	1.14	0.98
2(a)	3 September*	1.21	0.97	1.11	0.43	0.68	0.51
2(a)	6 September	0.03	0.25	0.09	0.18	0.83	0.69
2(a), (b)	16 September*	0.50	0.65	0.13	0.80	0.86	1.09
2(b)	19 September	0.53	0.55	0.12	0.40	1.31	1.06
2(b)	1 October	0.32	0.50	0.08	0.50	1.02	1.58

pressed emergence in treatment channels (Bell & Nebecker, 1969; Bell, 1971; Chemielewski & Hall, 1992), or avoidance of acid channels by adults (Sutcliffe & Carrick, 1973). In common with experiments employing sustained acid inputs or more severe pH depressions (>2 pH units below ambient; Hall *et al.*, 1980; Ormerod *et al.*, 1987), simuliid drift was enhanced by acid treatment, although only in the lowest acid treatment (pH 4.6) during a single acid pulse. Simuliids in our experiments did not respond to depressions of 1 pH unit below ambient, similar to the results of Bernard *et al.* (1990). Chemielewski & Hall (1992) have shown that simuliid resistance to moderate pH depressions can be both species and stage specific.

Enhanced macroinvertebrate drift is often an initial reaction to lowered pH, and drift in treatment and control areas generally converge to pre-acidification rates over time (Hall & Likens, 1980; Hall *et al.*, 1980; but see Ormerod *et al.*, 1987). Previous experimental designs have often used upstream control areas to compare with downstream treatment sites. An initial pulse of drift from the experimental section is likely due to killing of invertebrates in the experimental section by acid. Drift may move through control areas because the benthic environment is saturated with resident invertebrates, and upon entering the acidified experimental areas the drift is killed by the acid and continues to drift through. As a consequence, drift in experimental and control areas will converge. Convergence of control and treatment benthic densities after acidification has ceased may result from organisms colonizing denuded acidified areas from the drift (Pratt & Hall, 1981). Because larval invertebrate colonization was prevented during our experiments, significantly lower post-acidification baetid drift rates in acid *v* control channels probably resulted from reduced final benthic densities in treated channels. Reduced drift owing to decreased benthic density following acidification was observed by Ormerod *et al.* (1987). Without immigrants from upstream, low order alpine streams may suffer reductions in the abundance of some taxa as a result of catchment-wide episodic deposition events. Recolonization may be contingent upon aerial oviposition, and community recovery may be slow. Moreover, as the frequency of episodic events increases, and grades into chronic acidification, these communities may experience shifts in com-

position as more sensitive taxa are lost and more resistant taxa remain.

Rapid increases in drift associated with acidification may result from an active, behavioural response of organisms to acid stress (Hall *et al.*, 1987), or may be due to an increase in the insects' susceptibility to being accidentally dislodged. Alternatively, increased drift may be a passive phenomenon, where animals drift owing to being killed by the perturbation (Griffiths, 1987). Increases in drift in response to insecticide spraying have been shown to result from an increase in the number of dead invertebrates in the drift (Eidt & Weaver, 1983). The high percentage of dead *Baetis*, *Epeorus* and, in Experiment 2(a), chironomids in the drift during and immediately following acidification of stream channels indicates that killed drift may dominate observed drift responses. The mechanisms causing enhanced stream drift during acid pulses seem relatively consistent across species in this study and depend on exact pH levels, with killed drift increasingly dominating as pH is lowered.

The effects of acid inputs on the densities of individual benthic species seem to depend on the microhabitat preferences of these taxa (Allard & Moreau, 1984; Ormerod *et al.*, 1987). *Baetis* nymphs are epibenthic and active, spending most of their time on the upper surfaces of rocks directly exposed to acidified water. Weatherly *et al.* (1988) were unable to discount *Baetis* movement down into the substratum as a mechanism explaining reduced benthic densities following acidification. In contrast, our experiments show that benthic reductions represent mortality in the area treated with acid. Chironomids, on the other hand, often burrow into the substratum and so may be buffered from contact with polluted overlying water (Barmuta *et al.*, 1990). Our data suggest that chironomid drift may decline with acid inputs, possibly owing to active chironomid burrowing to escape deleterious conditions. Our chironomid assemblage was composed of at least a dozen species representing several different functional groups (grazers, collectors, predators). Sporadic effects of acidification on drift noted in this assemblage may represent the different responses of different dominant taxa at the time of acidification (Hall, 1990). Finally, the lack of significant differences in benthic chironomid densities between acidified and control channels was possibly due to the influx of chironomid

drift from the Marble Fork. Although our block net was sufficient to prevent the immigration of most macroinvertebrates to stream channels it did not restrict the passage of small chironomids.

Invertebrate responses to increased  $H^+$  concentrations may be enhanced by associated trace metals, such as Al and Fe (Bernard *et al.*, 1990; Hall, 1990; Rosemond *et al.*, 1992). As a result of the poor buffering capacities of the surrounding thin soils, headwater streams often show the greatest chemical changes in response to acid deposition (Johnson, 1979; Cronan & Schofield, 1979; Hall, 1990). Very small increases in trace metals have been observed in Sierra Nevada waters in response to acid events (Melack *et al.*, 1989; Barmuta *et al.*, 1990). Total Al concentrations determined from monthly water samples taken from the outflow stream of Emerald Lake (c. 2 km from the stream channels) range from undetectable to occasional winter peaks of  $2.9 \mu M$  (mean = 1.04, SE = 0.03; Melack *et al.*, 1989). In the experiments reported here, we did not include trace metals as independent variables in order to assess the capacity of Sierran substrate to contribute metal ions to lotic waters during acid events. Although Al and Fe concentrations were not increased by acidification, the area of our channels may have been too small to allow for significant quantities of trace metals to be mobilized during acidification. Therefore, the results of our experiments may be conservative estimates of both the lotic taxa affected and the magnitude of any effects relative to basin-wide acidification events in the Sierra which may be accompanied by increases in trace metal concentrations.

The effects of multiple *v* single acidic events on benthic communities are not well known (Ormerod & Jenkins, 1994). Increased rates of invertebrate drift associated with single acid episodes may decline with subsequent pulses (Merrett *et al.*, 1991), and could reflect a decline in benthic density. In these experiments reductions in benthic density and subsequent declines in invertebrate drift were noted in treatment channels following the single acid pulse in Experiment 1 and the initial pulse in Experiment 2(a). The second pulse in Experiment 2(b) had no additional effect on reducing benthic density (Table 9). Water temperature during the acid pulse in Experiment 2(b) ranged from 4.5 to 8.0°C, owing to rain and snow input, as opposed to 10–19°C and 11–19°C in Experiments 1 and 2(a), respectively. The

sensitivity of fish to acid exposure can vary with season as a result of inverse relationships between mortality and water temperature (Robinson *et al.*, 1976; Falk & Dunson, 1977; Korwin-Kossakowski & Jezierska, 1985; Gunn, 1986). Metabolic rates of some caenid mayflies varied with both temperature and pH resulting in survivorship of acid-stressed nymphs that was higher at 20°C than at 10 or 30°C over a pH range of 4.0–7.2 (Punzo & Thompson, 1990). Strong seasonal variation in acid sensitivity has been observed in the heptageniid mayfly *Stenonema femoratum*, with greater rates of wholebody ion loss and higher mortality in summer than in winter months (Rowe, Berrill & Hollett, 1988). The absence of any cumulative effect of acid inputs on benthic density in Experiment 2 may be related to the colder temperature during the second *v* earlier acid pulses.

Fish and invertebrates may show increased tolerance to metals or acid if they have been previously exposed to sublethal concentrations (Saliba & Krzyz, 1976; Frazer, 1980; Ormerod *et al.*, 1987; Baker *et al.*, 1990). In contrast, McCahon & Pascoe (1989) found no decrease in mortality of *Gammarus* in response to acid/Al additions following pretreatment with Al, nor was mortality different between two repeated acid plus Al pulses (McCahon & Poulton, 1991). Weatherly & Ormerod (1991) found little improvement in predicting trout density when using mean pH and aluminium concentrations rather than measures of fluctuation for these variables. However, in developing models of invertebrate assemblages relative to pH and Al concentrations, the inclusion of measures related to the episodic inputs of these variables moderately enhanced precision. It should be noted that mean pH and Al in their study streams were closely correlated with pH and Al variation so that the biological effects of episodic pulses could not be separated from chronic conditions.

Diel patterns in baetid drift varied across experiments with a pronounced nocturnal peak only in Experiment 2(a) (Fig. 3). We attribute this variability in diel patterns in drift to variation in benthic density among experiments. Baetid drift rates were positively associated with baetid benthic densities across these experiments (Fig. 9). Baetid benthic densities were over twice as high at the outset of Experiment 2(a) as at the end of Experiment 2(a), or at the beginning of Experiments 1 and 2(b) (Table 8). After a large number of baetids drifted from the channels at night

at the beginning of Experiment 2(a), benthic densities were reduced, and dispersal rates declined. Similarly, benthic densities and baetid drift rates at the beginning of Experiments 1 and 2(b) were low (Fig. 3). The reduced drift in acidified channels after acid additions also implies that drift rates depend on benthic densities. Further, baetid benthic densities and drift rates tended to decline with decreasing pH across experiments (Fig. 9) suggesting that reduced drift rates in acidified channels relative to controls following acidification resulted from decreased benthic densities in treatment channels. Benthic densities may account for variability in nocturnal drift peaks in other taxa although none showed as strong a relationship as *Baetis* (Table 7).

The taxa showing significant responses to acidification varied across our experiments. Although the effects of acidification on individual taxa and the community as a whole may be seasonal and linked to parameters such as discharge (Hall, 1990), temperature (Rowe *et al.*, 1988), or ontogenetic factors (Bell, 1971), we feel that some of the temporal variability in the results of our experiments was due to differences in benthic invertebrate densities across experiments. Because recolonization was prevented between the last two acid events, densities of most taxa were insufficient to permit rigorous statistical analyses in the last experiment. Cooper & Barmuta (1993) have discussed the importance of adequate numbers of individuals within samples for rigorous statistical analyses. Despite limitations on the power of some statistical tests, our experiments demonstrated a strong effect of episodic acidification on many stream taxa. Finally, in headwater streams where colonization from upstream is precluded, acid effects may consist of both benthic losses owing to drift as well as subsequent long-term benthic density reductions resulting from decreased oviposition by adults. Otto & Svensson (1976) have shown that reduced densities of a stage of a lotic invertebrate can translate into lower larval densities in the next generation. Episodic acid events over a single season may precede long-term chronic acidification and have effects on the abundance and composition of benthic communities.

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