

Small-scale movements of lotic macroinvertebrates with variations in flow

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SUMMARY

1. The small-scale movements and distribution patterns of invertebrates were observed in an attempt to identify the various mechanisms by which organisms may use flow refugia during flow disturbances. The microdistribution of lotic macroinvertebrates was examined in two replicate, non-circulating laboratory flumes with variations in flow among microhabitat patches ($\approx 0.015\text{--}0.035\text{ m}^2$). The discharge in one experimental flume was manipulated to mimic spates and alter near-bed flow patterns; the other flume acted as a control. After an initial settling period, the position and behaviour of animals within the flumes was recorded before, during and after a simulated spate. Three species with contrasting flow microhabitat preferences and movement behaviour were examined.
2. At low discharge, the microdistribution of all three study species in flumes was broadly consistent with field observations. In the field, the optimum current speed was lowest for adults of the dytiscid beetle, *Oreodytes sanmarkii*, and highest for mayfly nymphs, *Ephemerella ignita*, with nymphs of the stonefly, *Leuctra inermis*, most abundant at intermediate velocities. In the flumes, *O. sanmarkii* occurred only in very low velocity areas, *L. inermis* occurred widely throughout the flumes with highest density in low velocity areas and *E. ignita* also occurred throughout the flumes, but maximum density was in moderately high velocity areas.
3. Increased discharge did not reduce the total number of individuals in experimental versus control flumes for any of the three species studied, although total numbers did decrease over the observation period in both treatments. Simulated spates resulted in a change in the microdistribution of *O. sanmarkii* and *E. ignita*, but not *L. inermis*, such that numbers were reduced in very high velocity microhabitats and animals accumulated in lower flow areas, analogous to flow refugia. These distributional shifts were attributed to movements of individuals among microhabitats.
4. Both active and passive modes of movement contributed to the accumulation of *E. ignita* and *O. sanmarkii* in low flow microhabitats (i.e. flow refugia). Some nymphs of *E. ignita* actively crawled from high to low flow microhabitats. Both species drifted between microhabitats. Drift entry could be active or passive, whereas regaining the substratum was active: *O. sanmarkii* swam down and *E. ignita* altered its body posture to promote sinking.

Keywords: disturbance, flow refugia, macroinvertebrates, microdistribution, movement

Introduction

The persistence of populations and communities in

environments subject to physical disturbance has stimulated many ecological studies. It is almost axiomatic that the immediate impacts of disturbances are negative (but see Lancaster, 1996), in that there may be a reduction in the fitness of individuals and/or a reduction in population size (Lancaster & Belyea, 1997). Therefore, some advantage may be gained by

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individuals that are able to avoid or resist disturbances. At the most fundamental level, many organisms have morphological and/or physiological adaptations, evolved over evolutionary time scales, that permit them to resist the physical stresses of disturbance. On ecological scales, however, spatial and temporal heterogeneity within the environment may allow populations to maintain higher densities than would be possible through morphological or physiological adaptations alone. The mechanisms through which this might arise are diverse, but the common, underlying theme is that individuals can avoid the negative effects of disturbance by being in refugia. Refugia are broadly defined as places within the environment where the negative effects of disturbance are lower than in the surrounding area (Lancaster & Belyea, 1997). During adverse conditions, organisms in refugia have a higher probability of survival, and these organisms are subsequently available to recolonize or to provide recruits for areas affected more severely.

In streams, fluctuations in flow are an important source of disturbance to benthic communities, and much current research focuses on the impacts of spates and floods. High flow events are accompanied by increased velocity and hydraulic forces on the stream bed (e.g. Lancaster & Hildrew, 1993a), and often by sediment movement, that can have negative effects on individuals and generally can reduce population numbers (e.g. Giller, Sangpraduh & Towney, 1991; Matthaei, Uehlinger & Frutiger, 1997). Streams are heterogeneous environments, however, and within the environment there may be flow refugia places which are not subject to severe hydraulic stress or moving sediments during disturbances. Organisms in such refugia during spates would avoid the negative impacts of disturbance. The mechanisms of flow refugium use by stream organisms over the long-term (>1 generation) and large spatial scales (>1 habitat or stream) involve recruitment between generations and dispersal of individuals between habitats (Lancaster & Belyea, 1997). These mechanisms, involving several habitat patches (e.g. several stream tributaries or catchments) and whole habitat patches that act as refugia, are probably most important during catastrophic disturbances which virtually eliminate the entire community in a habitat patch, such as flash floods in desert streams (e.g. Fisher *et al.*, 1982). At smaller scales, flow refugia may exist as

microhabitat patches within a single stream (e.g. behind boulders, along stream margins and in the hyporheic zone), and mechanisms of refugium use may operate within a single generation and without recolonization from other habitat patches (Lancaster & Belyea, 1997). It is within this context of small-scale, environmental heterogeneity and refugium use that the present study is placed.

Empirical studies testing for flow refugium use within streams often search for changes in the microdistribution of benthic organisms among hydraulic microhabitats in association with changes in discharge (e.g. Lancaster & Hildrew, 1993b; Palmer *et al.*, 1995; Robertson, Lancaster & Hildrew, 1995; Palmer *et al.*, 1996a). As accurate measures of population densities in lotic habitats are difficult to obtain, the focus is often on patterns of relative differences in density among microhabitats. Such patterns, however, could arise via several different mechanisms depending on the flux or redistribution of individuals between microhabitat patches (Robertson *et al.*, 1995; Lancaster & Belyea, 1997). The concept of refugium use is consistent with observations that spate conditions are associated with a higher abundance of organisms in flow refugia relative to other areas of the stream habitat. Such data do not demonstrate conclusively that individuals accumulate in refugia during disturbances and that they redistribute after the disturbances. Additionally, changes in microdistribution could result simply from a disproportionate loss of individuals from non-refugium microhabitats compared with refugia. Indirect evidence exists to support the hypothesis that some invertebrates, especially small-bodied meiofauna, move vertically into refugia within the hyporheic zone in response to increased discharge (e.g. Marmonier & Cruézé des Châtelliers, 1991; Dole-Olivier & Marmonier, 1992; Dole-Olivier, Marmonier & Beffy, 1997), although there are exceptions (e.g. Palmer, Bely & Berg, 1992). Less evidence exists, however, for refugium use by invertebrates which do not use the hyporheic zone and that are restricted to flow refugia at the sub-stratum surface, such as larger-bodied macroinvertebrates. The existing empirical evidence that animals use flow refugia at the substratum surface comes from studies in the field (Lancaster, Hildrew & Townsend, 1990; Palmer *et al.*, 1996a; Winterbottom *et al.*, 1997) and in laboratory flumes (Borchardt & Statzner, 1990; Borchardt, 1993).

The details, however, of how animals move among microhabitats (e.g. actively or passively) remain poorly understood. There is a need for more information on the species-specific movements and behaviour of benthic invertebrates in response to changes in near-bed hydraulics (Lancaster, 1996; Palmer, Allan & Butman, 1996b).

The present study investigated whether lotic invertebrates move (by active or passive means) away from microhabitats (areas of $\approx 0.01\text{--}0.035\text{ m}^2$) where near-bed hydraulic forces increase with discharge towards areas that maintain lower flows, hence acting as refugia. The microdistribution and movements of macroinvertebrates were examined in laboratory flumes in which near-bed velocity varied along the length of the flume channel. Unlike laboratory flumes providing uniform flows (e.g. Lacoursière & Craig, 1990), there was a range of flows within each flume in the present study and animals could move among these. Discharge was manipulated to mimic increased discharge during spates and to increase near bed velocity. There were two nested hypotheses and predictions: (1) H_0' : Increased discharge and, hence, increased hydraulic forces in the flumes does not reduce the total number of animals in a flume. I predicted that discharge would not reduce total numbers of species able to resist dislodgement or to move from fast to slow flow microhabitats without being transported great distances. If the evidence supported this first hypothesis, then (2) H_0'' : Increased discharge has no impact on the microdistribution of invertebrates within a flume. Again, I predicted species-specific responses, based on individual morphology and behaviour. For example, species resisting dislodgement should show little or no change in microdistribution. In contrast, species unable to resist high flow forces may change their microdistribution such that density differences between low and high flow microhabitats are increased. Such distributional shifts could arise through behavioural avoidance of high flows (e.g. walking down a velocity gradient) or by dislodgement and drift. Whether individuals drift out of the flumes or regain the substratum in slower flowing microhabitats will depend largely on their behaviour once suspended in the water column. Three species with contrasting flow microhabitat preferences and motilities were chosen for the study.

Materials and methods

Animals

All three species used in the experiments are common in streams in the U.K. and were collected from Glencorse Burn ($55^\circ 51' 18''\text{ N}$, $3^\circ 12' 0''\text{ W}$), just south of Edinburgh. Adults of the dytiscid beetle, *Oreodytes sanmarkii* (Sahlberg), are small (length = 3.0 mm) and globular. These animals are active but poor swimmers, and tend to swim in short trajectories close to the substratum, avoiding areas of high flow (J. Lancaster, personal observation; Ribera & Nilsson, 1995). The tarsal claws of *O. sanmarkii* are well developed and adults can cling to some substrata. As air breathers, however, the animals must rise to the surface periodically. At such times, individuals may be particularly prone to being transported downstream as velocity is higher away from the boundary layer and there is no substratum on which to cling. In streams, *O. sanmarkii* typically occur in low flow microhabitats (see below) and individuals often leave the water to fly short distances before re-entering the stream. Nymphs of the stonefly, *Leuctra inermis* Kempney, are poor swimmers, moving predominantly by crawling, and are typically found in moderate flow (see below). The experiments used only late instar nymphs (mean ± 1 SE head capsule width = 0.90 ± 0.012 mm, $n = 30$). Nymphs of the mayfly, *Ephemerella ignita* (Poda), are active crawlers but poor swimmers, and occur most frequently in moderately fast flows (see below and Otto & Sjöström, 1986). Late instar nymphs, but without the black wing pads which indicate imminent emergence, were used in the present experiments (mean ± 1 SE head capsule width = 1.23 ± 0.015 mm, $n = 30$). All three species have been observed actively moving around on stone surfaces in natural stream channels during daylight hours (J. Lancaster, personal observations).

Flow response curves

The near-bed flow response curves of the three test species at baseflow in an upland Scottish stream which has a predominantly cobble stream bed (the Whiteadder Water, $55^\circ 53' 36''\text{ N}$, $29^\circ 35' 31''\text{ W}$) are illustrated in Fig. 1. Velocity was measured 2–3 cm above the substratum surface and averaged over 20 s using a 'mini' bucket wheel velocity meter (diameter = 5 cm) fitted with a photo-fibre-optic sensor to

ensure accurate measurements at low velocity. Within a 100-m stretch of stream, 100 velocity measurements were taken on transects spaced 1.5 stream widths apart, with three measurements per transect (one at the centre of the stream and at two points either side, equidistant from the edge and the centre point). At baseflow, the majority of velocities were low ($< 0.10 \text{ m s}^{-1}$), but ranged up to nearly 0.7 m s^{-1} (Fig. 1a). Benthic invertebrates were collected using a Surber sampler (0.1 m^2 , $200\text{-}\mu\text{m}$ mesh) at 30 places chosen at random from the 100 points at which velocity was measured. Samples were preserved in the field in 70% alcohol, and sorted and identified in the laboratory.

The densities of the three test species in relation to near-bed velocity are shown in Fig. 1b–d. Flow response curves were calculated following ter Braak & Looman (1986) and ter Braak & Prentice (1988). The species typically show unimodal (bell-shaped) response curves along environmental gradients (e.g. Whittaker, 1956); the Gaussian response curve (Gauch & Whittaker, 1972) is a simple bell-shaped curve in which the logarithm of abundance y is a quadratic function of the environmental variable x :

$$\log(y) = b_0 + b_1x + b_2x^2$$

where $b_2 < 0$. An alternative arrangement of the equation is perhaps more easily interpreted biologically:

$$\log(y) = a - 0.5(x - u)^2 / t^2$$

such that u is the optimum position of the species along the environmental gradient (the value of x at the peak), t is its tolerance (a measure of ecological amplitude or response breadth) and a is a coefficient related to the height of the peak. In some situations, a β -function describing skewed response curves provides a better description than the symmetrical curves of a Gaussian response (Austin *et al.*, 1994), but not in the case of the present data. Based on the response curves in Fig. 1b–d, the optimum for *O. sanmarkii* occurred at 0 m s^{-1} , that for *L. inermis* at 0.41 m s^{-1} and that for *E. ignita* at 0.66 m s^{-1} . The optimum for *O. sanmarkii* corresponded to the velocity regime encountered most frequently in the stream at this time (Fig. 1a), whereas optimum conditions for *L. inermis* and *E. ignita* were encountered much less frequently. The

rank order of the three species in terms of their flow preferences is clear. The absolute values of the optima and the response curves themselves, however, must be treated with caution. Near-bed velocity represents only one physical parameter which may influence the local densities of species and other factors may also be important (e.g. substratum particle size and heterogeneity, and food resources).

Artificial streams

The experiments were carried out in two identical fibreglass flumes (cast on a mould) in a semi-outdoor facility at ambient temperature and low light. The water circulatory system is closed (Fig. 2a). Water is aerated continuously in an overhead reservoir, falls by force of gravity through the flumes and then through a series of gravel filters, and finally, is pumped up into the reservoir to recirculate. The system was topped up with dechlorinated tap water to compensate for evaporation and spillage when necessary. Water temperature was close to and fluctuated in concert with mean ambient air temperature, but fluctuations were much smaller in amplitude. The flumes were arranged in parallel with one fixed inflow pipe on each flume. An additional inflow pipe could be directed to either flume to increase discharge in that flume, or it could by-pass both flumes and flow directly to the gravel filters.

Each flume consisted of a small head box (not illustrated), followed by a decelerating stretch of water as the flume increased in width and depth to form a pool, and then an accelerating stretch of water as the flume decreased in width and depth (Fig. 2b). Each flume was sloped at an angle of $\approx 1^\circ$ to the horizontal. At the most upstream end, water passed from the head box through a collimator to smooth turbulence before entering the flume. At the most downstream end, water cascaded over the end of the flume and through a drift net ($200 \mu\text{m}$ mesh) to catch any animals drifting out of the flumes and to prevent animals crawling upstream into the flumes. There was no natural substratum in the flumes; the bottom of the flumes was lined with a $200\text{-}\mu\text{m}$ mesh to which invertebrates could cling. Each flume was straddled by a mirror extending the length of the flume and angled at 45° . This allowed observation of animals within the flume without disturbing them.

Thus, the experimental arena was essentially two-

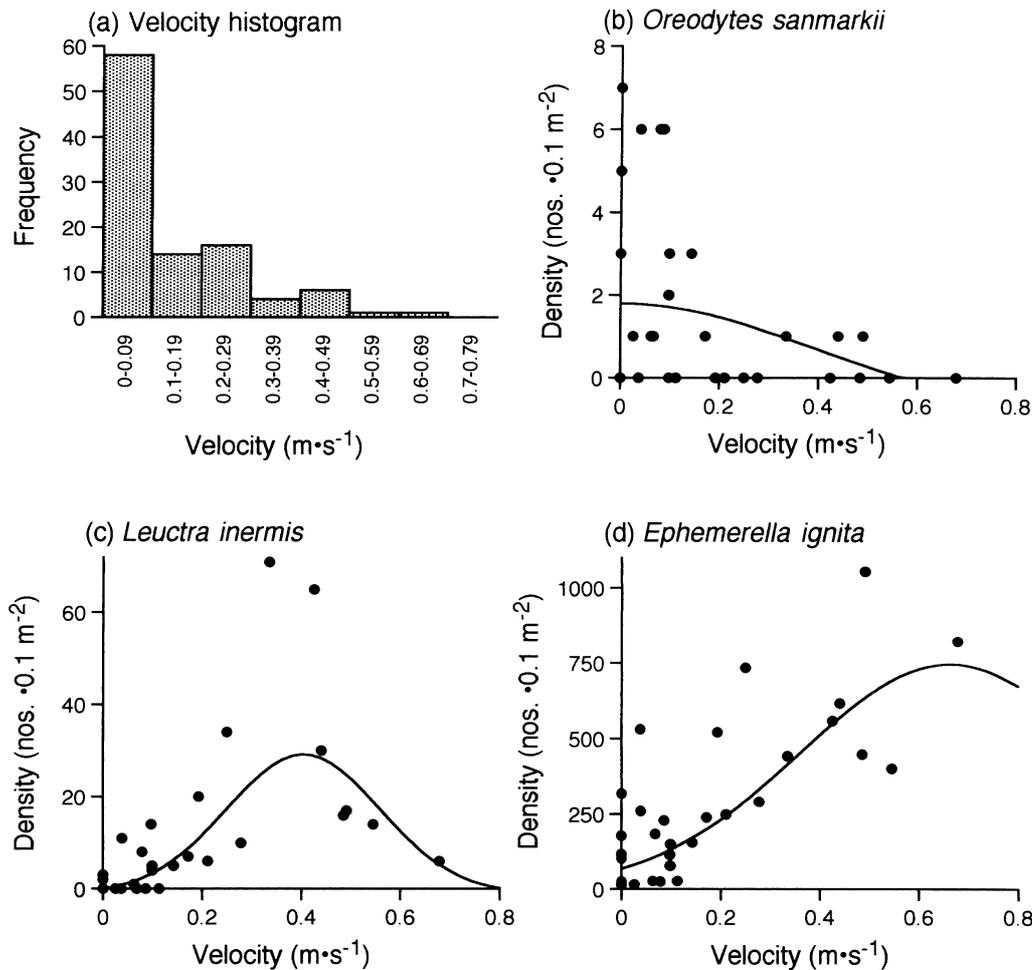


Fig. 1 (a) Frequency distribution of near-bed velocity at base flow in the Whiteadder Water on 3 June 1996 ($n = 100$). Density and Gaussian response curves of (b) *Oreodytes sanmarkii*, [$\log(y) = 0.447 - 1.383x^2$, $F_{1,28} = 6.64$, $P = 0.02$], (c) *Leuctra inermis* [$\log(y) = 7.324x - 9.064x^2$, $F_{2,28} = 84.69$, $P < 0.001$] and (d) *Ephemerella ignita* [$\log(y) = 1.832 + 3.143x - 2.372x^2$, $F_{2,27} = 11.03$, $P < 0.001$]. See text for a further explanation of curves.

dimensional with small-scale variations in near-bed flow associated with changes in flume width and depth. This was a highly simplified environment compared with the complex three-dimensional architecture found on the bed of natural streams. The advantage however, was that near-bed flow patterns were less complex than those in natural channels or in the presence of roughness elements (e.g. rocks). Therefore, any movements of animals between different parts of the flumes were more likely to be in response to flow, and not confounded by moving sediment particles nor obscured by preferences for crevices between stones, for example. It was not the objective of the present study to detail the hydraulic forces experienced by individual animals, as has been

done in other studies (e.g. Hart, Clark & Jasentuliyana, 1996). Indeed, such information would be difficult to interpret in an ecologically meaningful way given the highly simplified arena. Instead, the objective was to identify regions of the flumes that were broadly different (or similar) with respect to near-bed velocity. Then, more importantly, it would be possible to observe the distribution patterns of animals across the different velocity regions and to observe distributional changes associated with changes in discharge.

Velocity was measured within 1 cm of the flume bottom with a 2-cm discus, two-axis electromagnetic flow meter (Valeport Series 800, Valeport, UK) (Fig. 3). The sensing volume is a cylinder projecting

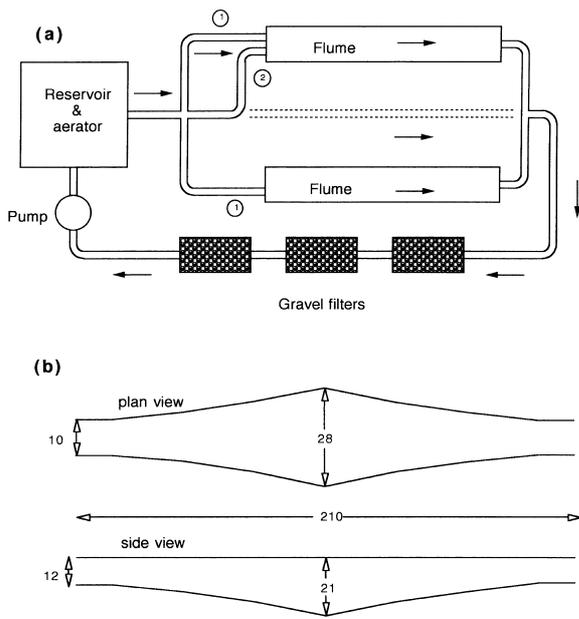


Fig. 2 (a) Schematic representation of the experimental flumes. The water is aerated in an overhead reservoir, falls by force of gravity through the flumes through a series of gravel filters and is then pumped up into the reservoir to recirculate. Each flume has one fixed inflow pipe (1). An additional inflow pipe (2) can be directed to either of the flumes or drained directly to the gravel filters. (b) Plan and side views of the flumes, showing an increase in width and depth in the centre to create a pool. All units are in centimetres.

1 cm below the sensor electrode face. The meter has a range of 0.003–2.000 m s⁻¹ with a detection limit of ± 0.001 m s⁻¹. Initially, flumes were divided longitudinally into 14 sections of equal length (15 cm). Within each section, depth and velocity were measured at six places: two transects across the flume, spaced evenly within the section, with three measurements per transect. One measurement was in the centre of the channel and two were at points as close to the left and right sides of the flume as possible, without distorting measurements (edge of sensor ≈ 1 cm from the flume side). This procedure was repeated at low and at high discharge.

In each flume, discharge was ≈ 3 × 10⁻⁴ m³ s⁻¹ with one fixed inflow pipe (low discharge) and increased to ≈ 7 × 10⁻⁴ m³ s⁻¹ over 30 s with the additional inflow pipe (high discharge, simulated spate). Fig. 4 shows near-bed velocity and water depth along a flume at low and high discharge. At low discharge, mean near-bed velocity in different sections ranged from 0 to 0.38 m s⁻¹, but increased to 0–0.62 m s⁻¹ at high discharge. The water

depth within flumes ranged from 1.5 to 7.8 cm at low discharge, and from 2.0 to 9.5 cm during simulated spates (high discharge). Velocity measurements at the flume sides were not systematically lower than those in the centre, suggesting that lateral velocity gradients at the flume sides were very steep and could not be detected accurately with the sensor. The biggest increase in velocity during spates occurred in the upstream decelerating stretch where the water was most shallow and turbulence appeared to be greatest (although it was not possible to quantify turbulence). Velocity remained very low in the central pool area with deep water and low negative values (upstream flow) were recorded at the upper end of the pool at low discharge. In the downstream decelerating stretch, near-bed velocity increased from low to high discharge, but the greater depth of water resulted in a lesser increase than in the upstream stretch. Velocity patterns did not differ significantly between the two flumes at either discharge (paired *t*-tests with velocity measurements paired between comparable sections of the two flumes: d.f. = 13; *t* = 0.493, *P* = 0.630 and *t* = 0.317, *P* = 0.756 for low and high discharges, respectively), but differences between discharge levels were significant (paired *t*-tests: d.f. = 13; *t* = 3.578, *P* = 0.003 and *t* = 4.171, *P* = 0.001 for each of the two flumes). For the purposes of measuring the distribution of animals within the flumes, the fourteen flume sections were combined in pairs to make seven, 30-cm sections referred to as microhabitats (a–g in Fig. 4) with broadly different near-bed velocity.

Experimental design

The microdistribution of insects within the flumes was observed before, during and after experimental

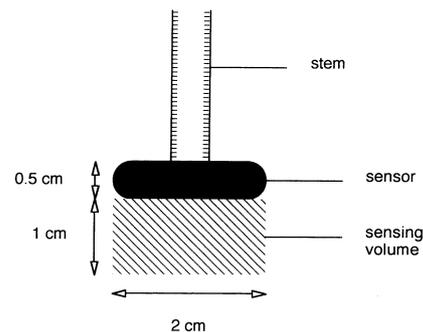


Fig. 3 Scale drawing (cross-section) of the discus sensor of the electromagnetic flow meter used in the laboratory flumes.

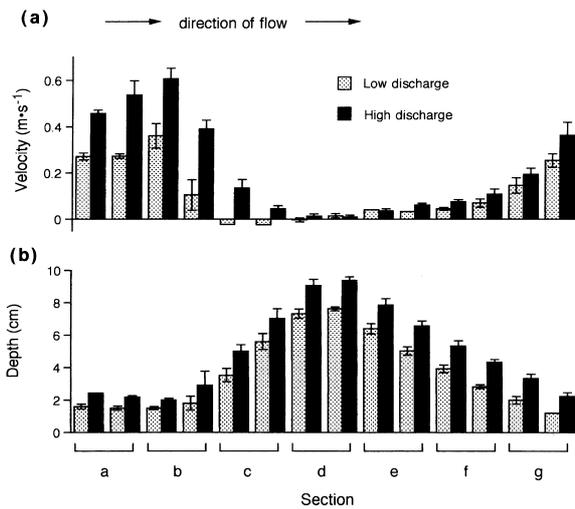


Fig. 4 (a) Near-bed velocity and (b) water depth in a flume at low and high discharge. Each bar represents the mean (± 1 SE) of six measurements (see text). The position of these bars along the x-axis corresponds to the views of the flumes in Fig. 2.

'spates'. Four replicate trials were carried out for each of the three species separately in late August and early September 1996. For each trial, animals were collected from the stream in the morning and returned to the stream at the end of each trial. Thirty individuals were placed in each of the two flumes, yielding a density (87 m^{-2}) much lower than those in the field, which can exceed 200, 2000 and 1000 m^{-2} for *O. sanmarkii*, *L. inermis* and *E. ignita*, respectively. This minimizes the possibility of intraspecific interactions confounding the response to flows. Once placed in the flume, animals were allowed a minimum of 2 h to settle; during the first hour only, any animals in the drift nets were returned to the flumes. After settling, the number of individuals within each section of the flume (a–g in Fig. 4) was recorded at 15-min intervals for 3 h of observation. One flume, the 'experimental' one, was chosen at random and subject to high flow (simulated spate) during the second observation hour by directing the additional inflow pipe to that flume (Fig. 2a). The first recording of the positions of the animals at high discharge was 15 min after the manipulation. Water from the additional inflow pipe flowed directly to the gravel filters for the remainder of the time, i.e. discharge in the experimental flume was low during the first and third hours, and was high during the second hour. Discharge in the other 'control' flume remained constant throughout. The

duration of each trial (including the settling period) is not very long, but it was adequate to observe short-term changes in microdistribution without the confounding effects of increased hunger (no food was provided in the flumes) or diel variations in behaviour.

Numerical and statistical analyses

The total number of animals in each flume was compared between treatments (experimental versus control) and over time using a univariate, repeated measures analysis of variance (ANOVA). The data were expressed as a percentage of the total number of animals present at the beginning of the observation period and were arcsin-transformed before statistical analysis. Repeated measures ANOVA has the capability to detect overall treatment effects, temporal trends (time effects) and differences among treatments in the slope or shape of temporal trends (interactions between treatment and time) (Winer, 1971; Milliken & Johnson, 1984). Temporal trends could be linear, quadratic, cubic or any higher degree polynomial up to a maximum of $n-1$, where n is the number of time intervals. For the data sets in the present study, Huynh–Feldt statistics (Milliken & Johnson, 1984) indicated that the assumptions of compound symmetry required by univariate tests were robust and, hence, a multivariate, repeated measures ANOVA was not required.

The microdistribution of animals in each flume was compared using three-way contingency tables based on log-linear models (Sokal & Rohlf, 1981). This is a non-parametric test, roughly analogous to a multi-way ANOVA, in which the primary interest is in the presence of significant interactions among factors rather than main effects. It allows comparisons of the microdistribution of animals between treatments and among time periods through a series of G-tests. The three factors were position within the flume (sections a–g), treatment (experimental versus control) and time (before, during and after simulated spates, designated 'pre', 'spate' and 'post' in the text to follow). Data for each trial were summarized as the number of animals recorded in each section of the flume summed over the four observations made within each level of the time factor (i.e. 'pre', 'spate' and 'post') and then summed over the four replicate

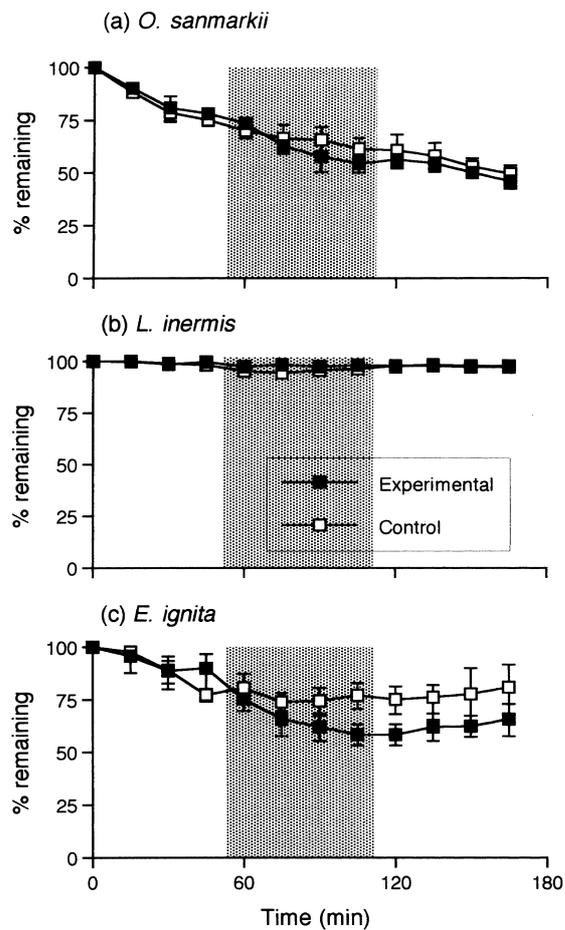


Fig. 5 Total number of (a) *O. sanmarkii*, (b) *L. inermis* and (c) *E. ignita* in experimental and control flumes over the 3-h observation period. Data are expressed as the mean percentage (± 1 SE) of the number present at the beginning of the observation period, averaged over the four trials. Mean and SE values were calculated on arcsin-transformed data and back-transformed to percentage values for the illustration. The stippled area indicates the 'spate' period in which the experimental flume was subject to high discharge, with the 'pre' and 'post' periods before and after, respectively. See Table 1 for statistical analyses.

trials. Contingency table analysis requires whole numbers, and therefore, sums rather than averages were used. Data from individual replicate trials were not used in the analysis, but summing data across replicates increased confidence that the patterns were real rather than chance events. Note that when comparing microdistributions, contingency table analyses and *G*-tests are relatively insensitive to differences between flumes in the total number of individuals present.

Results

Movement

During the pre-trial settling period, individuals wandered throughout the flumes and, initially, some individuals drifted out of the flumes. The number of drifting individuals and the degree of wandering decreased markedly after approximately 2 h, but animals did continue to wander and occasionally to drift out of the flumes throughout trials. There was no obvious preference of any species for the flume sides or centre. The beetles, *O. sanmarkii*, tended to move about the flumes in short swimming bursts, although a few individuals were observed crawling, occasionally against very fast currents. If suspended in the water column, individuals tended either to swim to the bottom and reattach to the substratum, or to rise to the surface and drift in the surface film. Some beetles were found flying about the flumes (and occasionally re-entering the water), although it is not clear whether such flights were initiated from the water within the flumes or from the drift nets at the outflow. When exposed to an increase in near-bed velocity during simulated spates, individuals in very high flow areas were observed entering the drift and swimming down to the substratum in more slowly flowing areas downstream. Once discharge declined again, some individuals moved upstream by crawling or swimming. The stonefly nymphs, *L. inermis*, were perhaps the least active of the three species, although they did wander throughout the flumes during the experiments. If suspended in the water column (through dislodgement by the current or by behavioural drift), they appeared powerless to influence their fate and drifted passively with legs, antennae and cerci spread out. These nymphs have moderately hairy bodies and, once caught in the surface film, appeared to have difficulty re-entering the water. Reattachment to the substratum usually occurred by sinking in areas of slow flow or once stranded against the flume sides. No obvious responses to changes in near-bed velocity were observed. Mayfly nymphs, *E. ignita*, were very active and crawled in all directions, including both upstream and downstream in fast currents. If caught in the drift, nymphs curled up with the legs and antennae tucked close to the ventral side of the body, and with the cerci folded over the dorsal side of the abdomen. This posture appeared to promote sinking so that the animal regained the substratum quickly.

Table 1 Summary of repeated measures ANOVA for each species, comparing the number of animals placed in control and experimental flumes

Source of variation	d.f.	<i>Oreodytes sanmarkii</i>			<i>Leuctra inermis</i>			<i>Ephemera ignita</i>		
		MS	F-value	P-value	MS	F-value	P-value	MS	F-value	P-value
<i>Between treatments</i>										
Treatment	1	0.008	0.28	0.61	0.043	0.19	0.68	0.262	1.30	0.30
Error (trial)	6	0.028			0.227			0.202		
<i>Within treatments</i>										
Time	11	0.419	53.66	<0.01	0.062	6.57	<0.01	0.294	8.62	<0.01
Time × treatment	11	0.003	0.36	0.97	0.007	0.73	0.70	0.024	0.72	0.72
Error (time)	66	0.008			0.009			0.034		

When exposed to increased near-bed velocity during spates, some individuals were observed walking downstream from the fast decelerating stretch towards the pool or drifting to moderate velocity areas below the pool before regaining the substratum.

Loss of animals from flumes

The total number of individuals in each flume decreased over the 3-h observation period (Fig. 5). This decrease was significant for all three species, as indicated by repeated measures ANOVA (time effect in Table 1). Although there may be some suggestion that more individuals of *E. ignita* and *O. sanmarkii* may have been lost from the experimental treatment than from the control, these differences were not statistically significant (treatment and time × treatment effects in Table 1). In the most extreme case of *E. ignita*, note that standard error bars overlap for control and treatment means on ten out of the twelve observation times. Comparisons of treatment means at each time interval indicated no significant differences among means at any time, although this test is not really justified in the absence of a significant interaction term (Milliken & Johnson, 1984). Power tests (J. Lancaster, unpublished data) indicated that repeated measures ANOVA could detect a manipulation impact as small as a loss of 8% of the total number of animals against the variation inherent in the data set. In Fig. 5c, there is an 'apparent' impact of 15% loss but, since this is within the detection limit of the analysis and since treatment and interaction effects were not statistically significant (Table 1), it must be concluded that there was no net loss of animals as a result of discharge manipulations.

Changes in microdistribution

At low discharge, the microdistribution of all three species was broadly consistent with field observations of flow preference (control and experimental flumes pre-spate, Fig. 6). The beetles, *O. sanmarkii*, were most abundant in the slowly flowing pool in the centre of each flume and rarely occurred in the very fast flows at either end. The microdistribution of the stonefly, *L. inermis*, was similar to that of *O. sanmarkii*, with maximum numbers in the pool, but some individuals were found in the fastest flows. Nymphs of *E. ignita* had the most uniform distribution and occurred in all parts of the flume. In control flumes, 96%, 78% and 50% of all observations of *O. sanmarkii*, *L. inermis* and *E. ignita*, respectively, were of animals in flume sections with mean velocities $\leq 0.04 \text{ m s}^{-1}$. *Oreodytes sanmarkii* was never observed in velocities $> 0.23 \text{ m s}^{-1}$, but both *L. inermis* and *E. ignita* were observed in near-bed velocities in excess of 0.50 m s^{-1} .

The simulated spate had an impact on the microdistribution of *O. sanmarkii* and *E. ignita*, but not that of *L. inermis* (compare the experimental and control flumes in the spate period in Fig. 6). Tests for a three-factor interaction (position × treatment × time) using three-way contingency table analysis were significant for both *O. sanmarkii* (d.f. = 12, $G_{\text{adj}} = 3214$, $P < 0.01$) and for *E. ignita* (d.f. = 12, $G_{\text{adj}} = 26.93$, $P < 0.01$), but not for *L. inermis* (d.f. = 12, $G_{\text{adj}} = 9.33$, $0.50 < P < 0.90$). For *L. inermis* only, further tests were carried for two-factor effects: time × treatment (d.f. = 14, $G_{\text{adj}} = 9.55$, $0.50 < P < 0.90$) and treatment × position (d.f. = 18, $G_{\text{adj}} = 24.50$, $0.10 < P < 0.50$) effects were not significant, but time × position was significant (d.f. = 12, $G_{\text{adj}} = 58.66$, $P < 0.01$). This result reflects a drop in the number of individuals in

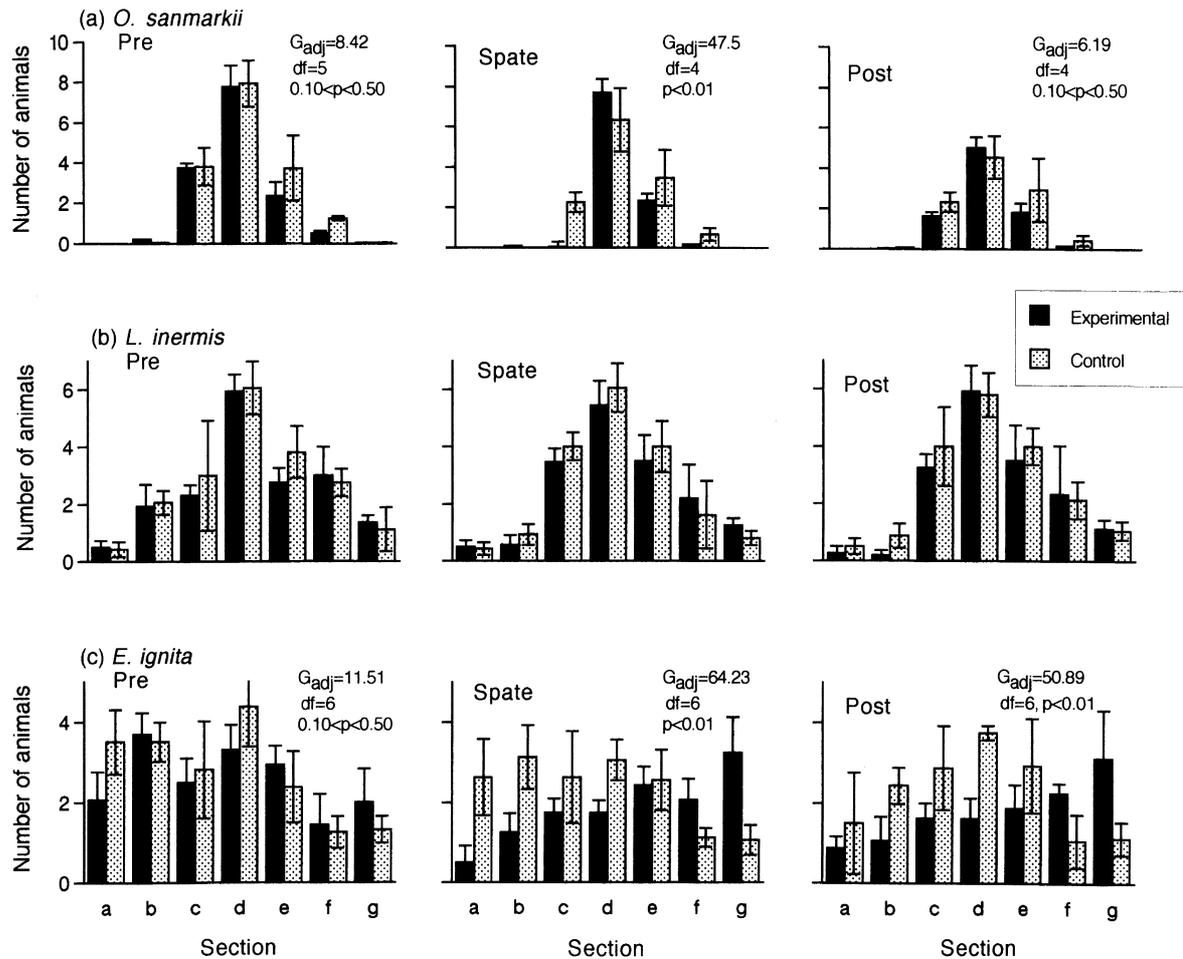


Fig. 6 Mean numbers (± 1 SE) of (a) *O. sanmarkii*, (b) *L. inermis* and (c) *E. ignita* in each section of the experimental and control flumes during the 'pre', 'spate' and 'post' periods. The numbers were summed within time periods in each trial and averaged over replicate trials. In (a) and (c), summary statistics are presented for *G*-tests of independence comparing the distribution pattern of animals between treatments during each time period. See the text for further explanation.

section 'b' of both experimental and control flumes after the pre-spate period. There is no obvious explanation for this pattern and it may indeed be a chance event. For *O. sanmarkii* and *E. ignita*, no further attempts were made to fit more simple models to the data within the framework of a three-way contingency table but separate two-way *G*-tests of independence were made within each level of the time factor, as is appropriate when three-factor interaction terms are significant (Sokal & Rohlf, 1981). The results of these *G*-tests are summarized in Fig. 6a,c. The microdistribution of *O. sanmarkii* was significantly different between experimental and control flumes during the spate, but not pre or post (Fig. 6a). During the spate, animals appear to have moved into the pool from the fast flowing water immediately upstream,

but returned during the post period. The microdistribution of *E. ignita* did not differ significantly between treatments before the spate, but differences were significant for both the spate and post periods (Fig. 6c). At high discharge, these mayflies appear to have moved (actively or passively) from the very fast flowing and turbulent water upstream of the pool to the slower flows down-stream, but there was no evidence of redistribution post-spate. For neither *O. sanmarkii* nor *E. ignita* was there any evidence that individuals moved away from the accelerating stretch below the pool during simulated spates.

Discussion

The observed small-scale changes in the distribution

of lotic invertebrates between hydraulic microhabitats during simulated spates are consistent with the hypothesis that some macroinvertebrates can accumulate in flow refugia at the substratum surface during disturbances. There was no net loss of total numbers of animals as a result of discharge manipulations, so observed changes in microdistribution cannot be attributed to disproportionate loss of individuals from high versus low velocity areas, and must be related to movements of individuals among microhabitats. The combination of such shifts in microdistribution without a net loss in total numbers makes it possible to begin identifying which mechanisms of flow refugium use might be applicable to individual species (Robertson *et al.*, 1995; Lancaster & Belyea, 1997). The change in distribution of the adult dytiscid beetles, *Oreodytes sanmarkii* and mayfly nymphs of *Ephemerella ignita*, appeared to result from an accumulation of individuals in low flow microhabitats analogous to flow refugia. Note that flow refugia do not have to be areas with the *lowest* flow and species may differ in their preference for refugium types. For example, *E. ignita* normally prefer fairly fast over slow velocities, and therefore, it is reasonable to predict that the ideal refugium for this species would have at least moderate velocity.

By using laboratory flumes with identifiable hydraulic microhabitats, it was possible to examine how variations in near-bed velocity influence the microdistribution and movement of invertebrates. Natural stream channels have highly heterogeneous substrata and, consequently, there is tremendous spatial and temporal variation in near-bed hydraulic forces over small scales. Out of necessity, field surveys generally involve rather coarse estimates of flows averaged over complex substrata and over spatial scales greater than that of individual organisms (but see Hart *et al.*, 1996; Sand-Jensen & Mebus, 1996). Artificial flumes can be more tractable systems for examining flows on small scales (e.g. Lacoursière, 1992; Lacoursière & Craig, 1993). It was not the objective of the present study however, to characterize the hydrodynamic environment around individual animals. Rather, I used flumes with very simple, homogeneous substrata (a smooth, flat bottom lined with fine mesh) to minimize the small-scale hydraulic heterogeneity normally created by three-dimensionally complex substrata and to create identifiable microhabitats ($\approx 0.015\text{--}0.035\text{ m}^2$) of contrasting flow

characteristics within the flume. The simple substratum also removed the possibility that individuals could take refuge underneath objects and, hence, considered only the possibility that animals might accumulate in refugia at the substratum surface. In addition, I was able to measure the velocity within 1 cm of the flume substratum, a scale more relevant to individual animals than is usually achievable in field conditions. Laboratory flumes are, however, artificial environments and this places limitations upon interpretation of the results.

At low discharge, the microdistribution of all three species conformed broadly to field observations of flow response curves. Adult beetles, *O. sanmarkii*, occurred almost exclusively in areas of the flumes where near-bed velocity was very low ($< 0.04\text{ m s}^{-1}$). Stonefly nymphs, *L. inermis*, occurred throughout the flumes with the highest density in the low velocity areas, and mayfly nymphs, *E. ignita*, also occurred throughout the flumes, but the maximum density was in moderately high velocity areas. The ratios of the density in sections of the control flumes with the velocity below or above 0.1 m s^{-1} were $\approx 1:2.4$ and $1.6:1$ for *L. inermis* and *E. ignita*, respectively, over the 3-h observation period. Some caution is required when comparing distribution with respect to flow in the field and in laboratory flumes, and it would be imprudent to attach too much importance to the absolute values of velocity since this is only one element of the hydrodynamic environment experienced by the animals and many other factors may influence small-scale distribution in the field.

For none of the three species studied was an increase in discharge and associated near-bed velocity accompanied by a significant decrease in the total number of individuals in a flume. Total numbers decreased over the duration of the observations in both the experimental and control flumes, indicating that individuals did wander and occasionally enter the drift, either voluntarily or through dislodgement, for long enough to leave the flumes. The lack of response of total numbers to increased discharge is contrary to observations in other flume studies of invertebrates (Allan & Feifarek, 1989; Palmer *et al.*, 1992; Richardson, 1992) and this difference may be attributed, in part, to variations in the magnitude of discharge manipulations and flume design. For example, the flumes of Allan & Feifarek (1989), in which the drift distance of mayfly larvae increased

with discharge, were straight rain gutters with none of the flow heterogeneity associated with more complex habitats. In contrast, Borchardt (1993) found that adding debris to flumes and, presumably, increasing hydraulic heterogeneity, reduced population losses of *E. ignita* and *Gammarus pulex* (L.). The lack of a reduction in total numbers in the present study is also contrary to expectations for at least some species, such as *O. sanmarkii*. This species typically occurs in slow flows in natural streams and also occurs in standing waters (Nilsson & Söderberg, 1996). It appears poorly adapted to high flow, i.e. it is a poor swimmer (Ribera & Nilsson, 1995) and, as an air breather, it must surface occasionally and hence risks transport downstream. Despite the very high near-bed velocities measured in some flume microhabitats at high discharge, all three species studied were able to resist dislodgement and/or exploit the low flow microhabitats and prevent a net loss in numbers.

Simulated spates resulted in a change in the microdistribution of two out of the three species of aquatic insect, *O. sanmarkii* and *E. ignita*, and thus the utilization of flow refugia may vary among species. At high flow, individuals of *O. sanmarkii* appeared to have moved into the slowly flowing pool from the fast flowing and turbulent water immediately upstream, but some individuals moved back upstream once discharge returned to low levels. The response of this species clearly fits the 'directed flux' model of refugium use (Lancaster & Belyea, 1997) in which individuals accumulate in refugia during disturbance, and redistributing subsequently. Some nymphs of *E. ignita* also moved away from the high velocity upstream of the pool to the more moderate flows below the pool. There was no evidence, however, of a return to their original distribution during the 1-h post-spate observation period, although recovery might have occurred with a longer observation period. Based on these data, it is difficult to discriminate with confidence between the 'directed flux' and 'undirected flux' models of refugium use (Lancaster & Belyea, 1997) for *E. ignita*. The latter involves accumulation in refugia during disturbances, but little or no redistribution once the disturbance has passed. The absence of a response by the stonefly nymphs, *L. inermis*, to increased discharge is difficult to interpret. It is possible that the maximum near-bed flow force during the disturbance was not sufficient to elicit a microdistributional shift. This is surprising since

stoneflies were observed in flume sections with mean near-bed velocities of 0.62 m s^{-1} . It has been suggested that flow refugia are particularly important to large nymphs of a congener, *Leuctra nigra* Olivier, (Lancaster & Hildrew, 1993b) and individuals do accumulate in refugia (Winterbottom *et al.*, 1997). These differences may be related to the low stability of substrata in some natural channels (such as Broadstone Stream which was the subject of these other studies) versus the high stability of experimental flumes, or these two species may indeed respond differently to hydraulic patterns. It is also possible that *L. inermis* (and perhaps *L. nigra*) is able to cling to the substratum and resist dislodgement during spates for periods longer than used in the present study (>1 h), but that changes in distribution would occur over the time periods (≥ 1 week) in the other studies (Lancaster & Hildrew, 1993b; Winterbottom *et al.*, 1997). *Leuctra inermis* was the least active of the three species examined and its poor ability to regain the substratum once suspended in the water column suggests that there may be some advantage for this species in not moving. Such behaviour is consistent with the 'no flux' model of refugium use (Lancaster & Belyea, 1997), where maintaining high population densities through refugium use requires that the majority of individuals remain in refugia at all times. These ideas are speculative, however, and require further corroboration.

Did *O. sanmarkii* and *E. ignita* accumulate in low flow areas (refugia) via active or passive modes of movement? Observations suggested that both processes may have occurred. Observations of *E. ignita* walking down the velocity gradient in response to increased discharge implicate an active, behavioural avoidance of very high near-bed velocity. Both species were observed drifting away from high velocity areas, but it is virtually impossible to determine whether individuals entered the drift passively through dislodgement or actively released their hold on the substratum. Once suspended in the water column both, however, species exhibited behaviours which might be interpreted as an active attempt to regain attachment before being transported great distances downstream. The beetles drifted passively at or near the substratum surface in fast flow, but actively swam down to the substratum in slower flow areas. Adult beetles also left the water and flew, suggesting that they could escape hydraulic disturbances on land and

might fly back upstream. In contrast, drifting nymphs of *E. ignita* modified their body posture in a way that appeared to promote sinking and increased the possibility of regaining the substratum. Otto & Sjöström (1986) also observed that drifting *E. ignita* curl up and sink passively, effectively reducing the time spent in the drift. Evidence suggests that behavioural adjustments to drift may be more common and/or more pronounced among mayflies than stoneflies (Ciborowski & Corkum, 1980; Otto & Sjöström, 1986), and this is consistent with the absence of changes in posture observed in *L. inermis*. Other stonefly species do alter their drift posture to promote sinking, as in some Nemouridae (Otto & Sjöström, 1986; Blum, 1989), but evidence of such behaviour in the Leuctridae is lacking. Indeed, field observations of drifting stoneflies suggested that drift distances may be under active behavioural control in the nemourids, but passively determined primarily by physical processes in the leuctrids (Lancaster, Hildrew & Gjerlov, 1996).

Two difficult questions are highlighted by observed changes in the microdistributions of *O. sanmarkii* and *E. ignita*, but not *L. inermis*, in response to increased discharge, and by a response magnitude which was perhaps less than might have been expected. Firstly, what magnitude of change in the physical environment is required before disturbance avoidance (e.g. accumulation in flow refugia) is apparent and/or disturbance resistance (e.g. by hooks and claws to resist dislodgement) begins to fail? Disturbance is a response phenomenon and responses are species-specific, so whether a species perceives a particular environmental event as a disturbance will also be species-specific. The increase from low to high discharge in the present study may not have been a disturbance for *L. inermis* since there was no net loss of individuals from experimental flumes and no evidence of flow refugium use. Both *O. sanmarkii* and *E. ignita* did change microdistribution in a way which is consistent with what might be predicted during a disturbance, so the physical change could be called a disturbance. Secondly, if changes in microdistribution in response to changes in the physical environment are not pronounced, is the disturbance a 'weak' one or are these responses ecologically 'trivial', i.e. could such distributional changes actually influence net population numbers in a habitat subject to frequent disturbances? This question is more difficult

to address without the benefit of longer-term studies of whole populations. I would suggest that the observed shifts in microdistribution of *O. sanmarkii* and *E. ignita* in this study may be important to population persistence, but an artefact of the experimental design reduced the magnitude of the response. In particular, the invertebrates may have been especially adept at clinging to the mesh bottom of the flume, and hence, individuals may have resisted dislodgement (or received insufficient stimulus to move) at a velocity higher than normal in field conditions with less coarse and perhaps unstable substrata (Lancaster & Mole, 1999). In short, animals were given a 'deluxe' substratum so the fact that changes in microdistribution actually did occur suggests that the discharge manipulation was indeed a disturbance. Evidence of the importance of substratum texture for aquatic invertebrates is largely circumstantial, yet some property of the substratum associated with texture may influence the distribution of at least some aquatic invertebrates (Minshall, 1984). Despite the difficulties, the present study did show that at least some species appear to accumulate in flow refugia during flow disturbances and both active and passive modes of movement are involved.

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