Euro-limpacs

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Macroinvertebrates as Indicators of Climate-Induced Change in River Ecosystems

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Integrated Project to evaluate the Impacts of Global Change on European Freshwater Ecosystems

WP2: Climate-hydromorphology interactions

Task 3: Hydrological changes and aquatic taxa

Subtask 3.2: Laboratory experiments

Deliverable No. 219

Macroinvertebrates as Indicators of Climate-Induced Change in River Ecosystems

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Aims and Objectives

The principle project aim is to assess the suitability of selected freshwater macroinvertebrate species as indicators of climate change in terms of the strength of their response to experimental low-flow treatments. It is hoped that as an outcome, suitable indicator species that occur in groundwater fed rivers will be identified for the climate change scenarios that have been predicted to occur in the UK.

The null hypothesis that will be tested is that the experimental manipulation of flows will have no effect on the abundance of any species of invertebrate, or any community level value such as total abundance, or diversity. It is possible to hypothesise that the control channels in this experiment, with normal discharge levels would be expected to have the most diverse macroinvertebrate fauna, with gastropods and crustaceans dominant (Armitage, 1995), whilst the low flow channels will have the least diverse fauna. The objectives of this study will therefore be to:

1. Determine which benthic invertebrate species found in the chalk rivers of southern England could act as positive indicators of extensive low flow periods.
2. Determine which benthic invertebrate species found in the chalk rivers of southern England could act as negative indicators of extensive low flow periods.
3. Monitor the habitat changes brought about by low flow treatments.
4. Relate these habitat changes to the changes in invertebrate numbers bought about by the flow treatments.

Methods

This experiment was carried out using artificial streamside channels located at the Freshwater Biological Association River Laboratory in East Stoke, Dorset, UK (50°40'48''N, 2°11'06''W) (Plate 1, Figure 1). These channels are directly fed with water from a side channel of the River Frome, known as the Mill Stream (Figure 1). The River Frome is a UK Biodiversity Action Plan (BAP) chalk stream priority habitat (EA, 2005).

Plate 1: The Freshwater Biological Association experimental site at East Stoke, Dorset. The artificial channels are on the right, and are fed by the Mill Stream on the left.

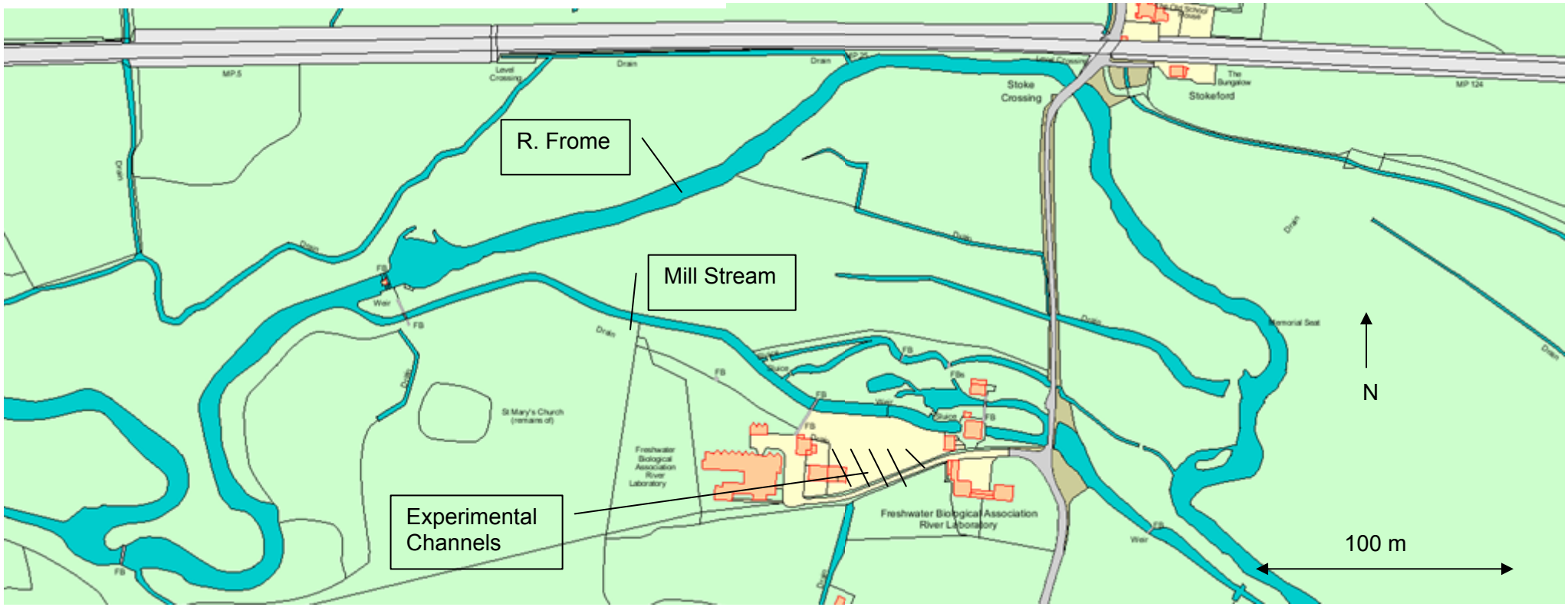


Figure 1: The site of the experimental channels.

The Mill Stream was used for over 500 years as the power source for the East Stoke Mill. The Mill was replaced in 1971 with an experimental fluvarium (a running water aquarium, seen on the upper left half of Plate 1) (Wood, 1997). This site was specifically chosen for the construction of the River Laboratory as the head of water in the Mill Stream is sufficiently powerful to passively fill the artificial channels using the force of gravity alone (Berrie, 1992b).

The main advantage of using these naturally fed, flow through artificial channels is that they are provided with realistic levels of biological recruitment, concentrations of nutrients and minerals, fluctuations of local temperature, suspended solids and other features from the water column (Guckert, 1993). The river system supplying the artificial channels has been the subject of much research. There is a large amount of literature on both the physicochemistry (Casey, 1975) and biology (Wood et al, 1999) of the Mill Stream, which can be classed as a typical chalk stream (Wood et al, 1999).

Previous work (Harris et al, 2006) has shown that the artificial channels provide a fairly accurate simulation of conditions in the Mill Stream. It is therefore valid to use these channels to conduct experiments whose theory is based on natural conditions. Water temperature, pH and conductivity in the artificial streams has been shown to be similar to that in the Mill Stream, and these variables correlate well over time between the two systems. The macroinvertebrate assemblage in the artificial channels has also been shown to be similar to that of the Mill Stream, with 63% of taxa found in both systems (Harris et al, 2006). These realistic conditions result from the streamside location of the artificial channels and their through-flow nature (Harris et al, 2006).

Design of the Artificial Channels

The artificial streams used are arranged in four blocks of three channels (Plate 2, Figure 2) roughly perpendicular to the Mill Stream, which provides the inflowing water, containing suspended algae, detritus and macroinvertebrates. There is an unused block between blocks three and four of this experiment that was not used because its inlet was larger than those of the other four blocks used (Figure 2). There are also two blocks that were too short for this experiment and were not used.

Water enters the artificial channels through branching 110 mm diameter pipes of approximately 6 m in length that pass under the Mill Stream bank. The individual channels are linear with aluminium walls that give them a dimension of 0.33 m wide, 12 m long and 0.30 m deep. The substrate composition in the channels consists of clean gravel of various particle sizes similar to that found in the Mill Stream. The flow level in the channels is controlled using a simple system of plastic boards that slot over the end of the inlet pipes, regulating the inflow of water. The lower end of each stream is open to allow discharge of water, detritus and macroinvertebrates into a small drainage stream. This means that these artificial streams provide a once-through system with no water recycling.

Plate 2: Block 1 looking upstream towards the inlet pipes. Note the bare gravel substrate as this photo was taken before the imposition of experimental treatments.

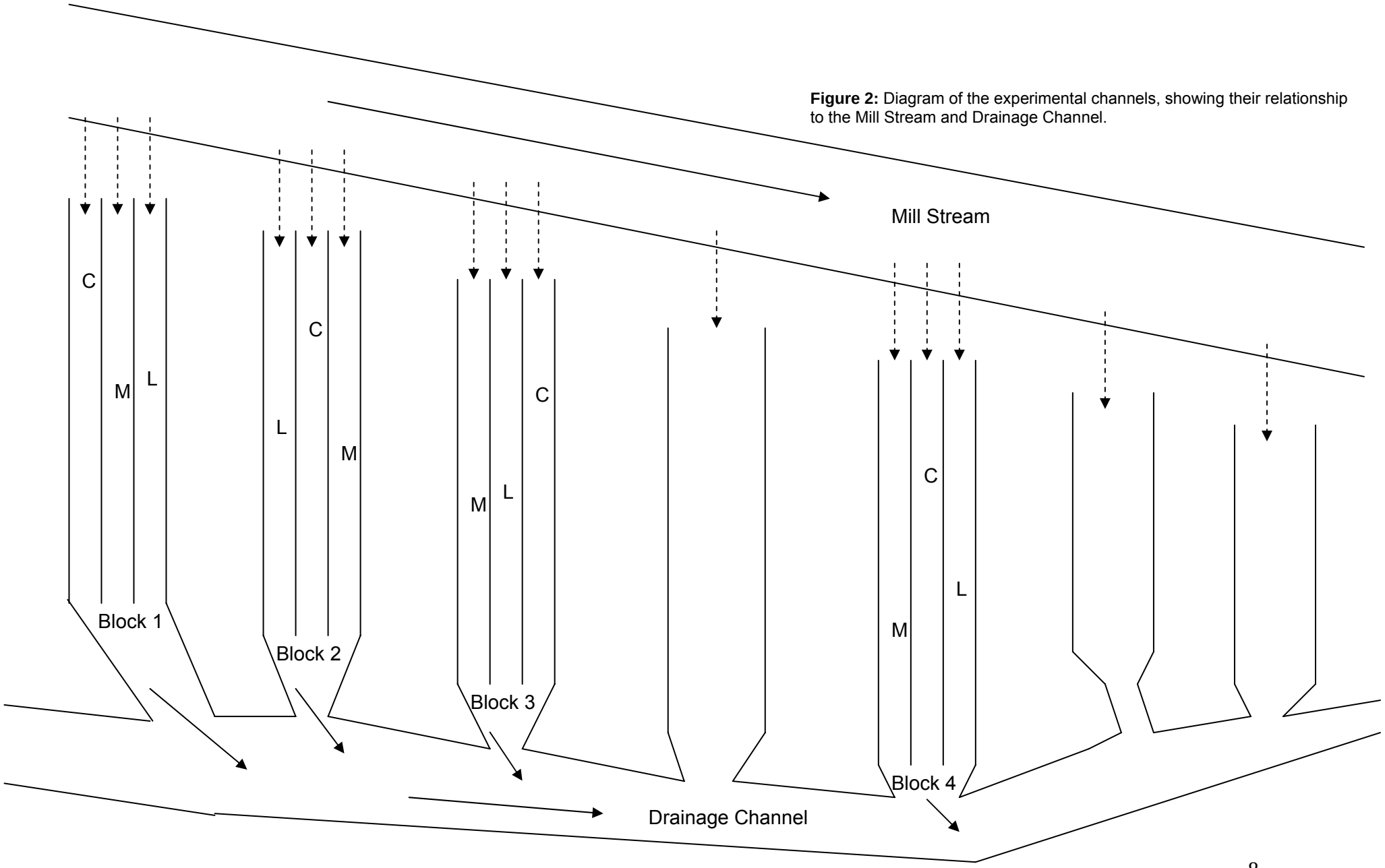


Figure 2: Diagram of the experimental channels, showing their relationship to the Mill Stream and Drainage Channel.

Use of Artificial Streams

There has been much debate over the applicability of using artificial streams for ecological experiments due to the difficulty of weighing up their advantages and disadvantages. An artificial stream is defined as “A constructed channel having a controlled flow of water, which is used to study some physical, chemical or biological property of natural streams” (Lamberti and Steinman, 1993). The obvious advantages of using artificial streams that will be exploited in this investigation is that they allow for better control over the environment than natural situations, they provide the opportunity to replicate treatments, they allow for the possibility of experimental designs that are compatible with the use of inferential statistics (McIntire, 1993) and they make it possible to examine the effects of a single factor, flow in this case, over a series of different magnitudes (Steinman, 1993). The use of artificial streams is limited by the extent to which the investigator is willing to depart from reality. If too much control is exerted then it may be impossible, or unwise, to infer that the results obtained from artificial stream experiments have any relevance to the natural world (McIntire, 1993). It has been suggested that when using artificial channels, the best an ecologist can do is to generate hypotheses about the mechanisms involved in a natural system that are based on changes that have been observed in the field (McIntire, 1993). Experiments using artificial channels may be particularly useful in isolating the effects of different mechanisms, as more than one mechanism can lead to similar observations in a natural system (Steinman, 1993).

Artificial channels have been utilised effectively in various experiments relating to invertebrate growth and survivorship, disturbance, colonisation dynamics, interactions between nutrients and macrophytes, algae and herbivores, predators and their prey, toxicity testing and ecological risk assessment (Harris et al, 2006). They have also been used to simulate high discharge events (Bond and Downes, 2003). Field experiments in biomonitoring, such as this one, have been shown to be of great value as they can be used in many ways. Examples of these uses include identifying sensitive species, which will be the primary aim of this project, predicting the responses of possible perturbations (such as low flows), disentangling direct and indirect effects of disturbances, determining the direct and interactive effects of a variety of variables on ecological systems, interpreting the observed response to environmental change, and calibrating biomonitoring programmes (Cooper and Barmuta, 1993).

Experiment Preparation

All twelve artificial channels were thoroughly agitated using a rake prior to the commencement of the project, in late April 2006, in order to remove any build up of silt that had covered the channel beds in the period since they were last used for scientific experimentation. The channels were also raked in order to level off the substrate, composed primarily of gravel, so that the channels were all in as similar condition as possible, without pools and sheltered, slow flowing areas.

Once the substrate conditions in the twelve channels had been equilibrated as much as possible, the discharge into each was set at roughly 5 m^3/s on the 9^{th} of May 2006 by adjusting the flow of water from the inlet pipes, using the plastic control barriers. Stream velocity was measured using a Valeport electromagnetic monitor. The channels were then left for a three week period under these conditions so that they were colonised by macroinvertebrates to a similar degree, giving them the same starting conditions for the actual experimental period. Colonisation occurred by macroinvertebrates drifting through the inlet pipes from the Mill Stream, by adult oviposition (Diptera) and by migration of winged

adults (Coleoptera) (Harris et al, 2006). Previous experiments using these particular artificial streams have shown that macroinvertebrate assemblages following a colonisation period did not differ significantly among blocks or by position within a block. Inter channel variability was found to be 6.1% for macroinvertebrates and 1.4% for physicochemistry (Harris et al, 2006).

Experimental Set-up

Within each block of three channels, each channel was assigned to one of three treatment levels. Hence there were four replicates of each flow treatment across the four blocks. The three different conditions used were the control channels with relatively high discharge rates, medium discharge channels, and low discharge channels.

It was important that the three experimental treatments were mixed between channels one, two and three in each block using a Latin squares-type experimental design (Figure 3), because the conditions in each of the three channels within a block could not be assumed to be equal. Due to the channel design there may have been potential differences in flow between the inner and outer channels in each block (Craig, 1993). Water entered the outer two channels via curved pipes, as opposed to entering through a straight pipe in the middle channel, and the outer two channels had a different cross-sectional profile from the central one, in that they had a trapezoid rather than rectangular shape. These differences are not thought to be substantial in terms of their impacts on biota, but to be safe, the treatments were distributed across all three positions within a block.

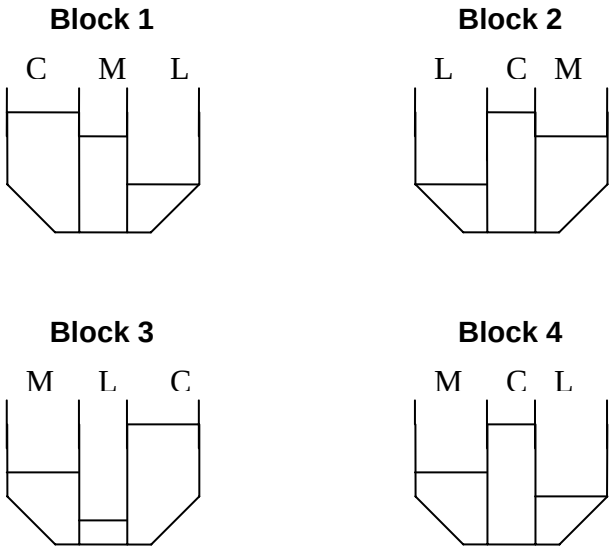


Figure 3: The experimental design. C denotes control flow, M medium flow and L low flow channels.

These three flow treatments were imposed on the 30th of May 2006 by adjusting the position of the plastic boards that restrict flow through the inlet pipes. The flow for each treatment was set by measuring the water velocity and adjusting the boards accordingly so that velocity was similar for all channels of the same treatment, and so that there was as distinct a difference in velocity between the low-flow and control treatments as possible. Velocity was used to set

the experimental treatments as the channels were all roughly the same size in cross section, so it was deemed unnecessary to calculate discharge.

Sampling Procedure

Surber Sampling

Three macroinvertebrate samples were taken from each of the twelve channels before treatments were imposed, on the 30th of May 2006, and after six weeks of experimental treatment, on the 11th of July, in order to discover what changes the various discharge regimes had brought about. When sampling was carried out, each channel was theoretically divided into three equal longitudinal sections of four metres, with a sample being taken from a random position within each of these sections. These pseudo-replicate samples were taken as there may be a gradient in physical conditions from the upstream to downstream end of each channel due to changes in flow as water moves through the channels. The sampling effort was therefore stratified to ensure that the three Surber samples taken from each channel were taken from the upstream, middle and downstream sections. Three pseudo-replicates were taken from each channel as this was thought to be a sufficient number to assess the invertebrate community, without taking exhaustive samples, which is implausible. All samples were taken using a standard protocol with a Surber Sampler (0.0225 m^2, 300 µm mesh) that was washed between samples to remove invertebrates that could contaminate subsequent samples (Plate 3). The substrate within the 0.0225 m^2 guiding frame of the sampler was disturbed by hand for thirty seconds to a depth of approximately 5 cm so that any sediment and benthic invertebrates present were carried by the water current into the downstream net. Each sample was taken for a standard time (thirty seconds) to ensure that equal sampling effort was used for each sample. The samples taken were double-bagged in polythene bags and fixed by adding a small amount of stream water along with 40% formalin, so that an approximately 4% formalin solution was created.

Plate 3: Surber sampling being carried out on 30/05/06.

Taxonomic Identification

Before taxonomic identification could be carried out, the invertebrates had to be sorted from organic material and sediment. Firstly, the samples were gently washed through a 0.25 mm mesh sieve using tap water to remove excess formalin. Sorting was carried out by placing the remaining material into a white flat-bottomed tray (35 cm x 25 cm), marked with a 4 x 4 grid to aid sorting, filled with a small amount of water to prevent drying. Invertebrates were transferred from the sorting tray into vials filled with a preservative made up of 70% industrial methylated spirit (IMS) and 5% glycerol. In the first set of samples, taken on 30/05/06, there was a superabundance of chironomidae (non-biting midge) larvae, so a sub-sampling procedure was carried out where only the first fifty were transferred into vials, and the abundance of the remaining specimens was estimated without removing them from the sorting tray.

Identification to the lowest feasible taxonomic level was then carried out, using a wide variety of standard invertebrate keys (Edington and Hildrew, 1995; Elliott et al, 1988; Elliott and Mann, 1979; Friday, 1988; Gledhill et al, 1993; Hynes, 1977; Macan, 1977; Nilsson, 1996; Wallace et al, 2003). Where damaged animals, or parts of animals were found, only the number of heads was counted to avoid multiple counting. Invertebrates that had obviously died prior to sampling (e.g. empty snail shells) were not counted. An experienced taxonomist occasionally checked the sorting trays to make sure that invertebrates were not being missed, and also checked that invertebrates were being identified correctly.

Habitat Monitoring

As a consequence of the wide spectrum of habitats discussed in the introduction, streams, or the artificial channels that will be used in this experiment, must be seen as a mosaic of patches of biota, their resources and their habitats, rather than as homogeneous units (Downes et al, 1993; McCormick, 1993). It is for this reason that changes in habitat imposed on each channel by the treatment that it was assigned were observed as the experiment proceeded. Habitat patch dynamics is an effective framework for the study of lotic systems as it integrates fine detail studies with coarser holistic perspectives (Armitage et al, 1995). Patches were recorded at the mesohabitat scale as this has previously been recommended as a useful structural unit to study (Pardo and Armitage, 1997). The accepted definition of mesohabitats is “medium-scale habitats that arise through the interactions of hydrological and geomorphological forces (which may include instream macrophyte growth)” (Wood et al, 1999).

Mesohabitats were selected by eye, with the instream habitat classified on the basis of observable substrate. Eight substrate classifications were used (Table 1, Plate 4).

Table 1: The habitat classifications used for mapping and statistics.

Mapping Classification	Statistical Classification	Details
Gravel	Mineral	Clean gravel
Gravel/algae	Mineral	Clean gravel covered with a thin layer of diatomous green algae
Sand	Mineral	Clean sand
Silt	Silt	Silt with no gravel edges visible
Gravel/silt	Silt	Clean gravel covered with silt, with the edges of the individual gravel particles still visible
Algae	Organic	Filamentous algae with no other substrate visible
Silt/algae	Organic	Filamentous algae with trapped sediment
Macrophyte	Organic	Either *Ranunculus* spp., or *Callitriche*

(a)

(b)

(c)

(d)

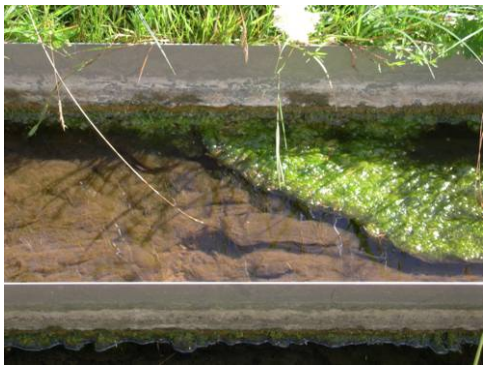


Plate 4: Examples of some of the habitat classifications used: (a) silt, (b) gravel, (c) gravel/algae and (d) algae.

Mapping of mesohabitats was carried out once a week during the experimental period using prepared grids drawn to scale with the dimensions of the channels (0.33 m x 12 m). The mesohabitats were mapped by eye using metre marks, defined by a tape measure, as a drawing aid. It was decided that this method was most applicable to the problem as the channels were narrow, so the channel walls acted as good scaling limits for the observer, and other, more methodical approaches (Wright et al, 1981) would have been too time consuming without yielding more information, as the channels were so narrow.

The mesohabitat maps (Figure 4) were analysed to quantify the proportional cover of each habitat type. This was done by first digitising the maps using a scanner to import .jpeg files into Adobe Photoshop 7. Contrast and brightness were then set to 100% in order to make the pencil drawn lines as clear as possible, before making sure that they were continuous using the black pencil tool in Microsoft Paint. Different habitat categories were then coloured with different shades of grey, with known numbers, using the fill tool, again in Microsoft Paint. Each individual channel was cropped from its surroundings before the Histogram tool of Adobe Photoshop 7 was used to obtain percentage scores for each shade of grey, and consequently, each mesohabitat type.

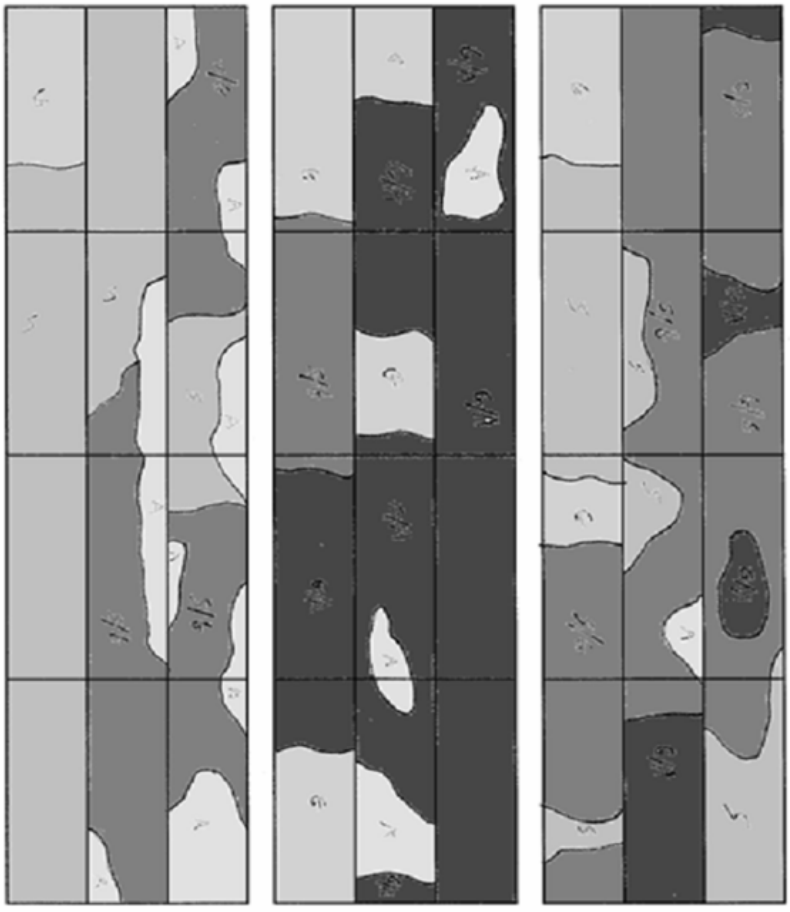


Figure 4: The three habitat maps for block 2 on the 20th June 2006 as an example of the mapping process. Each shade of grey represents a different substrate.

Water Chemistry Monitoring

The pH, conductivity and temperature of the water flowing from each of the twelve channels and the water feeding the channels in the Mill Stream was measured on several occasions. This was necessary in order to ascertain if the flow treatments had any effect on the channel environment other than a physical one, and to find out how well the artificial streams replicated the conditions in the more natural situation of the parent water body, the Mill Stream. These measurements were carried out by collecting water in a sampling pot as it left each of the channels, then placing Hanna Instruments pH and conductivity field meters in the water collected. Temperature measurements are a secondary function of both meters used.

Statistical Analysis

Identification of Potential Indicator Species

Principle Components Analysis (PCA) was carried out on the species data using the software package CANOCO v4.5 (Ter Braak and Smilauer, 2002) to assess the patterns of biotic variation between replicate channels and between sampling occasions, and to determine whether these patterns could be attributed to experimental treatments. PCA is an indirect method of gradient analysis that constructs axes from the total community data whilst assuming a linear relationship between species and their environment (Ter Braak, 1994). The axes that are constructed are latent variables that describe as much of the variation between samples as possible. These can then be compared with known environmental variables, such as flow treatment (Ter Braak and Prentice, 1988). PCA was used because gradient lengths on axes one and two of a preliminary Detrended Correspondence Analysis (DCA) were short, meaning that a linear ordination, rather than unimodal, method was applicable.

PCA was carried out first including species data for all twelve channels on both sampling occasions, and secondly using only the data from the post-treatment samples, after the experimental conditions had been running for six weeks. The data for the three Surber samples taken from a given channel were pooled to form one species list with abundances per channel. It was not necessary to convert the pooled data for each channel into average densities as PCA works with relative abundances. These analyses were performed in order to ascertain whether replicate channels from within a treatment had similar species composition, and to identify any species that responded strongly to a particular treatment, and so could be potential indicator species that should be subjected to more rigorous statistical analysis. PCA has been used previously to identify the species that can be used to make a distinction between environmental conditions, e.g. diatoms in clear water and brown water pools (Van Dam et al, 1981).

To reduce the excessive influence of rare taxa on the analysis, only taxa that occurred in more than 10% of samples were used in PCA. Where all 24 channels were used, only taxa that occurred in three or more channels were included in the analysis. Where12 channels were used, only taxa occurring in two or more channels were included in the analysis.

Confirmation of Indicator Species

For species that were abundant in both the first and second set of samples, a statistical analysis of the effects of the low flow regime on the abundance of existing populations could be performed.

The density of individuals of the species that were abundant in both sets of samples was analysed using a nested repeated measures Analysis of Variance (ANOVA). The nested model was used to account for as much of the variation within a replicate channel as possible to maximise the chances of discovering a significant result. The model used in the statistical programme SAS was: Block Treatment*Position (Treatment). This model was used to test for significant differences between treatments in the change in taxon richness, total density of individuals and density of selected taxa between the two sampling occasions. The model nested the variation between pseudo-replicate samples in a channel within treatment, and also assessed the extent to which the blocks differed in their treatment response. Repeated measures ANOVA was used in order to analyse the patterns of change from initial values to final values between treatments. All data were tested for normality, and appropriately transformed if necessary to meet the assumptions of parametric repeated measure ANOVA. For all ANOVAs, a *P* value of less than 0.05 was chosen as the region in which the null hypothesis (i.e. that there is no significant difference between the density of a species between different values of a factor other than that due to chance) should be rejected.

Analysis of Habitat Changes

Repeated measures ANOVA was also performed on the habitat data obtained over the entire six week experimentation period. Three such ANOVAs were performed, one on mineral substrates (clean gravel, gravel with a coating of diatomous algae and sand), one on silt substrates (silt and visible gravel with a heavy silt covering) and organic substrates (filamentous algae, algae with entrained silt and macrophytes). The habitat types were summed into associated groups (Table 1) with similar characteristics in order to aid the interpretation of any patterns that occurred.

Analysis of Water Chemistry Results

The water chemistry results were also statistically analysed using repeated measures ANOVA to find out if the experimental treatments had any effect on the channel pH, conductivity or temperature. This makes it possible to make a distinction about whether any treatments effects are due to the flow treatments themselves, or due to any changes in water chemistry that they bring about.

Results

Prior to the commencement of the experiment, a total of 8383 individuals across 37 different taxa were sampled, whilst a total of 3325 individual invertebrates across 46 different taxa were found after six weeks of experimental treatment. In total, 53 taxa were found in the two sets of samples. Chironomidae represented 68% of individuals prior to the experiment and 44% of individuals after six weeks of flow manipulation, indicating that the benthic community had developed considerably between the two sampling times. Approximately 17% of individuals were ephemeroptera (mayflies) in the first set of samples, compared to 14% in the second set. Crustaceans increased from 7% of individuals to 24%. Snails were rare on the first sampling occasion (0.0007%) and relatively common by the end of the experiment (2%). Densities of initial assemblages were greater than those of the final assemblages. This was largely as a result of the reduction in numbers of chironomids.

Identification of Potential Indicator Species

The macroinvertebrate community in the channels prior to the experiment was substantially distinct from that after six weeks of flow manipulation treatment (Figure 5a). All twelve pre-experiment sets of samples were positioned to the right of the post-experiment samples in PCA ordination space. The post-treatment samples were all grouped around the left side of the ordination space. There was no overlap between pre- and post-treatment samples along the first and most important ordination axis, which clearly corresponds with the occasion when samples were taken. Taxa that were relatively more abundant in one set of samples than the other have long arrows on the PCA biplot (Figure 5b), as the length of a species' arrow is proportional to the maximum variation in its abundance. Species with short arrows do not vary much across the ordination plot. Taxa such as Chironomidae, *Baetis scambus* group, *Baetis rhodani* (Pictet) and *Brachycentrus subnubilis* (Curtis) were relatively more abundant prior to experimental treatment, whilst taxa such as *Radix peregra* (Muller) and *Antocha vitripennis* (Meigen) were relatively more abundant in the samples taken after flow treatment had been imposed.

When we consider the post-treatment samples separately, we find that the samples are separated according to flow condition along the first ordination axis, showing that flow condition accounts for the greatest proportion of variation (Figure 6a). The low flow channels are on the right, with the control flow channels on the left of ordination space. The moderate flow channels are at the centre of the plot, with two (block 1 and 2) being associated with low flow channels and two (block 3 and 4) being associated with control flow channels. The larvae of the beetles *Nebrioporus depressus elegans* (Panzer) and *Hydrophilidae* sp., associate with low flow channels and could be potential negative indicators. Species that associate with control flow channels, such as the amphipod freshwater shrimp *Gammarus pulex* (Linnaeus), the mayflies *Ephemerella ignita* (Poda) *Caenis luctuosa* group, *Baetis muticus* (Linnaeus) and *Baetis scambus* group, the caddis flies *Hydropsyche contubernalis* (Mclachlan), *Polycentropus flavomaculatus* (Pictet) and Chironomidae sp. could be potential positive indicators (Figure 6b). *G. pulex*, *E. ignita* and *C. luctuosa* gp. all had distinctly higher abundances in control flow channels than low flow ones (Figures 7a, b and c), whilst *N. depressus elegans* larvae were much more abundant in low flow channels than in control ones (Figure 7d), showing why these flow associations were particularly strong.

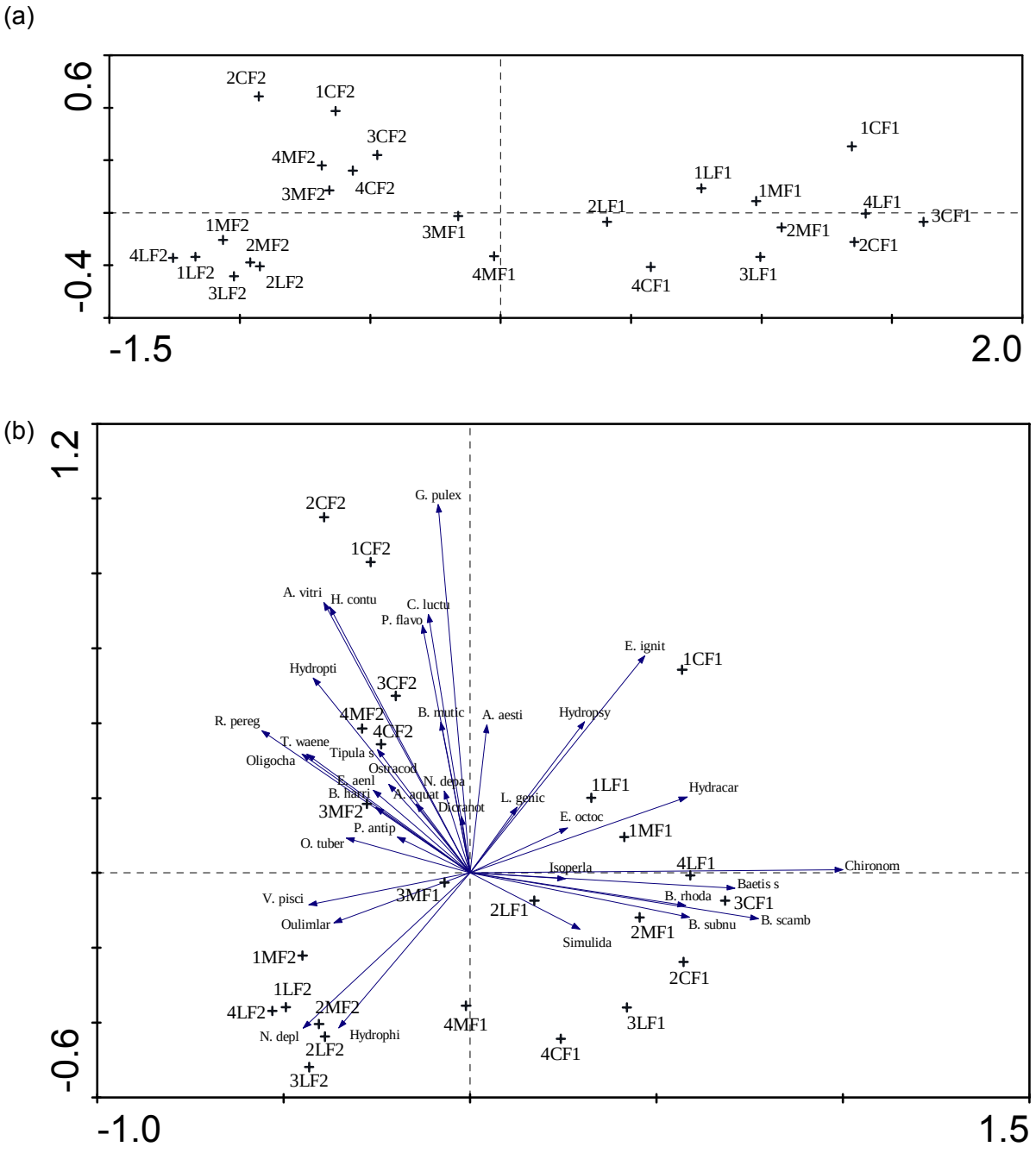
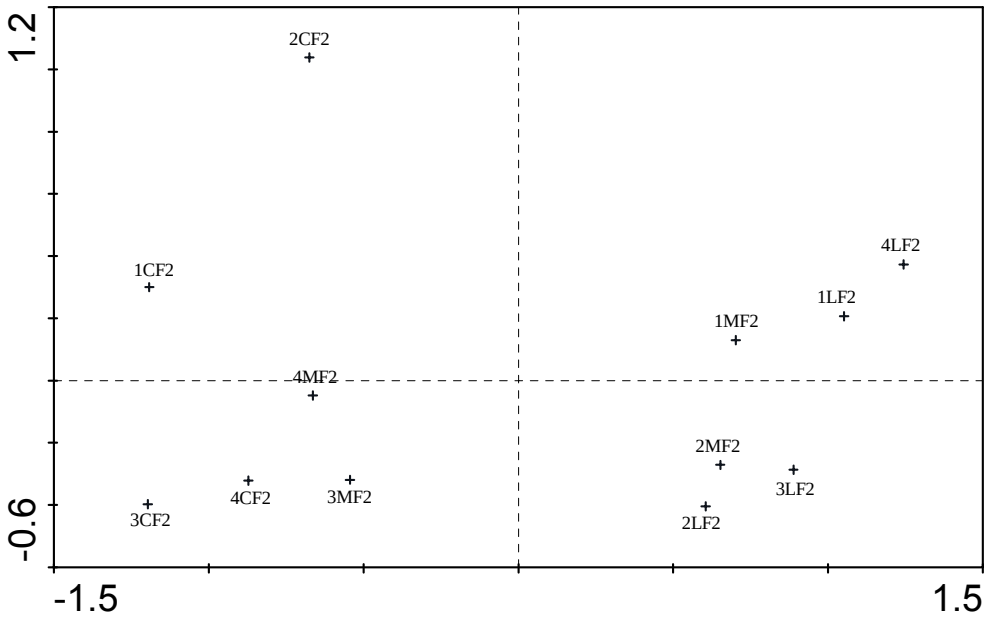


Figure 5: Principle Components Analysis ordination plot of sampling occasions one (e.g. 1CF1) and two (e.g. 1CF2). Plot (a) without species added, and (b) with species arrows added. The first number in each sample represents the block, whilst CF represents Control Flows, MF represents Moderate flows and LF represents Low Flows.

(a)



(b)

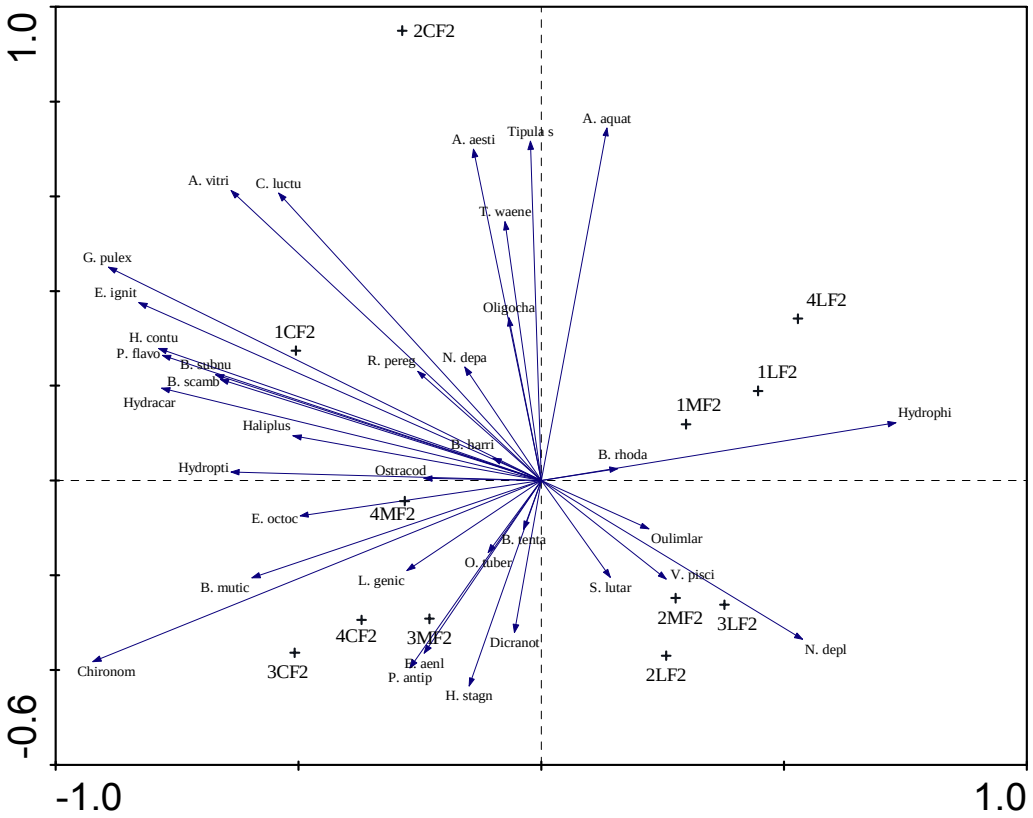


Figure 6: Principle Components Analysis ordination plot of sampling occasion two. Plot (a) without species added, and (b) with species arrows added. The first number in each sample represents the block, whilst CF represents Control Flows, MF represents Moderate flows and LF represents Low Flows.

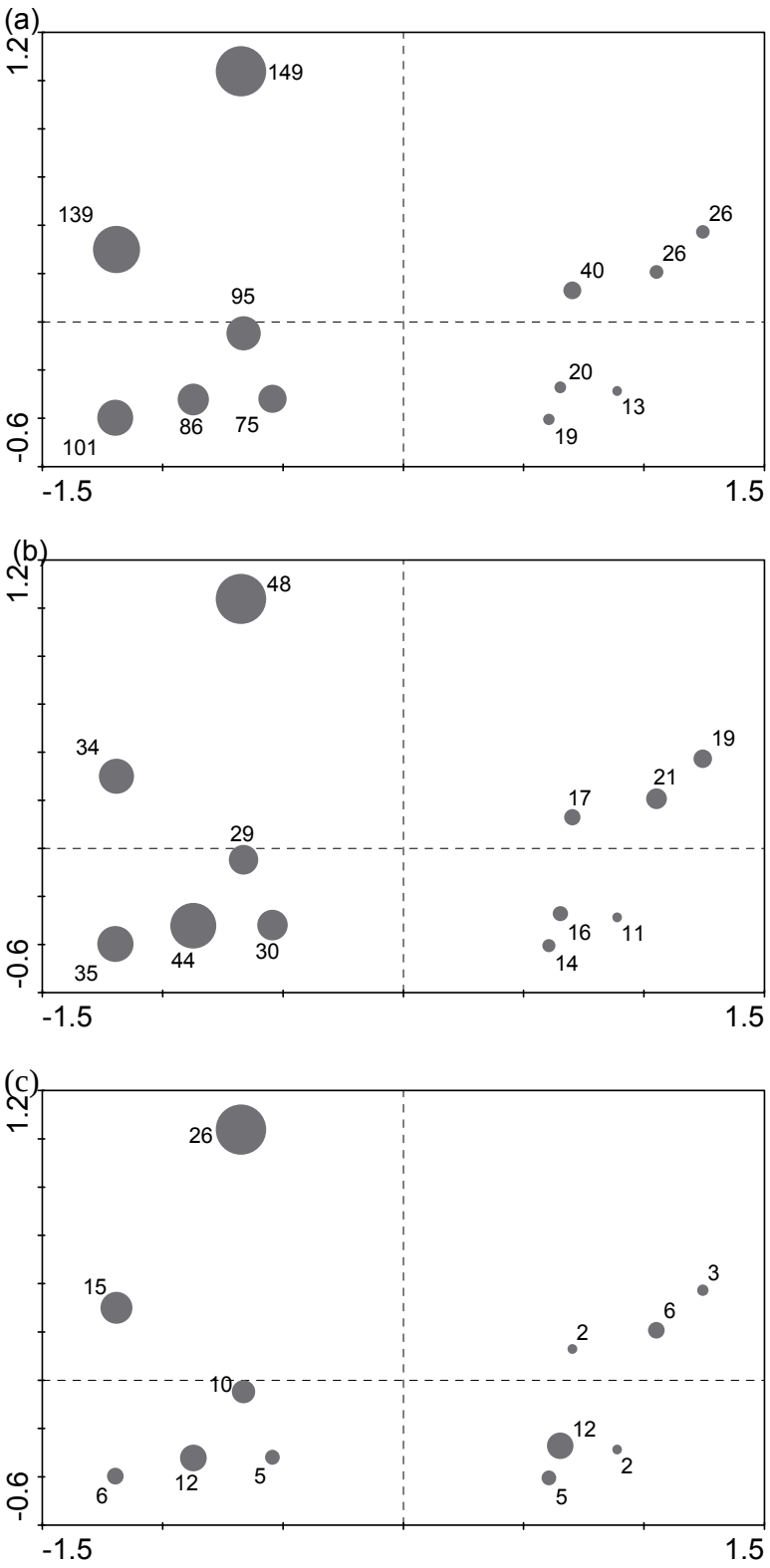


Figure 7: Attribute plots for the abundance of potential indicator species in particular channels after 6 weeks of flow treatment. Low flow channels are on the left and control flow ones on the right. Channel positions in ordination space can be seen in figure 6a. (a) *Gammarus pulex*, (b) *Ephemerella ignita*, (c) *Caenis luctuosa* group and (d) *Nebrioporus depressus elegans* larvae.

(d)

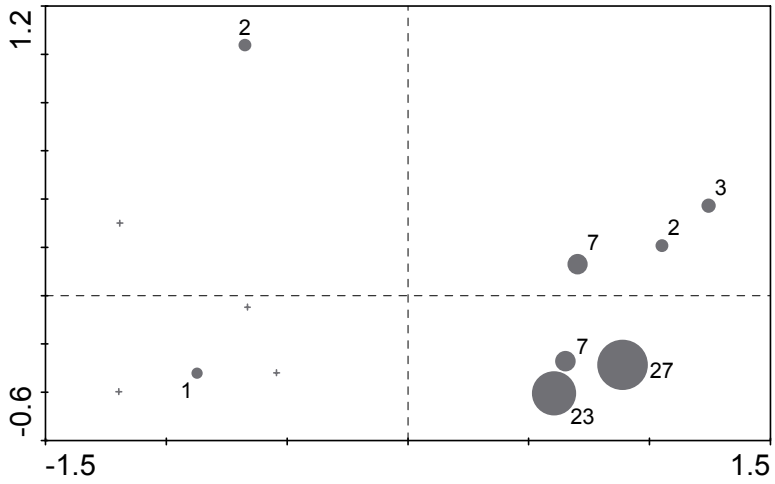


Figure 7 cont'd: Attribute plots for the abundance of potential indicator species in particular channels after 6 weeks of flow treatment. Low flow channels are on the left and control flow ones on the right. Channel positions in ordination space can be seen in figure 6a. (a) *Gammarus pulex*, (b) *Ephemerella ignita*, (c) *Caenis luctuosa* group and (d) *Nebrioporus depressus elegans* larvae.

The division of the moderate flow channels between control and low flow treatments in terms of species composition appears to have been on the basis of the number of invertebrate individuals (Figure 8). The moderate flow channels in blocks one and two have a similar number of individuals to the low flow channels, whilst those in blocks three and four have a similar number of individuals to the control flow channels.

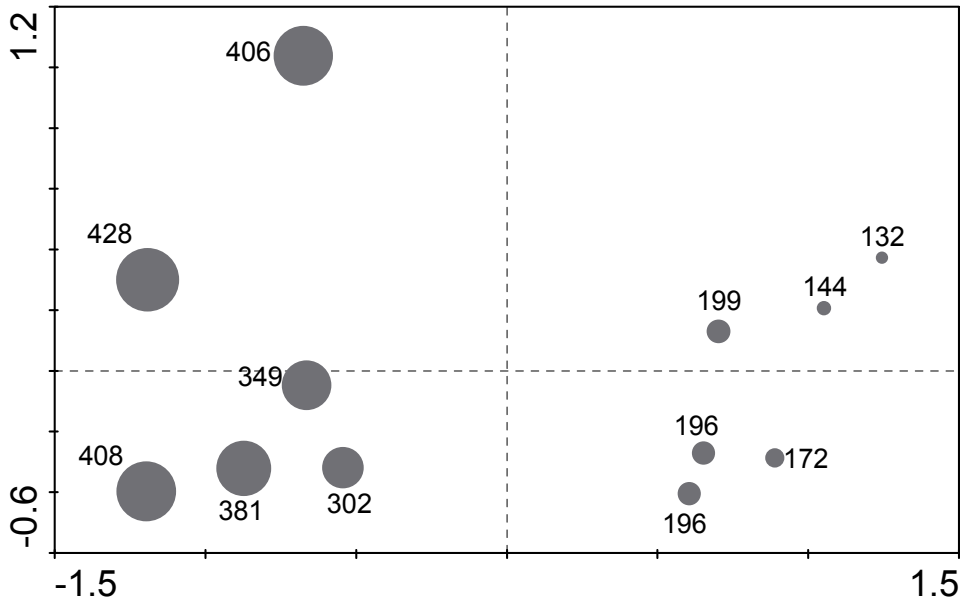


Figure 8: Attribute plot of number of invertebrate individuals per channel. Channel positions in ordination space can be seen in figure 6a.

Confirmation of Indicator Species

Repeated measures ANOVA can only be performed on data that are normally distributed. This unfortunately ruled out the possibility of statistically testing *Nebrioporus depressus elegans* larvae for indicator properties as none where present in the first set of samples, and they were only present in 15 out of 36 samples on the second sampling occasion. *Caenis luctuosa* group, another species that had been identified as a possible indicator of low flow conditions using PCA could not be analysed using ANOVA as although is was present in 27 samples on each sampling occasion, the data obtained could not be normalised using any common transformation. The following repeated measures ANOVAs were performed:

Taxon richness

The patterns of change in taxon richness between samples taken before and after flow treatment were not significantly different between treatments ($F = 1.46$, $P = 0.2527$). Tukey's Studentized Range post-hoc multiple comparison tests showed that there was no significant difference in taxon richness between treatments prior to the experiment, but there was a treatment effect after six weeks of flow manipulation, with the taxon richness being significantly lower in the medium and low flow channels than in the control flow channels (Figure 9). This was confirmed by an ANOVA performed solely on the post treatment set of samples that showed a highly significant treatment effect on taxon richness ($F = 8.18$, $P = 0.0019$).

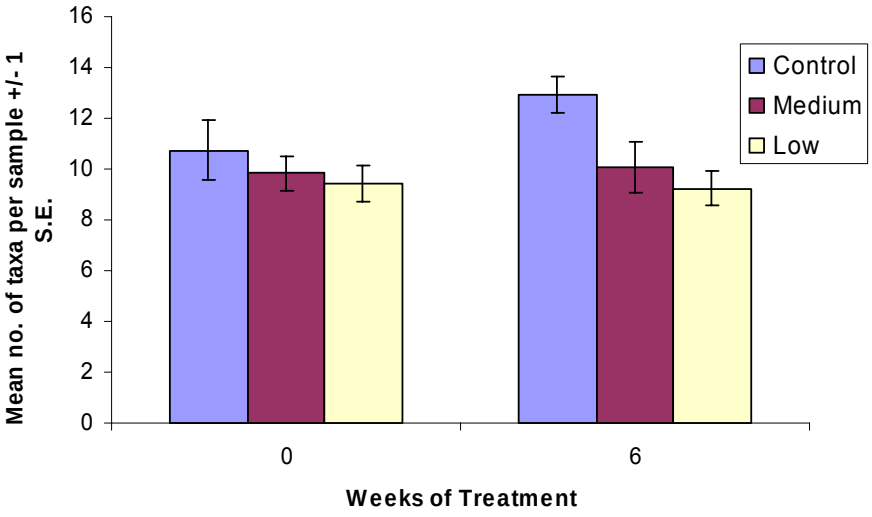


Figure 9: Mean taxon richness per sample in the two sample sets.

There was a significant effect of the longitudinal position within a channel from which a sample was taken on the taxon richness in both the samples taken prior to treatment and those taken post-treatment ($P = 0.0330$ and $P = 0.0150$). This effect was however different on the two sampling occasions, giving a significant ($F = 3.83$, $P = 0.0081$) interaction effect between sampling occasion and longitudinal position in a channel. The top position in the channel went from being the most taxon rich to the least taxon rich, whilst the bottom position went from least to most taxon rich.

Individual Density

There was no significant difference between treatments in terms of the patterns of change in the total density of individuals from the pre- to post-treatment samples (F = 2.86, P = 0.0770). The occasion on which the samples were taken had a highly significant effect (F = 97.83, P = <.0001). This was because there were significantly fewer individuals per sample in the post-treatment samples than in the pre-treatment samples (Figure 10). The rate of reduction in the number of individuals per sample was not significantly different between the treatments. Tukey's post-hoc test did however show that there was a treatment effect (F = 11.09, P = 0.0004) in the second set of samples with the number of individuals per sample being significantly lower in the medium and low flow channels than in the control flow channels. The expected ranking, with fewest individuals in low flow treatments, had developed after six weeks of treatment.

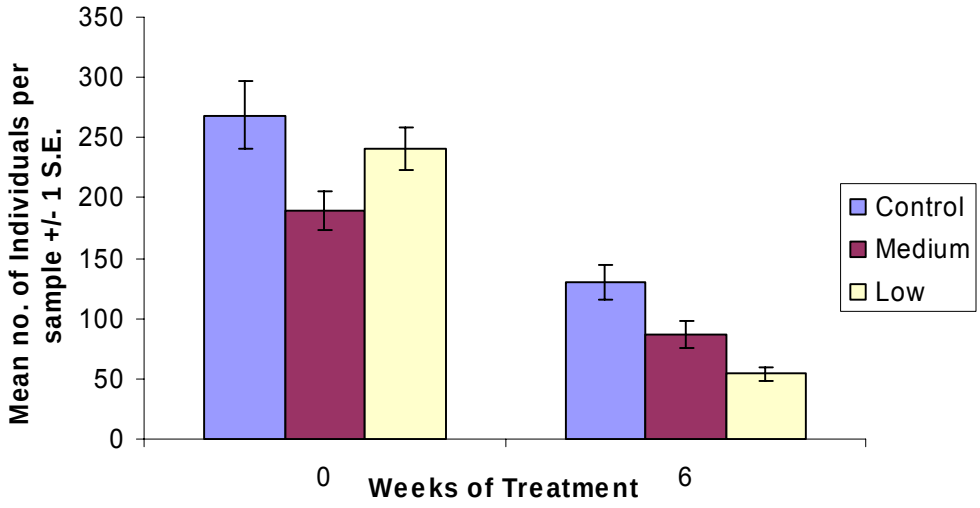


Figure 10: Mean number of individuals per sample in the two sample sets.

The results for the density of chironomids were very similar to the above results for the density of all individuals (Figure 11). This is because chironomids were the most dominant taxa, contributing 68% of individuals in the first set of samples and 44% in the second set. The difference in pattern of change between treatments was insignificant (F = 2.20, P = 0.1327), but there was a significant time effect (F = 155.17, P = <.0001) on the number of chironomids per sample due to this reduction. Again, the number of individuals decreased by the greatest amount in the low flow channels, so that a ranking from control, to medium, to low flows was established.

In order to establish whether the pattern for the number of chironomid individuals was masking a significant pattern of change due to treatment in individuals of all other taxa identified, a repeated measures ANOVA was performed on the overall number of individuals minus chironomids. The change in numbers of individuals of all taxa other than chironomids was significantly different between flow treatments (F = 3.69, P = 0.0402) and the occasion on which the samples were taken had a significant effect on this number (F = 5.42, P = 0.0287). The density of non-chironomid individuals in control flow channels decreased slightly, as did the density in moderate flow channels, whilst the density in low flow channels

decreased significantly. The ranking from control flows to low flows was established, and the changes were significantly different between treatments (Figure 12).

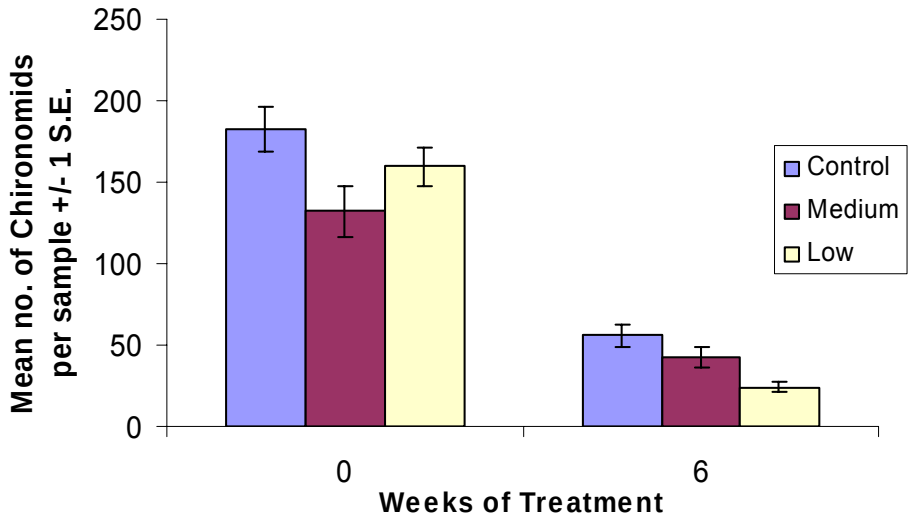


Figure 11: Mean number of chironomids per sample in the two sample sets.

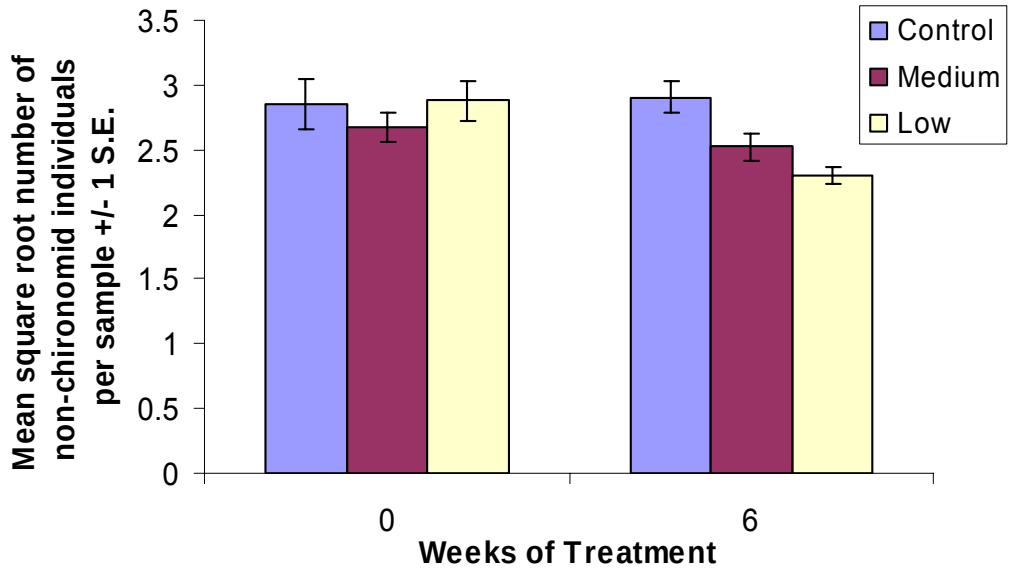


Figure 12: Mean square root number of non-chironomid individuals in the two sample sets.

Ephemerella ignita

The pattern of change in numbers of *E. ignita* per sample was not significantly different between treatments to the 5% level, but there was significance at the 7% level ($F = 3.02$, $P = 0.0678$). This reflects the fact that control flow channels saw an increase in this species, whilst medium flow channels saw a slight decrease and low flow channels a steep decrease after six weeks of flow treatment (Figure 13). As expected, there was no treatment effect on the number of *E. ignita* per sample before the treatments had been imposed ($F = 0.06$, $P = 0.9447$), but treatment had a significant effect on the number of *E. ignita* per sample after

treatment had been imposed for six weeks (F = 7.71, P = 0.0026). Tukey's post-hoc test confirmed that there was a treatment effect in the second set of samples with the number of individuals per sample being significantly lower in the medium and low flow channels than in the control flow channels.

Longitudinal position within a channel had a significant effect on the number of *E. ignita* per sample in the set of samples taken before treatment was imposed (F = 4.35, P = 0.0041) with the density recorded being lower in the most downstream samples. No position effect was seen in the post-treatment set of samples for *E. ignita* (F = 1.00, P = 0.4461).

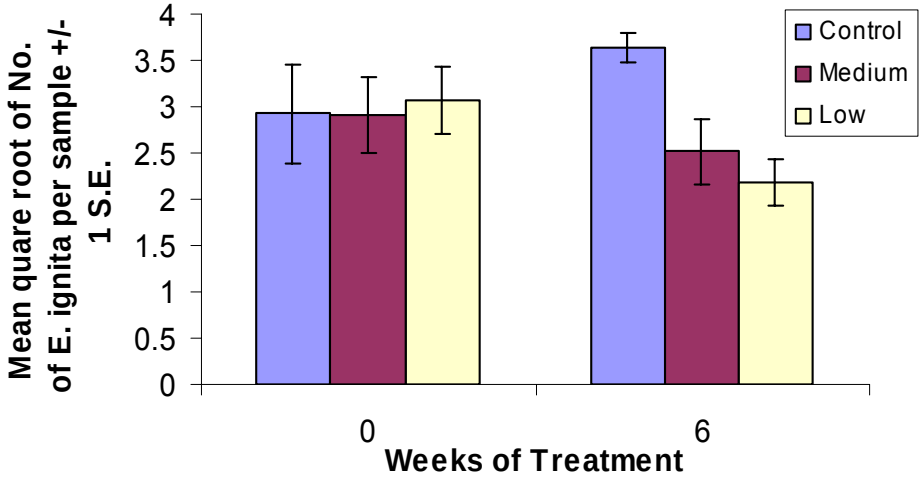


Figure 13: Mean square root abundance of *E. ignita* in the two sample sets.

Gammarus pulex

The patterns of change in the number of *G. pulex* per sample from pre- to post-treatment were significantly different between treatments (F = 4.79, P = 0.0177). The ideal pattern for species that acts as a positive indicator of low flow conditions was seen. The number of *G. pulex* increased in control channels, stayed roughly the same in medium flow channels and decreased in low flow channels. The flow treatment had a significant effect on the number of individuals per sample in the set of samples taken after six weeks of flow manipulation (F = 3.98, P = 0.0322). The number of individuals in low-flow samples (7 per 0.00225 m^2) is significantly lower than the number in samples taken from control flow channels (40 per 0.0225 m^2) (Figure 14).

In a similar fashion to the results seen for *E. ignita*, there was a significant longitudinal position effect in number of *G. pulex* in the set of samples taken before flow treatment was imposed (F = 3.96, P = 0.0068). Density was lowest in the most downstream samples. Again, no position effect was seen in the second set of samples for *G. pulex* (F = 1.49, P = 0.2248).

Oligochaeta sp.

The flow treatment imposed did not have a significant effect on the pattern of change in numbers of oligochaete worms per sample (F = 0.06, P = 0.9427). There was also no statistically significant treatment effect on the density of oligochaete worms in either set of samples. There was a significant difference in the number of oligochaetes per sample between the set of samples taken prior to treatment, and the set taken after six weeks of

treatment (F = 5.89, P = 0.0231). There were more oligochaete worms in the second sample set than the first (133 to 207) (Figure 15). The rate of increase was similar in all treatments, so flow treatment had no effect. There was no block effect on the number of oligochaetes per sample in the pre-experiment set of samples (F = 1.34, P = 0.2838), but there was a significant block effect in the post-experiment sample set (F = 3.06, P = 0.0476). This was a result of block 3 having about half (1.5 average) as many oligochaetes as block 4 (2.7 average). No other statistical analysis showed a block effect.

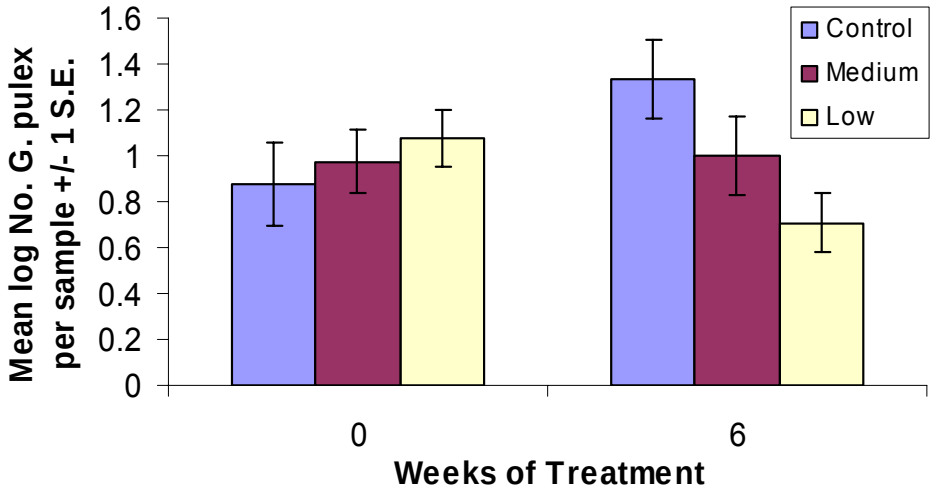


Figure 14: Mean log density of *G. pulex* in the two sets of samples.

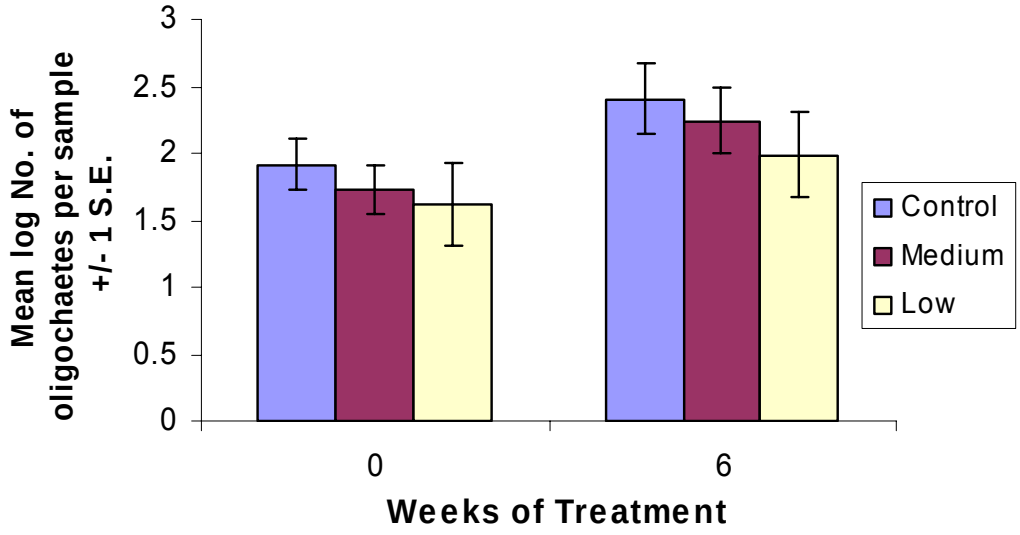


Figure 15: Mean log density of oligochaetes per sample in the two sample sets.

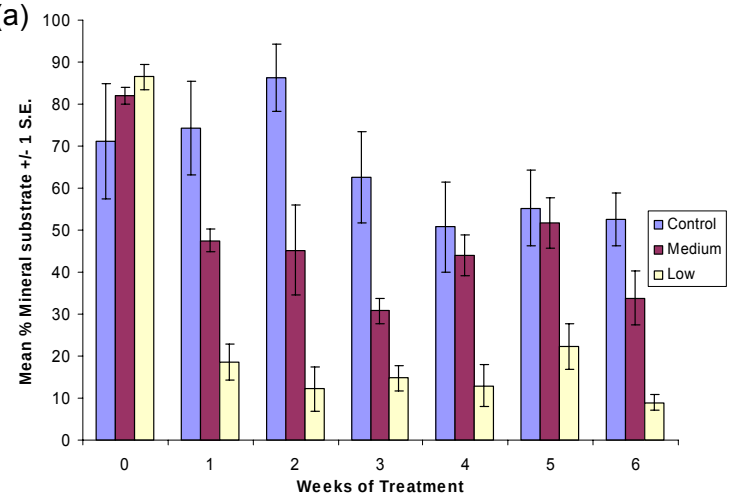
Habitat Responses

The experimental treatments clearly had an impact on the habitats present in the artificial channels. Plate 5 is a photo taken of block 2 at the end of the six week experiment that clearly demonstrates the impacts of the flow treatments that were imposed. The control channel in the centre has remained as clean gravel, with a thin cover of diatomous algae, the moderate flow channel on the right has a thin coverage of silt, and the low flow channel on the left has a think cover of silt, and plenty of filamentous algal growth.

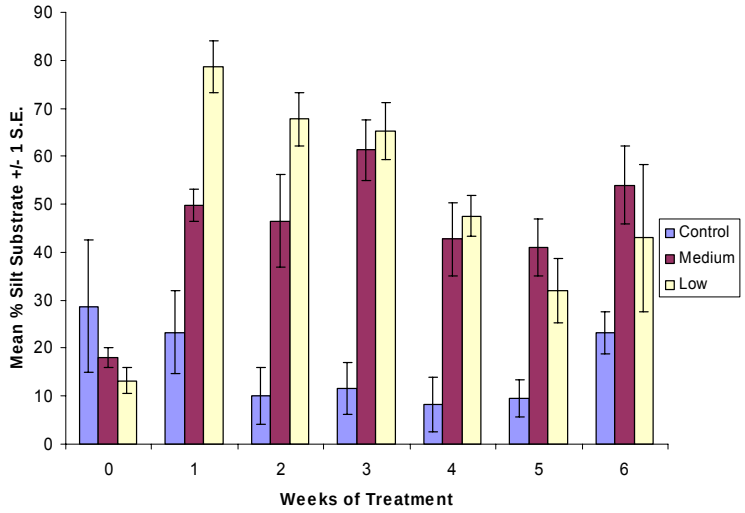
Plate 5: An illustrative photo taken on the 11/07/06 of block 2. From left to right, the treatments are low flow, control flow and moderate flow.

There was a significant difference in the patterns of change of mineral substrate coverage between the three flow treatment levels (F = 5.76, P <0.0001). There was also a highly significant time effect on mineral substrate coverage (F = 21.43, P <0.0001). This time effect was a result of the mineral habitats dropping in all channels over the six week period, due mainly to the encroachment of silt and the arrival flora that were not present before treatment. As percentages were used, the results must be considered together (Figure 16). One substrate cannot increase without another decreasing. There was a significant treatment effect as the proportion of mineral substrates decreased in all treatments, but it decreased significantly more in low flow channels, so that a ranking was established by the end of the first week of treatment, with low flow channels having least clear mineral substrate and control channels having most. From week two of habitat recording onwards, the low flow channels had significantly less mineral habitats than the control flow channels.

(a)



(b)



(c)

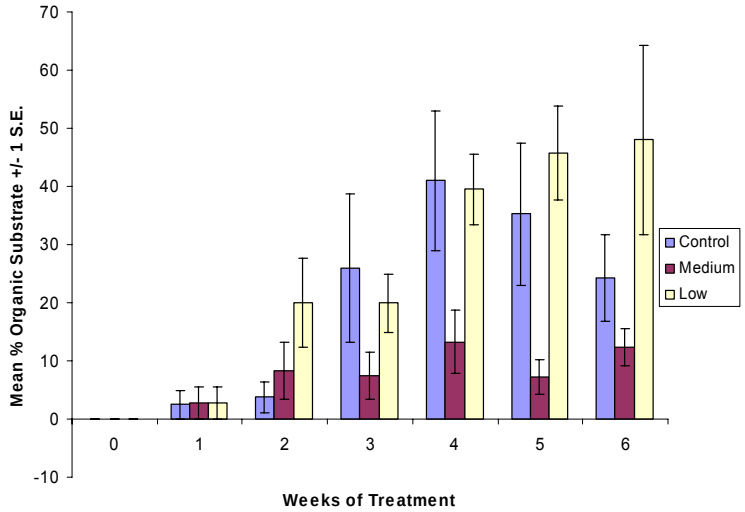


Figure 16: Mean habitat data for the six week experimental period. (a) mineral habitats, (b) silt habitats and (c) is organic habitats.

The silt habitats classification also showed a treatment effect on the changes in substrate composition as the experiment proceeded (F = 6.18, P <0.0001), as well as a significant time effect (F =9.95, P <0.0001). These significant effects were seen because both low and medium flow channels showed an increase in silt substrate over the experimental period, whilst control channels showed a slight decrease. The average silt coverage in low-flow channels rocketed from 13.25% before treatment to 78.75% after one week of treatment. On week two of monitoring the three treatments showed significantly different silt coverage, so that the hypothesised ranking with most silt in low flow channels was established. This ranking had disappeared after six weeks of treatment when medium flow channels showed the highest silt coverage. Control channels did generally have significantly lower silt coverage than the other two channels. Interestingly, all three treatments saw an approximately equal increase in silt substrates between week six of measurements and week seven.

Observation of the artificial channels showed that filamentous alga was the dominant type of organic habitat. A few macrophytes of the species *Ranunculus* and *Callitriche* were also observed, but the experimental period probably was not sufficient for extensive macrophyte establishment. Macrophytes and filamentous algae did not colonise the artificial channels until week three or four of the experiment, so the values for the first four sets of measurements were too low to achieve a normal distribution. To counteract this problem, the first four sets of organic habitat data were averaged so that ANOVA could be performed. The organic coverage increased significantly (F = 9.44, P = 0.0006) in all treatments, as it started from zero. The pattern of change in organic habitat coverage was not significantly different between flow treatments (F = 2.12, P = 0.1018) as there was so much variation within each treatment that the treatments were not significantly different on most weeks. The block in which a channel was situated may have been a more important factor governing the flora coverage than experimental treatment, although there was no overall block effect of the percentage cover of organics (F = 3.67, P = 0.0824). However, the organic coverage of blocks three and four was significantly different after five weeks of treatment, and showed a big, but not significant difference after six weeks. Block three had more organic substrate than the other blocks on all recording occasions.

Water Chemistry

The mean water pH, conductivity and temperature within the artificial channels were similar to that of the parent water body, and variation in these characters clearly correlates well with the Mill Stream (Table 2). There was no significant effect of flow treatment on the conductivity in the channels (F = 2.43, P = 0.1684) and no significant treatment effect on water temperature (F = 0.90, P = 0.4564). There was however a treatment effect on the pH of the water in the experimental channels (F = 14.38, P = 0.0051). This was a result of the pH in the low flow channels being significantly higher than the pH in the other two treatments on three of the four occasions on which monitoring took place. The low flow treatment channels had the highest pH on all four sampling days.

Table 2: Summary of the water chemistry results obtained from the 12 experimental channels and the Mill Stream on four separate monitoring occasions. Figures are shown with +/- 1 S.E.

Date:	27/06/2006	Conditions:	Overcast, morning
	pH	Conductivity (µSv)	Temperature (^{o}C)
Control	8.14 ± 0.010	524.8 ± 1.19	14.2 ± 0.115
Medium	8.14 ± 0.014	524 ± 0.82	14.1 ± 0.029
Low	8.24 ± 0.037	523.8 ± 0.73	14.3 ± 0.047
Mill Stream	8.13	520	14.3

Date:	03/07/2006	Conditions:	Very Sunny, afternoon
	pH	Conductivity (µSv)	Temperature (^{o}C)
Control	8.58 ± 0.010	524.5 ± 1.11	21.6 ± 0.120
Medium	8.58 ± 0.003	522.8 ± 0.55	21.5 ± 0.087
Low	8.59 ± 0.011	517.5 ± 3.04	21.6 ± 0.202
Mill Stream	8.56	520	21.7

Date:	07/07/2006	Conditions:	Overcast, morning
	pH	Conductivity (µSv)	Temperature (^{o}C)
Control	8.17 ± 0.017	543.5 ± 3.35	17.5 ± 0.207
Medium	8.17 ± 0.007	545.5 ± 1	17.3 ± 0.047
Low	8.25 ± 0.019	544.3 ± 0.99	17.4 ± 0.067
Mill Stream	8.14	544	17.4

Date:	11/07/2006	Conditions:	Sun/Cloud, morning
	pH	Conductivity (µSv)	Temperature (^{o}C)
Control	8.18 ± 0.011	528.3 ± 1.52	17.1 ± 0.141
Medium	8.18 ± 0.006	529.8 ± 2.72	17.1 ± 0.191
Low	8.31 ± 0.050	526 ± 4.19	17.3 ± 0.271
Mill Stream	8.16	526	17.3

Discussion

Invertebrates

The results of this experiment have, to a varying degree, suggested that the mayflies *Ephemerella ignita* and *Caenis luctuosa* group, *Baetis* mayflies of the species *muticus* and group *scambus*, Chironomids, and caddis flies such as *Polycentropus flavomaculatus* and *Hydropsyche contubernalis* could all act as positive indicators of low-flow conditions in chalk streams as they all associated strongly with control flows. *E. ignita* and *G. pulex* have been most strongly confirmed as positive indicators of low flows. Beetle larvae, particularly those of the species *Nebrioporus depressus elegans* and family Hydrophilidae, could act as negative indicators of low flows as they associated strongly with low flow samples after six weeks of experimental flow treatment. Reduced abundance of invertebrate individuals could also be used to indicate low flows, as could a decline in taxon richness. The hypothesis that channels with the highest discharge should have the most diverse invertebrate fauna was confirmed.

There was greater variation between the samples taken prior to treatment and those taken after six weeks of treatment than that seen between the different treatments after six weeks of treatment. This may be because the channels were not fully colonised after the colonisation period. The invertebrate community was still developing, with novel species arriving. Species such as the *Baetis* mayflies that were very common in the first set of samples and not in the second may have completed the aquatic stage of their life cycle and moved on.

By the end of the experiment, the lower flowing channels supported less taxa, whilst the highest number of taxa were recorded in the control channels. This decline in the number of taxa under low flow conditions has been seen in previous experiments that focussed on low flows resulting from abstraction (Bickerton et al, 1993) and in experiments based on flow manipulation (Armitage, 1995). These results also correspond with those of Pinder et al (1987) who found that the greatest diversity was found in gravel as opposed to soft sediment or macrophyte habitats. Slower flowing sections may have been less diverse because sediment deposition is more prevalent. This would have filled the interstitial spaces, reducing the range of habitats available and hence the invertebrate diversity. The deposition of sediment must be the most important factor in the reduction of habitat diversity as, unlike experiments using natural streams, the artificial channel design meant that channel width was not reduced, and whilst depth was reduced by low flow conditions, or increased by large stands of filamentous algae, this is thought to be of minor importance in itself, as the sampling method aimed for benthic invertebrates. The position effect seen in the data for taxon richness indicates that there may have been a gradient within the channels, as predicted. The expected result was seen in the first set of samples, but the second set showed that the bottom positions had the most diverse invertebrate community. This could result from species moving down the channel as time progressed.

The reduction in the number of individuals per sample may to a large extent have been mediated by the reduction in chironomid density. There were far fewer chironomids in the second set of samples than in the first, perhaps due to a more complete community development, meaning that there were more predators of chironomids and more competitors for their ecological niche, or as a result of chironomids having completed the aquatic stage of their life cycle. However, this pattern is unexpected, as chironomids tend to be ubiquitous and increase in number as time passes. They are a well known opportunist group (Cowx et

al, 1984) and tend to be among the first colonisers after a disturbance (Harrison, 1966) as the adults are less discriminating in their oviposition than other species. Previous studies have suggested that there is no definitive relationship between chironomid abundance and flow at this low level of taxonomic resolution (Extence, 1999). The results from this investigation however show that chironomids decreased significantly more in low flow channels than in control and moderate flow channels. This may be because chironomid larvae do well when there is a good supply of diatoms (Wright and Symes, 1999), which extensively covered the gravel in the control channels.

The number of individuals of all taxa other than chironomids stayed roughly the same in control flows and decreased significantly in low flow conditions. Cowx et al (1984) found that drought in a Welsh stream caused a reduction in invertebrate numbers to 40% of normal levels. This reduction was probably due to the encroachment of silt that would fill the interstitial spaces and therefore reduce the total amount of habitat space available, so that fewer individuals were supported. Stable substrates, such as gravel are thought to support a high invertebrate density (Erman and Ligon, 1988).

E. ignita was classified as being associated with moderate to fast flows for the LIFE index that seeks to determine the flow level using the invertebrate community (Extence et al, 1999). This corresponds with the results obtained here. The impact of reduced flow may be due to the feeding nature of *E. ignita*. It is a collector-gatherer that gathers fine organic detritus (Elliott et al, 1988). Lower flows may mean that less suitable detritus is carried, so feeding is less efficient. *E. ignita* nymphs may also have been more abundant in control channels as it has been experimentally demonstrated that those fed on diatoms had higher growth rates and adult fecundity than those fed on detritus (Rosillon, 1988). The position effect in the first set of samples possibly occurred because *E. ignita* is a species that is poor at swimming (Elliott et al, 1988). The flow was less strong in the channels than in the Mill Stream, so the mayflies would have been deposited in a similar fashion to sediment, when the settling velocity exerted upon them exceeded the velocity of the instantaneous vertical velocity components in turbulent flow. Most *E. ignita* individuals would have been deposited at the top of the channels, with progressively fewer deposited as the water flowed down them. The lack of a similar pattern in the second set of samples is probably because colonisation had been more complete, with more time for this species to disperse evenly throughout the channels, through the movement of water and the movement of individuals to exploit the maximum available habitat. The same pattern was seen for the amphipod *G. pulex*, for similar reasons, with position being determined by water currents.

G. pulex could act as a positive indicator of the low flow conditions that are likely to result form continued climate change as it is relatively intolerant of low flow conditions. *G. pulex* is well-known as a widespread species that is abundant in many types of standing and flowing water. It is however known to prefer well oxygenated waters (Gledhill et al, 1993), so its preference for the control channels may result from better mixing of oxygen into the water due to increased turbulence. The preference of this species for control channels may also result from its feeding behaviour. It is a known grazer of algae, which feeds by shredding, so it may have been restricted where there was heavy silt coverage that made algae inaccessible (Gledhill et al, 1993). It is a species classified as being associated with moderate to fast flows according to the LIFE index (Extence et al, 1999). *G. pulex* is known to be found in highest abundances on gravel as opposed to silt substrata (Armitage and Cannan, 2000). In marked contrast to all of these findings, it has been suggested that low flows can be beneficial to *G. pulex* (Wright, 1992), although this opinion is not prevalent.

The larvae of the *Caenis luctuosa* group (*Caenis luctuosa* and *Caenis macrura*) are collector-gatherer feeders, so they may do poorly in lower flowing channels where less detritus is being carried so there is low food available, giving the results seen here. However it has been suggested that they have a preference for living in silt as they are adapted for such habitats by way of features such as their gill covers (Elliott et al, 1988). The observation that they are typically associated with slow flowing and standing waters (Extence, 1999) is not supported by the results obtained in this experiment.

The larvae of caddis flies of the family Hydropsychidae have a known preference for coarser substrates (Bickerton et al, 1993), so it is not surprising that they are seen in greater abundance in control channels where the high discharge provided flushing flows that prevented sedimentation. They are filter feeders (Wright et al, 2003) so they probably do better where high current velocities make filter feeding more effective (Wright and Symes, 1999). In contrast, it has previously been suggested that the other caddis fly species that showed a strong association with control channels, *Polycentropus flavomaculatus* may have a preference for fine substrates (Bickerton et al, 1993) so its preference for the control flow channels that had relatively stable, clear gravel substrates is surprising. *P. flavomaculatus* is however a predator (Pardo and Armitage, 1997) and so may have shown a preference for control channels as there was more prey available. Both of these caddis fly species have been classified as taxa primarily associated with moderate to fast flows for the LIFE index (Extence et al, 1999).

Nebrioporus depressus elegans larvae may act as negative indicators of low flow conditions as, whilst the adults are well adapted for swimming with a streamlined shape and well adapted leg movements, the larvae are not good swimmers. Many Dytiscidae (diving water) beetle species have, as a result, been noted to prefer the more stagnant, sluggishly flowing parts of lotic systems where silt mesohabitats are more common (Nilsson, 1996; Extence, 1999; Wood et al, 1999). *Nebrioporus depressus elegans* has itself been named as a species that prefers slow flows, or even lentic conditions (Extence, 1999; Wood and Armitage, 2004).

There was no flow treatment effect on the density of oligochaete worms. There was also no ranking change between treatments. This lack of any interesting result could be because such a low taxonomic resolution was used. Separate species may have responded to the treatments, but the taxa as a whole did not show a relationship between abundance and flow. It has been suggested previously that the number of oligochaetes is dependent on the occurrence of macrophytes (Armitage, 1995), so the lack of macrophytes in this experiment may have meant that there were insufficient suitable habitats for significant oligochaete colonisation.

The attribute plot for the number of individuals per sample (Figure 3.4) shows that two moderate flow channels were similar to low flow conditions, whilst two were similar to control flow conditions. This suggests that there was insufficient discrimination between the three treatments, and that for interpretation of other statistical analyses it may be better to look at the differences between low and control flow treatments, with less focus paid to moderate flow treatments as they may not be significantly different from one of the other two treatments. More easily controlled flow inlets and more accurate discharge measuring equipment may make it possible to create a distinctly unique moderate flow treatment in future investigations.

Habitat Changes

The six weeks of experimental conditions have caused great variation in substrate and habitats available. There were different patterns of change in percentage cover of mineral habitats in different treatments, with the proportion of mineral habitats in low flow channels decreasing by the most over the experimental period. This reduction in mineral habitats in low-flow channels was largely driven by the encroachment of silt and the arrival of filamentous algae. As expected, low-flow channels had a great deal more settled silt than the control channels after the experimental treatment had been imposed. Armitage (1995) gained similar results when manipulating flow. In slow-flow zones the gravel was overlain with silt and in fast flow zones the fine particles were washed away leaving clean gravel. The significant time effect seen for the silt habitats category was expected, as there was very little silt in the channels at the start of the experiment, so a significant amount of deposition was expected. This was probably a result of the lower water velocities in medium and low flow channels relative to the velocity in these channels during the colonisation period. There was insufficient turbulence to keep sediment suspended in the water, so it was deposited. The upturn in proportion of silt substrate seen in the final week of the experiment in all treatments was most likely the result of a spell of heavy rain that would have washed more sediment into the Mill Stream and consequently into the experimental channels.

Although no significant treatment effect was seen for organic habitats, three out of the four low flow channels clearly had more organic substrate than the other treatments after six weeks. This may show that algal establishment is favoured in low flow conditions, as establishment is easier with less force exerted on propagules, but this has not been statistically proven in this investigation. The filamentous alga in the experimental channels was most probably *Cladophora*, which is known to be favoured by slower flowing conditions (Armitage, 1995).

The algal coverage in the various blocks was visibly different. This may have confounded any treatment effects for both the habitat and invertebrate data. The large amount of algae in some channels would have had two main effects that reduced the chances of clear patterns in invertebrate communities relating to flow regime being observed. These are: 1) damming the channels, so reducing flow in channels that may not have been low flow channels in the experimental design, and 2) Affecting the second set of invertebrate samples with a ‘net’ effect. This ‘net’ effect occurs where algae acts in a net-like fashion to trap invertebrates, giving higher abundances than would be present on clean gravel. This was a problem when clumps of algae were inadvertently passed into the Surber sampler. These algal affects would not be a problem if the algal coverage clearly correlated with flow regime, they would be part of the suite of factors created by the regime that brings about the community results. However, filamentous algae were in statistical terms randomly distributed between treatments and blocks. An option considered was to clear away the algae that established on the vertical channels walls, which are not present in natural chalk streams. This was not carried out as it was decided that to remove algae would cause an excessive divergence from the natural situation.

The fact that the best gradient from control to low flows was seen in the results for the proportion of mineral substrates suggests that looking at this factor may be the best way of observing physical changes in streams that result from varying flow regimes. The numbers of all of the taxa that responded strongly to the flow treatments also correlated well (In Spearman’s Rank Correlation and Pearson’s Correlation where appropriate, results not shown) with the percentage coverage of mineral habitats and not with coverage of silt or organic substrates. However, it is important to remind the reader that any ranking seen in this

investigation from low to medium to control flows is slightly unreliable, as the invertebrate results suggest that separation of medium treatments from the other two was not definitive.

Water Chemistry

The water chemistry results show that the different flow regimes had no effect on the conductivity and temperature of water in the experimental channels, but treatment did have a significant effect on water pH. The pH was significantly higher in low flow channels than in the channels of the other two treatments on three out of four sampling occasions. The only day on which pH was not higher in low flow channels relative to the other channels was when the pH was elevated in all channels as a result of an extended dry period when a higher proportion of the river water was aquifer derived and therefore more alkaline. This pattern was probably a result of there being substantially more algae in low flow channels when the measurements were taken. These algae would have been photosynthesising to gain nutrition, so carbon dioxide would have been removed from the water and oxygen produced. Carbon dioxide is an acidic gas, so removing it from the water would have reduced the concentration of hydrogen ions, and so increased the pH.

The results also show that the artificial channels provided a good approximation of the conditions seen in a more natural stream such as the Mill Stream. Under all of the climatic conditions during which sampling was carried out, the three factors measured were always close to the values obtained for the Mill Stream, despite the great variation between the results obtained on the different days. The water in both the Mill Stream and artificial channels was cooler in the morning and under cloudy conditions than when it had been exposed to strong sunlight for a whole day. The water pH was alkaline as a result of its groundwater source, and highest following consistent dry periods, when more river water was aquifer derived. Conductivity was highest following a period of rainfall. This is possibly due to increased run off adding more solutes to the river water.

Recommendations and Future Work

Although the artificial channels at the Freshwater Biological Association River Laboratory are a rare and very valuable resource, several changes in the basic design would improve their utility in similar experiments to this one. Firstly, the conditions in each block were not exactly identical due to the slight variation in the gradient of the stream beds between blocks, and due to the nature of the pool into which the water in the channels flowed, before entering the drainage stream. Where these pools were not very big, there was a possibility of water flowing back into the channel under heavy flow conditions, such as during a rain storm, for example in block two. Secondly, the outer channels in each block of three had angled walls around the water level, as opposed to the vertical walls in the middle channel. This would have created different conditions in the outer channels from the central one. However, statistical analysis (not shown) on the results obtained here show that the channel from which a sample was taken did not affect invertebrate densities by way of its cross-sectional structure, but did through the experimental treatment that had been imposed. Thirdly, it would be desirable to seal the channels off from each other totally so that there was no possibility of water flow under the channel walls into an adjacent channel. Fourthly, if more resources were available, it would make flow experimentation a lot easier if there were calibrated valves at the inlet to each channel, so that the flow into each one could be set accurately. Finally, in an ideal world, the channels would have been wider, so that there was a better width to depth ratio to minimise the influence of wall turbulence effects. The width to

depth ratio should preferably be around 10, as opposed to the ratio of 4/5:1 used in this experiment (Craig, 1993). This would also have made the conditions in the channels more similar to those in a natural chalk stream. The channels in their current condition are however still a very valuable experimental resource. One possible approach to test the hypotheses generated in this project, and definitively confirm indicator species status would be to take invertebrate samples from several similar natural and fully colonised chalk streams, then restrict the flow in some streams for an extensive period and re-sample the invertebrate community.

If more time had been available to extend the scope of this project, a study into the patch dynamics of streams under low-flow stress could have been made. Upstream habitat patches will obviously have impacts on downstream ones (McCormick, 1993). Patches of habitat, as well as species, should not be regarded as isolated systems, so it may have been useful to gather some data on the habitat from which an invertebrate sample was taken, and the habitat upstream from the sample. According to the 'river continuum' concept, producer and consumer communities are established in harmony with the prevalent physical conditions, whilst downstream communities rely on the inefficiencies of upstream processing for a resource input (Petts et al, 1995). A more complete study of the interaction between the habitats available and the species composition within each of the three experimental treatments could yield results that are easier to interpret and therefore enable a more precise identification of suitable indicator taxa based on knowledge of their responses. Much work has previously been carried out on the habitat preferences of invertebrates found in chalk streams (e.g. Armitage and Cannan, 2000; Wright et al, 2003). There are many interactions whose effects can not be isolated using this simple, but effective experimental set-up, as natural biological systems are highly developed and contain many complex interactions.

Conclusions

The mayflies Ephemerella ignita, Caenis luctuosa group, Baetis muticus and Baetis group scambus, Gammarus pulex, Chironomids, and caddis flies such as Polycentropus flavomaculatus and Hydropsyche contubernalis could all act as positive indicators of low-flow conditions in chalk streams as they all associated strongly with control flows. Beetle larvae, particularly those of the species Nebrioporus depressus elegans and family Hydrophilidae, could act as negative indicators of low flows as they associated strongly with low flow samples after six weeks of treatment. In particular, it has been proven that *G. pulex*, and to a lesser extent *E. ignita* could act as positive indicators. The density of all individuals other than chironomids could also be an indicator of potentially ecologically damaging low flow conditions. The observation seen here that species richness declines under low-flow conditions has lead other ecologists to conclude that an index could be created to assess the impact of low flows (Bickerton et al, 1993). This project has tried to identify suitable indicator species that could be monitored more easily than calculating an entire index score, whilst giving a similar result.

This experiment has also shown that different levels of flow directly alter the physical environment in a stream, and so alter the habitats present. Invertebrates will be seen at different densities under different discharge levels because they select their preferred habitats. Species that prefer stable clean gravel habitats will decrease under low flow conditions, whilst those that prefer fine sediment habitats will increase under similar conditions.

As human-induced climate change will cause major changes in the frequency and severity of extreme disturbance events such as drought, it is vital that we are aware of the links between such disturbance events and their results, such as changes in faunal diversity (Downes et al, 1993). Identification of highly responsive invertebrate indicators will help us to monitor the extent of serious ecological damage, and hopefully prevent it.

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