THE HYPORHEIC ZONE OF SCOTTISH RIVERS:

ITS ECOLOGY, FUNCTION AND IMPORTANCE

BY

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A Descriptive Poem on the Silvery Tay

Beautiful silvery Tay,
With your landscapes, so lovely and gay,
Along each side of your waters, to Perth all the way;
No other river in the world has got scenery more fine,
Only I am told the beautiful Rhine,
Near to Wormit Bay, it seems very fine,
Where the Railway Bridge is towering above its waters sublime,
And the beautiful ship Mars,
With her Juvenile Tare,
Both lively and gay,
Does carelessly lie By night and by day,
In the beautiful Bay
Of the silvery Tay.
Beautiful, beautiful silvery Tay,
Thy scenery is enchanting on a fine summer day,
Near by Balnerino it is beautiful to behold,
When the trees are in full bloom and the cornfields seems like gold -
And nature's face seems gay,
And the lambkins they do play,
And the humming bee is on the wing,
It is enough to make one sing,
While they carelessly do stray,
Along the beautiful banks of the silvery Tay,
Beautiful silvery Tay,
Rolling smoothly on your way,
Near by Newport, as clear as the day,
Thy scenery around is charming I'll be bound...
And would make the heart of any one feel light and gay on a fine summer day,
To view the beautiful scenery along the banks of the silvery Tay.

William Topaz McGonagall (1825-1902)

DECLARATION

I declare that this thesis is a presentation of my original work that has not been submitted for any other degree or award. All additional sources of contribution have been acknowledged accordingly.

The work was completed under the supervision of Professor David J. Gilvear, Dr. Nigel Willby, Prof. Phil Boon (SNH) and Dr. Simon D. Rundle (University of Plymouth) and was conducted at the University of Stirling, United Kingdom.

David James Pryce

ABSTRACT

The hyporheic zone (HZ) has been defined as an active ecotone lying between the river bed and underlying groundwater; it is chemically and biologically distinct from these over- and underlying zones. Research into the chemistry, faunal composition and ecological relationships within the HZ have received relatively little attention until recent years and it has since been shown to have an important impact on riverine ecology.

Initial work centred on the development of a simple, robust sampling methodology that could be used to obtain discrete, analysable samples of both invertebrates and water for faunal and chemical analysis. No single sampling methodology was found that fulfilled these criteria, consequently two separate methodologies were used in parallel: Karaman-Chappuis pits excavated in exposed river gravels were used to obtain a shallow (10 cm) sample; modified Bou-Rouch pumping was used to extract a deep (50 cm) sample from below the pit. Initial trials at three sites were used to determine that four replicate pit-pipe samples would extract a representative sample from a site.

A total of 25 sites were surveyed across Scotland, these were selected to cover as wide a range of river types, water chemistries, geographical diversity and physical structure as possible. A degree of clustering within the samples was used to help assess between-site differences.

The survey found that a well developed hyporheic fauna is present across Scotland. Over 92% of all invertebrates recovered were from pit samples indicating that the fauna is primarily shallow. The composition of the fauna differs from the benthos and is dominated by oligochaetes, cyclopoid copepods, nematodes and dipteran larvae; these four groups accounted for 77% of invertebrates from pits samples and 78% from pipe samples. The pipe samples were not faunally distinct from the pit samples. It appears that the HZ is an important nursery area for the smallest Plecoptera instars.

The number of invertebrates recovered and taxonomic diversity were patchy at the local scale but regionally uniform; both tending to decrease northwards.

Trends in chemical parameters from montane to lowland sites indicate that considerable changes in environmental chemistry occur along Scottish rivers. Trends at a local (site) scale are less clear, but seem to indicate a degree of within riffle processing, particularly with respect to DO; patterns were broadly similar in pit and pipe samples. While the total number of invertebrates and taxonomic richness in pits decreased downstream through bars this was not evident in pipe samples. It is suggested that the compactedness of sediments acts as a filter so that invertebrate assemblages are at their most developed in the downwelling zone at the head of the bar where the most intense chemical processing occurs.

The key drivers of community composition were found to be distance to source, conductivity and source altitude in pits; site altitude, longitude and total alkalinity in pipes. Dissolved oxygen was found to be a key determinant of taxonomic richness.

BMWP scores from taxa present in the samples were used to back-calculate scores for their role in bioindication within the HZ. While the revised invertebrate scores ranged from 2.1 to 12.4, the back-calculated results ranged from 5.50 to 7.12. The relationship between the two scoring systems was significant at the $P < 0.05$ level and indicates that within the HZ high- and low-scoring macroinvertebrates have a higher probability of co-occurring than they do in benthic communities.

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1 INTRODUCTION

1.1 Historical background and definition of the zone

The earliest papers to identify the hyporheic zone (HZ) dealt mainly with the development of sampling techniques and its characterisation as a separate ecological entity (Karaman, 1935; Chappuis, 1942). It was first named as a distinct biotope by Orghidan (1959); Schwoerbel (1961, 1967) was the first author to describe the HZ as integral to the function of the fluvial ecosystem.

The name itself is derived from the Greek *hypo*- (under) and *rheos* (stream). Several definitions of the zone have since been postulated. Triska *et al.* (1989) proposed a rigid definition based on the percentage of advected surface water present. A more flexible approach was taken by Danielopol (1980, 1991) who suggested that the individual researcher define the system according to the questions being asked. The most widely used current definition is that of Bolton *et al.* (1998) who described it as:

> "a spatially fluctuating ecotone between the surface stream and deep groundwater where important ecological processes and their requirements and products are influenced at a number of scales by water movement, permeability, substrate particle size, resident biota, and the physiochemical features of the overlying stream and adjacent aquifers."

A characteristic feature of the HZ is the presence within it of multiple gradients in both physical and chemical parameters that change both spatially and temporally. This differentiates the zone from the underlying groundwater where these gradients are generally much weaker and the overlying fluvial system which is more homogeneous due to rapid mixing (Figure 1).

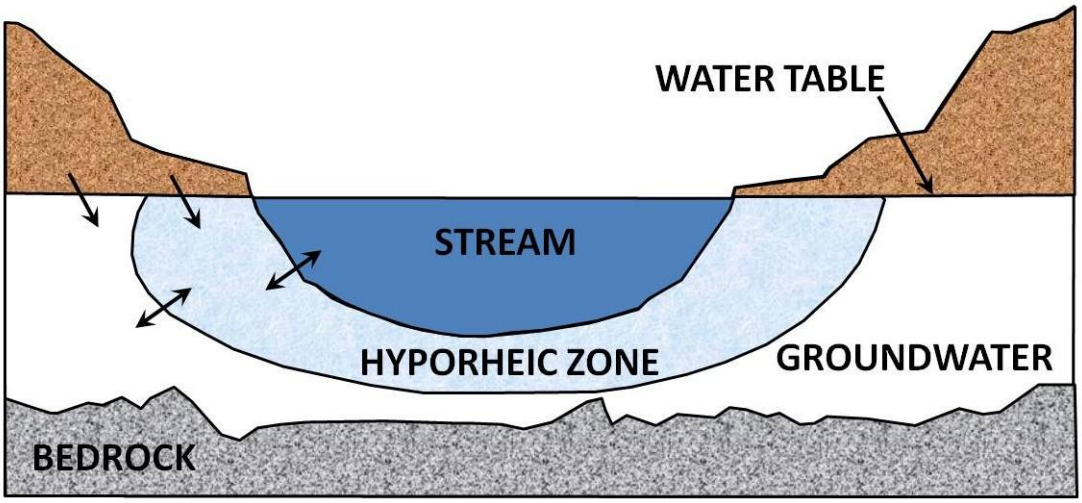

Figure 1 *Schematic representation of the hyporheic zone*

The HZ varies in vertical and lateral extent depending primarily on the nature of sediment present within which the zone can develop. It is spatially complex with hydrological pathways operating at multiple scales from the individual particle to the reach and even up to catchment scale where extensive alluvial deposits are present. The structure of the zone is disturbed by reworking of sediment through time, creating a dynamic three-dimensional matrix of contrasting porosity, permeability, morphology and connectivity. Flow variability as a result of seasonal or day-to-day fluctuation is also an important driver.

The invertebrate fauna present within the zone is distinct from the epibenthic fauna of the river bed, consisting mainly of meiofauna and the early instars of the benthic macrofauna; this fauna has been termed the 'hyporheos' (Brunke & Gonser, 1997). There has, however, been relatively little research into the ecology and distribution of epibenthic meiofauna as traditional ecological sampling almost without exception follows the traditional macroinvertebrate methodology; rare exceptions include Fryer (1993) and Robertson *et al.* (1997). Identification of the true hyporheos is further complicated by the presence of a separate groundwater fauna termed 'stygofauna' (or 'stygobionts' when referring to obligate species) that will intergrade with the hyporheos at the interface of the two

zones. Due to a lack of data on these organisms it is often difficult to determine the ecological niche they inhabit, a task further complicated by the categorisation of otherwise benthic and hyporheic organisms inhabiting cave pools and stream beds as stygofauna (Galassi *et al.*, 2009a).

1.2 Spatial scales

One of the most important factors when examining the hyporheic zone is the question of scale. Researchers have often restricted their investigation to a single scale such as an individual riffle (Godbout & Hynes, 1982; Davy-Bowker *et al.*, 2006) or a river reach (Mermillod-Blondin *et al.*, 2000; Malard *et al.*, 2003b). It is important to understand the processes occurring within the HZ in terms of the different scales within which they operate. These are discussed below in terms of local-, reach- and catchment-scale processes. Local scale is defined as a scale that is equal to or less than an individual feature such as a pool, glide or riffle. Reach scale is defined as a length of river sharing a similar suite of habitats; in Scottish rivers this could range from a few hundred metres to several kilometres.

1.2.1 Sediment-scale processes

The majority of physical and chemical processes that occur within the HZ are determined by the precise composition of the sediments present. The size, shape and sorting of sediments are the primary determinants of porosity and permeability. Reworking of sediments over time results in a three-dimensional matrix with the potential for extreme contrasts over short distances. This means that even in sites with well a developed HZ flow paths will be complex and areas of no flow (dead zones) will occur. As soon as river waters enter the HZ the physical changes (loss of light, change in temperature, etc.) will affect the chemistry of the water. Ammonification, nitrification and denitrification all occur and are controlled by oxygen availability (Jones & Holmes, 1996) (Figure 2).

Intense nitrogen processing has been reported to occur, in particular at the interface of aerobic and anaerobic zones (Brunke & Gonser, 1997). In a theoretical paper Storey *et al.* (1999) argued that the heterogeneous nature of the HZ allows the stepwise processing of nutrients (nitrogen in particular) as it provides a series of environments through which different conditions prevail. It is even suggested that biofilm dynamics could allow anaerobic respiration pathways including nitrate, ferric ion, sulphate and methanogenic processes to take place in aerobic sediments.

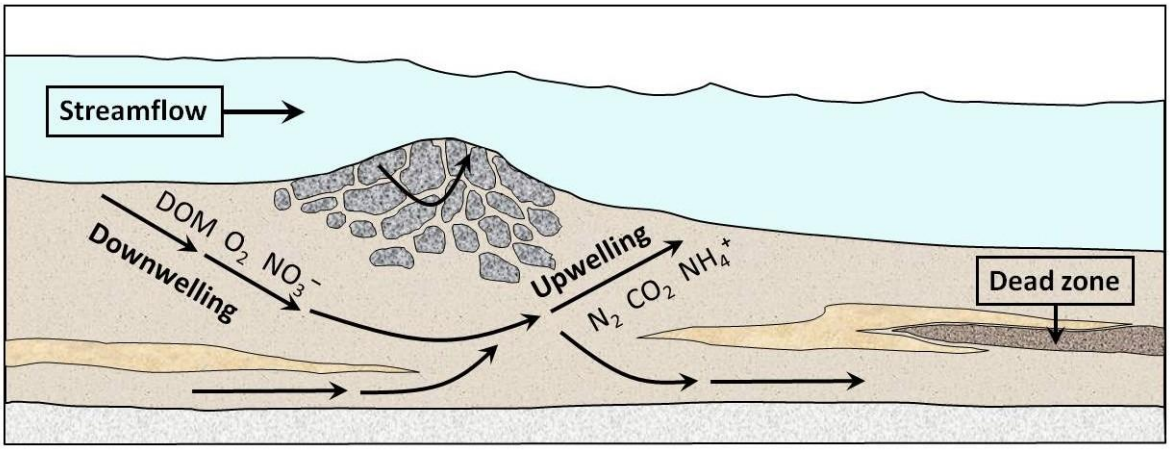

Figure 2 *Flow paths and water chemistry changes at a hypothetical riffle*

Chemical processes within the hyporheic zone are further influenced by microbial activity. An investigation by Feris *et al.* (2003) found a diverse microbial community at three streams, the composition of which exhibited greater in-stream similarity between riffles than between streams; there were clear trends in seasonal abundance with all taxa peaking in autumn.

At the sediment scale one of the most important factors affecting the HZ is pore clogging. If fine particles enter the system, or microbial biofilm production increases to the point that pores in the sediment become choked, then velocities will decrease. This is of particular importance in downwelling zones where water is entering the stream bed – filtering of fine particles will occur and microbial activity is likely to be at its highest in transition zones (Brunke & Gonser, 1997). If clogging

of the top layer of sediment (colmation) occurs then hyporheic processes will either slow or cease entirely. Colmation is alleviated when sufficient bedload movement occurs during spates. Upwelling zones are less likely to clog as the vertical hydraulic gradient naturally removes sediment settling from above.

The importance of these processes to the ecology of the overlying stream was highlighted by Doering & Uehlinger (2006) in an investigation of biofilms on the bed of the Tagliamento River in Italy. It was found that biofilm production was twice as high in a reach where hyporheic water was upwelling than in a downwelling reach (25.3 and 12.2 g m^{-2} ash-free dry weight respectively).

1.2.2 Reach-scale processes

The majority of research in the field has concentrated on reach-scale processes, particularly those of upland streams, braided rivers and riffle-pool-riffle sequences, this being the most manageable scale for study in the field (sites are close together). At this scale many researchers concentrate on key processes such as the effect of upwelling and downwelling water (Franken *et al.*, 2001; Malard *et al.*, 2003b), the effect of floods (Olsen & Townsend, 2005; Hancock, 2006) or impacts of river restoration (Sarriquet *et al.*, 2007). Relatively few workers have studied multiple factors in an attempt to determine overall drivers in the system; notable exceptions are Boulton *et al.* (1997) and Mermillod-Blondin *et al.* (2000).

As a general rule with regard to hydrology, it has been found that decreasing stream depth and increasing velocity at the end of a pool forces surface water down into the sediment which then moves through it before upwelling to the surface some distance downstream due to a vertical head gradient (Boulton *et al.*, 1998). In reality the heterogeneity of the surface and subsurface sediment means the picture is much more complex, with variations in pathways from location to location and

temporally in relation to changes in flow conditions (Thorp *et al.*, 2008; Käser *et al.*, 2009). Hydraulic conductivity and retention of water within the sediment is directly related to geologic setting and alluvial characteristics. In a study of three streams on contrasting geology in New Mexico Morrice *et al.* (1997) found that residence time (*i.e.* rate of water turnover) was lowest on a bed derived from fine-grained sandstone and highest in a stream bed of poorly sorted boulders, cobbles, gravel and sand derived from granite and gneiss; a third stream with sediment derived from weathered tuff had intermediate retention.

The complex nature of the substrate results in a network of flow paths of contrasting lengths, velocities and directions. Temporal changes can be considerable. In a high resolution study of spatio-temporal change of hydraulic conductivity and vertical hydraulic gradient (VHG) on a single riffle Käser *et al.* (2009) found that spatially VHGs changed from upwelling to downwelling both laterally and longitudinally over a six week period.

The longer the hyporheic retention time the greater the interaction between the water, biofilms and macroinvertebrates. These complex interactions have been likened to the workings of an ion chromatograph with differential retention and segregation of ions as water passes through the sediment (Freeman *et al.*, 1995).

1.2.3 Catchment-scale processes

Analysis of hyporheic flow paths at the catchment or drainage basin scale has led to the development of the 'hyporheic corridor concept' (Stanford & Ward, 1993) which stresses the links between the hyporheic zone and the catchment within which it operates. Flow paths and residence time are proposed as the main determinants of biodiversity and metabolism in the HZ (Boulton *et al.*, 1998). The subsurface continuum extends laterally to link riparian areas, anabranches,

palaeochannels and floodplain aquifers. These environments provide a varied range of landscape features whose spatial and temporal diversity relates to the extent of connection and discharge regime of the watercourse (Boulton *et al.*, 1998). Vertical hydrolgical exchanges occur at relatively discrete points between the stream channel and the hyporheic zone.

In summary, the hyporheic corridor concept highlights catchment scale concepts whereby:

- Upwelling water largely affects production in the stream channel;
- Riparian zone structure and dynamics mirror hyporheic flow pathways.
- Diversity in hydro-exchange processes and linkages encourages biodiversity within the catchment.

1.3 The ecological significance of the HZ

Until recently stream ecologists have concentrated almost entirely on the benthic zone. This has been a result of its ease of sampling, established role in bioindication of water quality, well resolved taxonomy of most organisms and importance as a habitat for the food source of economically important higher trophic level organisms such as fish.

The HZ has been shown to be a very important habitat for aquatic invertebrates. The hyporheos itself can be divided into three main groups – the obligate hyporheos that live within the zone through all of their life stages; the occasional hyporheos that spend a portion of their life within the zone; and the accidental hyporheos that enter it by chance. As a result of lack of sunlight detritivores dominate and the number of larger top predators is also reduced by the physical necessity for movement through the medium in order to locate prey, coupled with small pore sizes and periodic bed mobilisation. The resulting fauna has truncated functional biodiversity which will consequently

react differently from the benthic fauna to environmental stressors (Dahm *et al.*, 2007). The HZ also acts as a refuge for some benthic macroinvertebrates during times of drought and flood (Dole-Olivier *et al.*, 1997; Hancock, 2006; Stubbington *et al.*, 2009a). Most of the obligate hyporheos are poorly known in terms of their distribution, life history and ecology and almost any information that can be recovered about these organisms will add significantly to the body of knowledge about them.

Recent work on the HZ has greatly expanded our knowledge of the functioning of stream ecosystems and our understanding of the three-dimensional utilisation of habitats by stream organisms. For many fluvial habitats it has been shown that invertebrate production within the HZ can equal or even exceed that of the benthos (Stanford & Ward, 1988; Smock *et al.*, 1992).

Interactions between water chemistry, bacterial primary production and invertebrates within the HZ have been shown to have major consequences for the overlying benthic community. In a study in the catchment of the Flathead River Wyatt *et al.* (2008) showed that upwelling hyporheic water enhances benthic algal production in comparison to downwelling or neutral zones. This enhancement is in response to nutrients being brought to the surface and can result in downstream nutrient spiralling (Poole *et al.*, 2008); this varies both spatially and temporally (McClain *et al.*, 1994). Downwelling water rich in dissolved oxygen has been shown to be of importance in the survival of salmonid eggs (Malcolm *et al.*, 2004). Riparian land use and man-made stream discontinuities (*e.g.* dams) have been shown to have a considerable impact on the biota of the HZ (Brunke & Gonser, 1997; Boulton *et al.*, 1997).

A large body of research on the HZ has focused on the processing of solutes and the effect of this on stream metabolism. Rates of nitrogen, phosphorus and carbon cycling have been shown to be strongly influenced by processes occurring in the HZ and the residence time of water within it (Grimm & Fisher, 1984; Triska *et al.*, 1993; Brunke & Gonser, 1999). Dissolved oxygen carried by

downwelling water is one of the primary drivers of productivity within the HZ, so any metabolism that reduces oxygen in the stream water above or within the zone itself will have a profound effect on the organisms within it (Malard & Hervant, 1999). The cycling of nutrients within the HZ has also been shown to have an influence on the development of riparian vegetation (Harner & Stanford, 2003).

As a consequence of all these (and many other) factors, the exchange of waters between the benthic and hyporheic zones can have a profound impact on the distribution and structure of the biotic community in streams and rivers, stream metabolism, the processing of nutrients and even the structure of riparian vegetation.

1.4 The hyporheic fauna

The hyporheos is dominated by meiofauna, a group classified as those invertebrates smaller than would be defined as the lowest size class of macrofauna and are typically the organisms that would pass through a 1 mm or 500 μm sieve and be retained by a 40 μm sieve (Higgins & Theil, 1988; Palmer *et al.*, 2007).

Due to their small body size the HZ can hold huge numbers of invertebrates. Williams & Hynes (1974) estimated the density of such invertebrates in the hyporheos of the Speed River (Ontario) to be between 184,760 and 797,960 animals per cubic metre, representing a dry weight biomass of between 31 and 253 g. Invertebrate processes (burrowing, ingestion of bacteria and other organic matter and excretion of wastes) will in turn affect the physical and chemical processes taking place within the HZ. The greatest number of invertebrates within the zone has been shown to generally occur just under the bed of the stream in the top 10 cm of sediment with numbers decreasing

downwards from there (Williams & Hynes, 1974). However, some invertebrates in this study were found regularly down to 70 cm, their numbers generally decreased beyond this depth.

Several studies have reported a correlation between the distribution of hyporheic invertebrates and upwelling zones (Ward, 1989; Boulton, 1993). Nutrients brought to the surface create productivity hot spots which can, in turn, drive algal and macrophyte growth on the stream bed and can lead to downstream nutrient spiralling.

The fauna is dominated by crustaceans (usually copepods, cladocerans, and ostracods), nematodes, smaller segmented worms, flatworms, mites and the earliest life stages of the aquatic invertebrate fauna (most commonly Diptera, Ephemeroptera, Plecoptera and Coleoptera). These are predominantly grazers of microbial biofilms so that larger and more diverse assemblages of invertebrates should reflect the density of aerobic microbes within the HZ. It has been proposed that grazing of biofilms may enhance their productivity (Montagna, 1995). Another important food source is buried coarse particulate organic matter (CPOM) such as leaves, twigs, *etc*. It has been postulated that CPOM buried in the form of leaf packs may act as hot spots for primary producers (microbial breakdown), grazers and consequently predators. Despite anecdotal evidence there are few data to support this theory (Boulton & Foster, 1998). Further investigation is required into the dietary requirements of the hyporheos and the food web dynamics of the system (Robertson *et al.*, 2000).

The eggs of some invertebrates (a British example being the dragonfly *Cordulegaster boltonii* (Donovan)) and several species of fish are deliberately deposited in the hyporheic zone (Tillyard, 1917; Hansen, 1975). As well as concealment from opportunistic predators it has been shown that salmonid eggs benefit developmentally when deposited in downwelling zones where the water brings a constant supply of oxygen to the eggs (Malcolm *et al.*, 2004). The smallest instars of many

benthic macroinvertebrates enter the hyporheic zone to escape the shear stress of faster currents (Boulton *et al.*, 1998) and it is also used by numerous species as a refuge for diapausing or pupating stages of the life cycle (Pugsley & Hynes, 1986).

One key biological aspect requiring more in-depth investigation is the extent to which commonly kick-sampled invertebrates actually belong to benthic or hyporheic communities. It appears that some commonly observed species from traditional benthic kick samples, particularly among water mites and crustaceans, may in fact belong more properly to the shallow hyporheic assemblage (Fernández, 2004).

In Europe the stygofauna has been found to be unexpectedly diverse (Gibert & Culver, 2009). Counterintuitively, overall species richness is greatest in porous as opposed to karstic aquifers (Malard *et al.*, 2009), however, species richness in karstic local communities varied linearly with the richness of the surrounding region while that for porous communities levelled off beyond a certain point. All areas of Europe surveyed were found to contain regional endemics, the proportion varying from 19% in the Rhône corridor to 86% in Cantabria. One study of the Lassinian Massif of northern Italy found 89 stygobiotic species with 35 of these new to science (Galassi *et al.*, 2009b).

The high degree of regional endemism, presence of relict populations and occurrence of taxonomically isolated groups in the European stygofauna has not been reported from hyporheic communities in this region. This is probably a result of greater connectivity and ease of colonisation of the zone. A study of upstream colonisation of benthic and hyporheic zones in the valley of a retreating glacier, the Val Roseg in Switzerland, was undertaken by Malard (2003). He found that upwelling groundwater was a major source of species and that in groundwater-fed channels both hyporheic and benthic zones had a significantly higher densities of invertebrates. He postulated that the hyporheic corridor acts as the main upstream migratory pathway in this system.

1.5 The HZ in an environmental policy context

The EU Water Framework Directive (WFD) (Directive 2000/60/EC) calls for a coordinated programme of actions to secure the protection, enhancement and sustainable use of European freshwaters. This has made it one of the major drivers of aquatic policy and associated research in the UK and much of continental Europe over the last 10 years. The WFD makes no specific reference to hyporheic habitats *per se* and, while the protection of groundwater is one of its main objectives, the major emphasis here is on chemical contamination and quantitative resource assessment.

Further to this the Groundwater Directive (Directive 2006/118/EC) was subsequently established with the aims of clarifying the criteria for good chemical status and specifications related to the identification and reversal of pollution trends (European Commission, 2008). Ecological aspects receive minimal attention in the Groundwater Directive but form part of its underpinning case. Decontamination processes associated with subsurface attenuation are particularly emphasised due to their likely influence on surface water quality.

Significantly the WFD recognises for the first time the environmental importance of groundwater, as distinct from its value as a strategic societal resource, and requires the ecological assessment of associated aquatic and directly groundwater-dependent terrestrial ecosystems, whose status is assumed to be linked inextricably to the quality of groundwater. It also requires the evaluation of supporting hydromorphological (HM) elements as part of the overall process of ecological classification of water bodies. In the case of rivers these HM elements include 'connectivity to groundwater', and 'structure and substrate of the river bed'. One of the biological quality elements required for classifying the ecological status of rivers is the benthic invertebrate fauna. Although this has generally been interpreted to represent the shallow macrozoobethos, the hyporheos or

microzoobenthos is not specifically excluded. Thus, while the WFD does not directly address the protection of the HZ and its associated fauna this is encompassed indirectly through several assessment requirements and the recognition that linkages between ecological, hydrological and hydrogeological components of the catchment must be respected to achieve integrated protection of freshwaters.

The Habitats Directive (Council Directive 92/43/EEC) on the conservation of natural habitats and of wild fauna and flora does not specifically include hyporheic zones, although it includes riverine habitats ("Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation"), a number of riverine fish (*e.g.* salmon and lamprey) and invertebrate taxa (*e.g.* pearl mussel and white clawed crayfish) whose status could be considered directly or indirectly dependent on the condition of the HZ and its linkage with surface waters.

In Europe the PASCALIS (**P**rotocols for the **AS**sessment and **C**onservation of **A**quatic **L**ife **I**n the **S**ubsurface) research project has been initiated to assess the biodiversity of groundwater organisms and provide policy guidance for their conservation. The work is supported by the European Commission under the Fifth Framework Programme and has three main objectives:

- To create an integrated approach of groundwater biodiversity.
- To identify and promote standard methods for assessing and predicting regional biodiversity.
- To produce a scale-oriented conservation strategy of groundwater biodiversity to support the EU Energy, Environment and Sustainable Development programme on assessing and conserving biodiversity.

The key outcomes of this project were reported in a special issue of the journal Freshwater Biology (Vol. 54, No.4, 2009), and the recommendations are currently being assessed by EU regulators.

1.6 Hyporheic research in the UK and Ireland

Despite the large number of articles published on the HZ many of the biological characteristics and environmental processes within it are still poorly understood. Work in the UK has lagged behind that of other countries for a number of reasons:

- The UK lacks the extensive alluvial plains that have stimulated work in the USA and Canada.
- There are no extensive karstic zones in the UK; these have been investigated in southern and central Europe where links to the diverse stygofauna have promoted interest.
- Intermittent stream systems that have promoted study of the hyporheos in Australia are scarce in the UK.
- The dominance of surface water resources in much of the UK means that little information has been gleaned from borehole studies compared to other regions.
- There has been no active research group investigating the zone such as that at Lyon, France.

Some of the earliest work on British meiofauna assessed the fauna of streams at the benthic / hyporheic interface in the Ashdown Forest in southern England (Rundle, 1990; Stead *et al.*, 2003) and streams in upland Wales (Rundle & Ormerod, 1992; Rundle & Ramsay, 1997). Robertson *et al.* (2000) stressed the need for further research into even the most basic aspects of fauna in this region. A summary of the current knowledge of the hyporheic and stygofaunal communities of England and Wales is given in Robertson *et al.* (2009).

Most recently the creation of the Hyporheic Network (a knowledge transfer network focusing on groundwater-surface water interactions and hyporheic zone processes) has spurred interest in the

zone and improved communication of research findings between workers in the field. A series of papers stemming from this collaboration are now beginning to appear (Käser *et al.*, 2009; Krause *et al.*, 2009; Stubbington *et al.*, 2009b). A key output from this project is the Environment Agency Hyporheic Handbook (Buss *et al.*, 2009) which summarises the current state of knowledge of the HZ and promotes consideration of it in an environmental management context.

An important contribution to the knowledge of the HZ of Ireland was made by Kibichii (2009). The key findings of this research were that:

- Dissolved oxygen decreased rapidly away from the stream bed.
- That two distinct shallow (0.2 m) and two distinct deep (0.5 m) faunal assemblages could be identified.
- Faunal differences between spring and summer indicated dynamic spatio-temporal changes.
- At streams impacted by intensive agriculture taxonomic richness was depleted, although this was ameliorated to a degree where riparian woodlands were present.

1.7 The history of hyporheic research in Scotland

The HZ has received very limited attention in Scotland. The first paper to deal with hyporheic fauna in the country was a short note on the accidental discovery of the microcrustacean *Antrobathynella stammeri* in river gravels of the Altquhur Burn, a tributary of the Endrick, in 1960 (Maitland, 1962). The first survey specifically to target the fauna of the zone was undertaken in 1972, but was restricted to water mites from the catchments of the rivers Tay and Almond in Perthshire (Gledhill, 1983). Benthic microcrustaceans (an important component of the hyporheos) from two streams in the Rhinns of Kells, southwest Scotland, were recorded as part of a larger study by Robertson *et al.* (1997). More recently a series of papers considered the chemical and thermal variability of the

hyporheic zone and the consequent implications for salmonid egg survival (Soulsby *et al.*, 2001; Malcolm & Soulsby, 2002; Malcolm, *et al.*, 2003, 2004). These papers are based on work conducted at two small sites on the Girnock and Newmills Burns in north-east Scotland.

Prior to the current research a pilot study (Gilvear *et al.*, 2007) investigated the hyporheos at six sites in upland Scotland (a single site each on the rivers Feshie and Spey and two sites each on the Tummel and Dee). The key findings of this survey were that:

- A hyporheic fauna consisting of macrofaunal and meiofaunal components was present. Numbers were generally low but diversity was high and was likely to have a significant nature conservation value.
- There were significant differences between surface water (benthic) and hyporheic communities and that a diverse benthic community did not imply a diverse hyporheic community and vice versa.
- Subsurface depth was the main correlate with the composition of the community therein.
- The hyporheic fauna is patchy in distribution and requires research into the sampling technique in order to obtain a representative sample.

1.8 Objectives

Following on from the pilot study (Gilvear *et al.*, 2007) that proved the presence of a distinct hyporheic community in Scottish rivers the current research was undertaken with four main objectives:

- To develop a simple, robust method for sampling the invertebrates from the HZ of rivers in Scotland.

- To conduct a survey of hyporheic invertebrates from rivers across Scotland in order to assess their distribution and composition.
- To determine through field measurements and chemical analysis the key drivers influencing the hyporheos.
- To assess the importance of the hyporheos in terms of conservation and to define potential threats to it.

2 SAMPLING TECHNIQUE

2.1 Comparison of methods used to sample the hyporheos

It is widely accepted that obtaining quantitative samples of invertebrates from the hyporheic zone is challenging (Fraser & Williams, 1997; Scarsbrook & Halliday, 1992); this is likely to be particularly true in coarse gravels. The overriding difficulty is the necessity to extract a representative sample of invertebrates and interstitial fluid while avoiding disturbance of the sample zone. All methods used to sample the hyporheic zone are intrusive and the very act of sampling is liable to cause bias due to the nature of that extraction. Two examples of this are that the act of hammering a pipe into the sediment is liable to disturb the invertebrates therein, or that suction of fluid through the sediment will have a size-dependent filtering effect. The method chosen depends to a certain extent on the focus of the work; for example, whether it is to examine the physical characteristics of the zone, water quality, microbial activity, invertebrate assemblages or vertical connectivity. To date five main sampling methods have been developed to extract samples. These are:

- Karaman-Chappuis pits;
- Bou-Rouch pumping;
- Sediment coring;
- Freeze coring;
- Colonization pots;

Each method has its advantages and disadvantages; they are described and discussed separately below.

2.1.1 Karaman-Chappuis pits

This is the simplest method for obtaining a hyporheic sample. A pit is excavated in exposed sediments to such a depth that its base is below the piezometric water level. A sample is then removed (Chappuis, 1942). Despite the simplicity of the method few workers have adopted it (Danielopol, 1976; Gledhill, 1983; Dahm *et al.*, 2007). This method has several advantages:

- As samples are being extracted from exposed sediments there is a higher probability that invertebrates will be obligate hyporheos as accidental vertical colonisation by stream bed fauna is excluded.
- At equilibrium the water level in the pit equates to the piezometric water level within the sediment so the sample is always extracted from the same position vertically within the hyporheic zone.
- The technique does not require personnel to enter the stream thus decreasing health and safety concerns.

However, several drawbacks are apparent:

- Samples can only be taken from exposed sediment, limiting the number of possible sample points.
- The need to remove sediment down to the piezometric water table further restricts sampling locations to those where this is near the surface.
- The technique is limited to the shallowest portion of the hyporheic zone and is usually restricted spatially to the active channel.

2.1.2 Bou-Rouch pumping

This is the most commonly used technique for the extraction of samples from the hyporheic zone. It involves the insertion of a pipe into the sediment and extraction of interstitial fluid and invertebrates by pumping (Bou & Rouch, 1967). The technique is flexible and allows extraction from different depths and theoretically from all locations within the stream corridor. Studies employing this technique use the same basic method, but the equipment (pipe type, pump type, *etc*.) has not been standardised and is often poorly described. Several studies have used large, heavy, custom-built samplers based on the design of an old-fashioned village water-pump (Williams & Hynes, 1974; Malard *et al.*, 2003b; Kibichii, 2009). Various proprietary pumps have also been used including bilge pumps (Fowler & Scarsbrook, 2002; Boulton, pers. comm.). However, the pump type is not mentioned by most authors. The type of sample pipe, selected sampling depths, number of litres extracted and filter size vary considerably from study to study.

2.1.3 Sediment coring

This technique involves the physical extraction of a sample of sediment. By its very nature it can only be employed in finer-grained environments such as sands and the finest, well sorted gravels. It has been used extensively in the prairies of the USA and Canada where this habitat is more common (Williams & Hynes, 1974; Godbout & Hynes, 1982; Williams, 1989).

2.1.4 Freeze coring

In this technique a pipe is driven into the sediment and cryogenic fluid (usually liquid nitrogen) poured in. The water in the sediment freezes progressively outwards from the pipe and, when sufficient time has elapsed, the pipe and frozen core are winched out of the surrounding unfrozen

gravels. The technique is particularly suited to studies considering the physical structure of the sediments themselves such as that of Olsen & Townsend (2003). However, while it may be advantageous to obtain a quantitative physical sample of the hyporheic sediments there are several disadvantages:

- It is difficult to separate any invertebrates present from the very large volume of sediment recovered.
- The use of liquid nitrogen on the river bank raises several health and safety concerns.
- A large tripod and heavy duty winch are required in order to lift the sample out of the sediment.
- As a consequence of the previous two points the manpower required on site in order to transport the equipment and operate it in a safe manner raises the cost of sampling significantly.
- Repeated sampling at a site is liable to lead to destabilisation of the river gravels sampled so that they may be stripped out by scour during subsequent spates (Paul Wood, pers. comm.).

The technique has been used in several studies (Adkins & Winterbourn, 1999; Olsen *et al.*, 2002; Scarsbrook & Halliday, 2002), but not widely adopted.

2.1.5 Colonisation pots

The final technique employed to sample hyporheic invertebrates involves the emplacement of a chamber containing material suitable for colonisation. One version of this method uses a pipe hammered into the sediment with an opening that can be either operated from the surface (Hynes, 1974) or closed prior to extraction of the sample (James *et al.*, 2008). A second variation on this method involves excavation into the sediment and emplacement of a pot followed by site

restoration (Scarsbrook & Halliday, 2002). Once emplaced it is necessary to leave the pots *in situ* to allow colonisation – at least 28 days are required for full colonisation (Coleman & Hynes, 1970). The pots are then removed or re-excavated and invertebrates extracted.

2.1.6 Discussion of sampling methods

Only two papers have been published directly comparing combinations of these techniques (Fraser & Williams, 1997; Scarsbrook & Halliday, 2002), but neither compared all five. Fraser & Williams (1997) compared sediment coring, colonisation coring (9 week settling time), freeze coring and modified Bou-Rouch pumping. Their key findings were:

- Taxonomic richness did not differ between the different methods.
- Colonisation coring significantly underestimated invertebrate density.
- Modified Bou-Rouch pumping did produce a filtering effect on the larger invertebrates in terms of both the proportion of insects collected and mean chironomid body size.
- The two coring techniques most effectively characterised the hyporheic fauna, but the other two techniques were also acceptable where coring was impractical.

Scarsbrook & Halliday (2002) compared colonisation pots, Bou-Rouch pumping and freeze coring at six sites in spring and autumn. Their main findings and conclusions were:

- The total number of invertebrates collected in colonisation pots ranged from 2 to 425 per 1500 cm^3; 55 taxa were collected.
- The total number of invertebrates collected in pumped samples ranged from 0 to 131 per 6000 cm^3; 33 taxa were collected.

- The total number of invertebrates collected in freeze core samples ranged from 0 to 42 per 140 cm^3; 37 taxa were collected.
- The most useful of the three methods for discriminating between sites was pump sampling, which exposed significant differences in both spring and autumn; colonisation pots discriminated significantly in autumn, but not in spring; differences between sites in freeze coring were not significant.

In summary, while pump sampling produces the lowest number of invertebrates and lowest taxon richness it is, however, the best technique for discriminating between sites. In order to compare the five techniques and inform a choice of method a matrix (Table 1) was prepared to assess their relative advantages and disadvantages.

Attribute	Karaman-Chappuis	Bou-Rouch	Sediment core	Freeze core	Colonisation pot
Extent of use	Occasional	Widely used	Occasional	Occasional	Occasional
Type of sample	Semi-quantitative	Semi-quantitative	Quantitative	Quantitative	Quantitative
Ease of sampling	Easy	Easy	Medium	Difficult	Medium
Rate of sampling	Quick	Quick	Quick	Medium	Slow
Sample extraction	Easy	Easy	Difficult	Difficult	Difficult
Equipment requirements	Simple	Medium	Medium	Complex	Medium

Table 1 *Summary of the relative advantages and disadvantages of the five sampling methods*

Summarising the results from the matrix, it can be seen that Karaman-Chappuis pits and Bou-Rouch pumping are the two easiest methods to employ as a result of the relative simplicity of the equipment; samples from these techniques are also the most rapid to collect. However, results are

semi-quantitative – although the volume of water extracted from the sediment is identical in each sample, the volume of the hyporheic zone sampled will vary as a result of the porosity and permeability of the sediment and the connectivity of flow paths therein. While the other three methods do provide quantitative results they all have at least one major disadvantage:

- Sediment coring cannot be used on most rivers in Scotland as substrates are generally too coarse.
- Freeze coring is complex, difficult and, as a result of the weight of the equipment and use of cryogenic fluid on the river bank, health and safety and manpower issues (and hence cost) are raised.
- Colonisation pots are difficult to install and would require at least two visits to each site.

Bou-Rouch pumping was initially chosen as the preferred method for this study as a result of its flexibility, low cost and ease of sample extraction. Following discussions with Andrew Boulton it was decided to complement this with Karaman-Chappuis pit sampling. Each sample would therefore consist of a paired pit (shallow) and pipe (deep) sub-sample, the pipe sample being taken through the base of the pit. This method has the added advantage that the piezometric water level in the sediment is revealed during the excavation of the pit and it is therefore possible to extract the deep sample from a precise distance below it. Previous studies have been restricted to depth below sediment surface.

It is to be lamented that there is as yet no standardised method for sampling invertebrates from the HZ as there is, for example, for sampling benthic macroinvertebrates (ISO 7828:1985; BS EN 27828:1994). Individual teams have conducted research using one (sometimes more) of the above techniques, but even within a single method there is often variation in the precise application of the technique. There is a real need for research to be carried out using multiple techniques across a

wide latitidudinal gradient in order to assess the variability of the fauna and the best methods with which to sample it. Only then will data be readily comparable across large spatial scales.

2.2 Development of sampling methodology

Having selected the best two sampling methods, it was necessary to define the sampling protocol and to develop and test the equipment required.

The pilot project (Gilvear *et al.*, 2007) used standard, galvanised, screw-assembly piezometers inserted into the river bed with a fence-post driver. Interstitial fluid was extracted with a hand-operated water pump. Initial trials in the present study found this type of piezometer to be difficult to install as a result of the highly compacted nature of the river gravels in this region. Once installed, it was also found that they were extremely difficult to remove, with extraction often resulting in shearing of joints, bending of the pipe or the requirement to unscrew the top section leaving the tip, filter and associated screw collars in situ. This had important cost and logistic implications for the survey work. The available pumps were also found to be of very inefficient. Other authors (Hunt & Stanley, 2000; Boulton, pers. comm.) have used plastic electrical conduit inserted into the bed and this was methodology was subsequently adopted.

2.2.1 Selection of key sampling variables

The three most important variables to determine when sampling the hyporheic zone using the Bou-Rouch method are the depth from which the sample is to be taken, the volume of interstitial fluid to be extracted and the size of mesh through which to pass the sample in order to extract the invertebrates. A summary of values reported in the literature is given in Table 2.

Depth(s) (cm)	Volume (L)	Filter size (μm)	Reference
50	10	125	Bolton *et al.*, 2003; 2004
30	Not stated	50	Cooling & Boulton, 1993
Not stated	10	50	Danielopol, 1976
50, 100, 150, 200	10	300	Dole-Olivier *et al.*,1997
30, 60	8	300	Fowler & Death, 2001
20, 40, 60	1	53	Fraser & Williams, 1997
50	2	63	Gilvear *et al.*, 2007
40	6	125	Hancock, 2006
37.5	2.5	63, 120	Hunt & Stanley, 2000
20, 50	10	60	Kibichii, 2009
30	10	100	Malard *et al.*, 2003b
50	10	300	Marmonier *et al.*, 2000
20, 50, 100	10	200	Mermillod-Blondin *et al.*, 2000
20, 40	6	63	Scarsbrook & Halliday, 2002
20	6	90	Stubbington *et al.*, 2009b

Table 2 *Sample depth, volume and filter size reported from 15 papers utilising the Bou-Rouch sampling method*

The average depth sampled in these studies was 54.9 cm, the average volume withdrawn was 7.25 L and the average mesh size 129 μm. Andrew Boulton (pers. comm.) recommended the use of a 63 μm filter as this would result in the retention of the greatest number of organisms.

Three papers have assessed differences in sampling methods (Hunt & Stanley, 2000; Boulton *et al.*, 2003; Boulton *et al.*, 2004). In a study comparing well design, pumping rate and sample volume Hunt & Stanley (2000) are the only authors to have compared different designs of sample pipe. They examined the effects of using a permanent well with 6 mm pores and four different types of temporary well: 37.5 cm pipes with no pores and 45 cm pipes with pores of 4, 6 and 8 mm drilled in the lowest 15 cm (in four rows, evenly spaced, eight holes in each row). No statistically significant differences were found between these designs. It was also found that increasing the sample

extraction rate from 1.5 L min^{-1} to 4 L min^{-1} produced a statistically significant increase in density estimates at two of the three sites and a statistically significant increase in taxon richness at one of the sites.

Boulton *et al.* (2003) conducted experiments in order to optimize sampling using the Bou-Rouch method. Ten consecutive 1 L samples were extracted from each of nine wells in a 3 m x 3 m grid. It was found that the number of invertebrates collected was consistently higher in the first litre than in subsequent samples. At one of the two study sites the first litre consistently recovered more taxa than subsequent samples, but this was not the case at the second site. Overall the trend was for a steady increase in the number of taxa recovered which reached a plateau after 3 to 5 L. However, in many wells additional taxa were collected in later samples with nine of the 18 wells producing new taxa in the tenth sample. They concluded that, given the nonlinear relationship between sample volume, the number of individuals and the number of taxa recovered, comparisons of studies quoting the number of invertebrates per litre were not possible when different sample volumes were collected. It was stressed that researchers should conduct similar exercises to determine the optimum sampling strategies for their own region.

Boulton *et al.* (2004) again assessed sampling volume issues but more specifically in relation to invertebrate composition; they also addressed the level of taxonomic resolution required to assess between-sample differences. It was found that a filtering effect preferentially collected small-bodied taxa (*e.g.* nematodes, ostracods and cladocera) in early samples. Larger taxa (*e.g.* isopods) were equally likely to be collected in earlier and later samples. Identification of invertebrates to order level rather than species did not significantly alter the ordination patterns of community composition. Their conclusions were that a sample volume of 5 L and identification of taxa at a broad level would be sufficient to discriminate between sites and, at a finer level, between sub-samples at a site.

2.2.2 Sample collection procedure

Pits were excavated using an iron bar and trowel. It was found necessary to remove most of the loosened gravel by hand. Due to the abrasive nature of the sediment and occasional presence of sharp objects (*e.g.* broken glass) it was necessary to wear neoprene gloves with Kevlar® lining for protection. Pits were excavated to a depth sufficient for approximately 10-15 cm of water to accumulate in the base at equilibrium level and wide enough so that the resultant pool was 25-30 cm in diameter. A sample of 20 L was then extracted from the pit in 4 aliquots of 5 L (Figure 3A). The pump used was a Sealey TP69 oil and fluid extractor with a capacity of 6.5 L (Figure 4D). It is lightweight, easily portable, simple, robust, graduated in litres and relatively inexpensive. In order to standardise the methodology a consistent strategy was employed when extracting samples. Once the pit was excavated water was extracted at the maximum rate possible so that the water drained down to the base. Finer sediments were extracted with the last of the water, leaving the coarser grains behind. This should produce the maximum number of invertebrates while minimising the quantity of sediment in the sample. Once the pit had refilled this process was repeated until 20 L had been collected. Pit refill rates varied from extremely rapid, requiring the deployment of two pumps simultaneously, to very slow (approximately 20 minutes to collect a sample). At one site the hyporheic throughflow was so rapid that standing ripples were visible on the surface of the water. At two sites the sediments were so compacted that no water entered the pit after excavation to a depth of 40 cm. Each 5 L aliquot was passed through a 63 µm sieve and the resultant sample placed in a 125 mL bottle so that there was approximately $^1/_3$ sediment, $^1/_3$ water and $^1/_3$ air present. The bottle was labelled with site abbreviation (Table 3), sample type and number. Standard physicochemical variables (DO, temperature, conductivity and pH) were measured immediately after the collection of the invertebrate sample. A sample of water was retained for chemical analysis.

Figure 3 *Sample extraction*
A – extraction of sample from a pit; B – insertion of sample pipe; C – extraction of pipe sample. River Allan at Bridge of Allan (site ALL)

Andrew Boulton (pers. comm.) suggested the use of heavy-duty plastic electric conduit for sampling pipes rather than the more rigid piezometers or specially designed metal sample tubes more widely employed in the literature. His rationale for this was that the narrower bore allowed the pipe to flex and more easily work its way through coarse substrates. The equipment developed (Figure 4) proved reliable, robust, lightweight and effective. A length of conduit was slid over the pipe driving rod (Figure 4B) and a heavy-duty driving cap (Figure 4C) fitted over the top of that. A sledgehammer was then used to drive the entire assembly into the sediment. When the 50 cm mark on the pipe reached the water level in the pit the driving cap was removed and the pipe insertion rod carefully removed to avoid disturbing the sediment. Although the fit of the rod in the pipe was very good occasionally gravel would be forced between the two when driving them into the sediment, locking the one inside the other. A hole was drilled through the head of the driving bar allowing the use of a lever to free and extract the rod when this occurred. The pump was then attached to the pipe with a sealing bung and water extracted under vacuum.

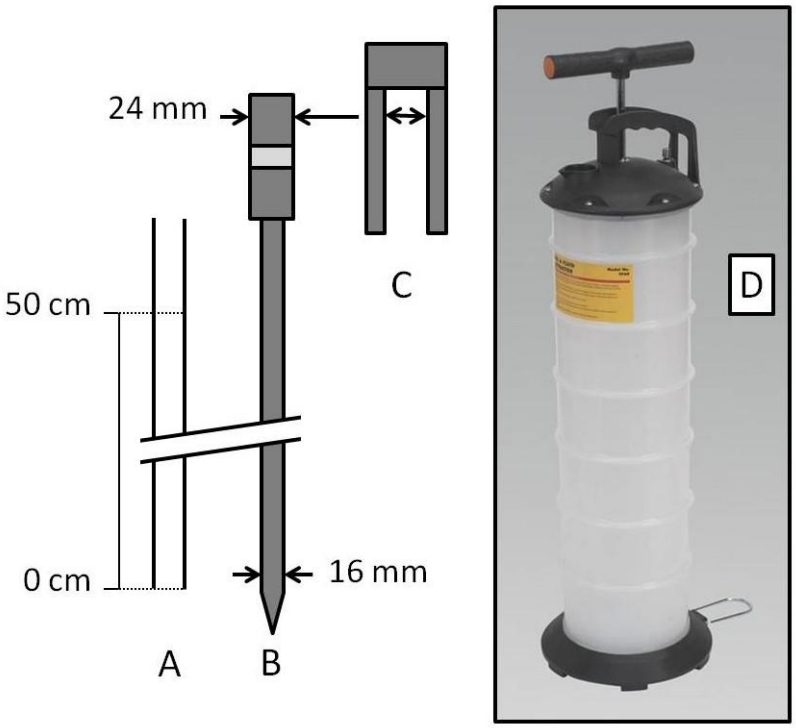

Figure 4 *Sampling equipment*
A –pipe; B – driving rod; C – driving cap; D – Sealey TP69 pump

In initial trials glitter was used as a tracer to determine potential contamination from the pit into the pipe. No flakes were found in the resulting samples indicating that the disturbance of the substrate caused by pipe insertion was not creating a significant downwards pathway for hyporheic fluids. The efficiency of this pump is extremely high and it was found that it often extracted considerable quantities of sands and finer gravels along with the water and invertebrates, particularly when inserted into finer sediments. This made sample sorting more difficult than for the pits, so it was decided to extract a smaller sample volume (10 L). The standard physicochemical variables were again measured immediately following extraction of the sample and a sample retained for chemical analysis. The piezometric difference between the water in the pipe and that in the pit was measured before removal of the pipe and restoration of the site to its original condition. The same four physicochemical variables were measured in the river water and a sample retained for analysis. All samples were kept refrigerated until analysis (including during transport). Invertebrates were

removed from the sample by live sorting under a stereomicroscope within 48 hours of sample collection.

2.2.3 Determination of the number of replicates

Once the sampling procedure had been finalised it was necessary to determine the appropriate number of replicates to be taken in order to obtain a representative sample. Multiple samples were collected at three contrasting sites:

- AFTON: Afton Water / Montraw Burn above Afton Reservoir (NS639032 and NS639035) – two adjacent acidic upland (altitude 415 m) streams with exposed gravel midstream bars approximately 30 m long by 4 m wide.
- TEITH: The River Teith below Doune Castle (NN729006) – an area of exposed gravel approximately 100 m long and 10 m wide along the side of a large, lowland, moderate alkalinity river.
- TUMMEL: The River Tummel / Tay confluence at Richard's Island (NN979510) – a large (700 x 250 m) gravel island with partially vegetated and wooded zones on a large low alkalinity piedmont river.

Eight samples were collected at the Teith and seven each at the Afton and Tummel. Invertebrates were sorted into broad taxonomic groups and counted. A summary of the invertebrates found in this survey is given in Table 3.

Taking into account the difference in sample volume it can be seen that the number of invertebrates found in the pits and pipes is comparable in both the Afton and the Teith. However, in the Tummel the number of invertebrates found in the pipes was greatly reduced. The fauna was dominated by

oligochaetes (22%), nematode worms (21%) and cyclopoid copepods (20%). A full breakdown of these data is given in Appendix 1.

	AFTON		TEITH		TUMMEL		Total
	Pits	Pipes	Pits	Pipes	Pits	Pipes	
Cyclopoid Copepoda	57	129	104	259	311	2	**862**
Harpacticoid Copepoda	85	62	92	26	166	4	**435**
Ostracoda	15	3	13	9	98		**138**
Cladocera	87	22	3	1	10		**123**
Gammaridae			65	73	12		**150**
Asellidae			7	13	11		**31**
Plecoptera	98	5	5	2	2		**112**
Ephemeroptera	42	5	3	1			**51**
Trichoptera	8		1	1			**10**
Coleoptera	5	2	1	1	2		**11**
Chironomidae	90	12	72	16	104		**294**
Collembola	1				4		**5**
Acari	12	47	46	3	43	1	**152**
Oligochaeta	341	129	53	31	407	13	**974**
Tricladida	19	2	14	2	35	4	**76**
Nematoda	104	76	283	42	423	4	**932**
Hydrozoa			2		2		**4**
Total	963	494	764	480	1630	28	**4359**

Table 3 *Number of invertebrates collected during the replicate determination survey*

The EstimateS statistical package (Colwell, 2009) was used to generate synthetic species accumulation curves from the data. These were then used to determine the optimum number of samples to be taken in order to obtain a representative sample of the biota from a site (Figure 5). The three sites exhibited different trends. The Teith sample was the most diverse with 19 groups

present. However, it was calculated that there would be negligible further increase in richness with increasing samples. The Afton site had the lowest diversity, and it was calculated that increased sampling would add only a few groups to the total. The River Tummel site was of intermediate richness, but it was calculated that increased sampling would produce a considerable number of extra groups.

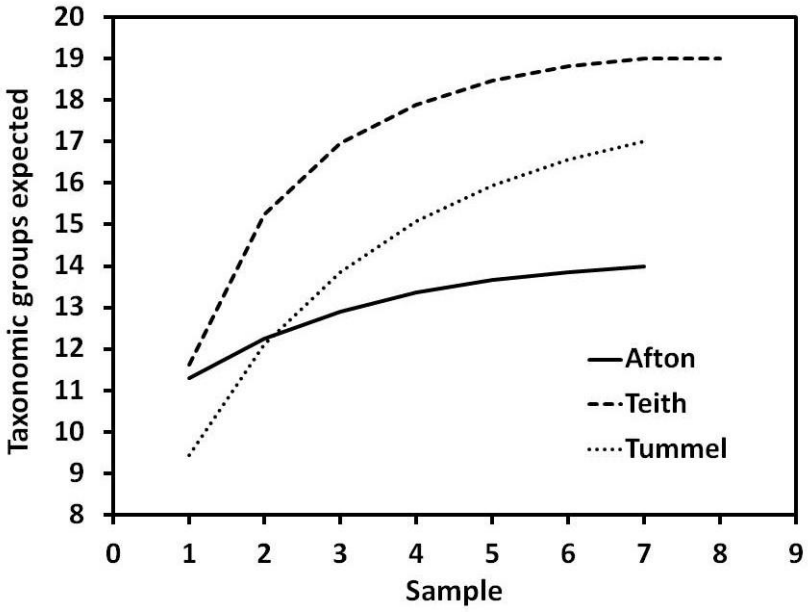

Figure 5 *Synthetic species accumulation curves derived from multiple replicates at three sites*

The number of samples to be taken was then assessed. Three paired samples was deemed too low to be representative. In order to assess the improvement in sampling efficiency the percentage increase in the number of groups predicted was compared. Increasing the number of samples from three to four would give an increase in the number of groups of 3.3, 5.0 and 7.2% respectively at Afton, Teith and Tummel. Increasing the number of samples to five would produce increases beyond that of 2.1, 3.0 and 5.1% respectively. After discussion it was decided to take four paired samples. Increasing the number of sub-samples to five would also have caused logistical problems for the main survey: by taking four sub-samples it was possible to collect two complete samples per day; increasing the number to five would have meant that only one site could be sampled per day.

In order to reduce potential human bias in sub-sample point selection it was decided that samples would be collected from four specific points on an exposed bar, these were:

US: The upstream end;
MU: Middle-upstream – approximately $^{1}/_{3}$ of the distance down the bar;
MD: Middle downstream – approximately $^{2}/_{3}$ of the distance down the bar;
DS: The downstream end.

These are illustrated in a near-ideal example in Figure 6. Here it was only possible to sample the edge of the bar along the smaller of the two channels as the side of the bar adjacent to the main one was too steep and tall to permit pit excavation. It was not always possible to collect samples from these precise points, however, all sub-samples were allocated to one to allow comparison (*e.g.* at site ALL it was only practical to sample at sites classified as US, MD, DS and DS).

On 13 occasions (out of 100 pipe samples) no fluid could be extracted. These were treated as null samples – permeability in the substrate was so low that no viable hyporheic community was likely to be present. On two occasions (out of 100 pits) there was no ingress of water after considerable sediment extraction (despite there being standing or flowing surface water within 2 m in each case). These were also treated as null samples and a pipe was inserted to 50 cm below the lowest point of excavation before attempting extraction of a sample; in both cases this was successful.

Throughout this thesis three different terms (site, sample and sub-sample) are used to refer to the three distinct levels of sampling, these are defined as:

- Sub-sample – an individual pit or pipe, or the four combined samples of each from a site.
- Sample – a pit and pipe combined.

- Site – a combined sample (four pits and four pipes) from one of the 25 sample sites.

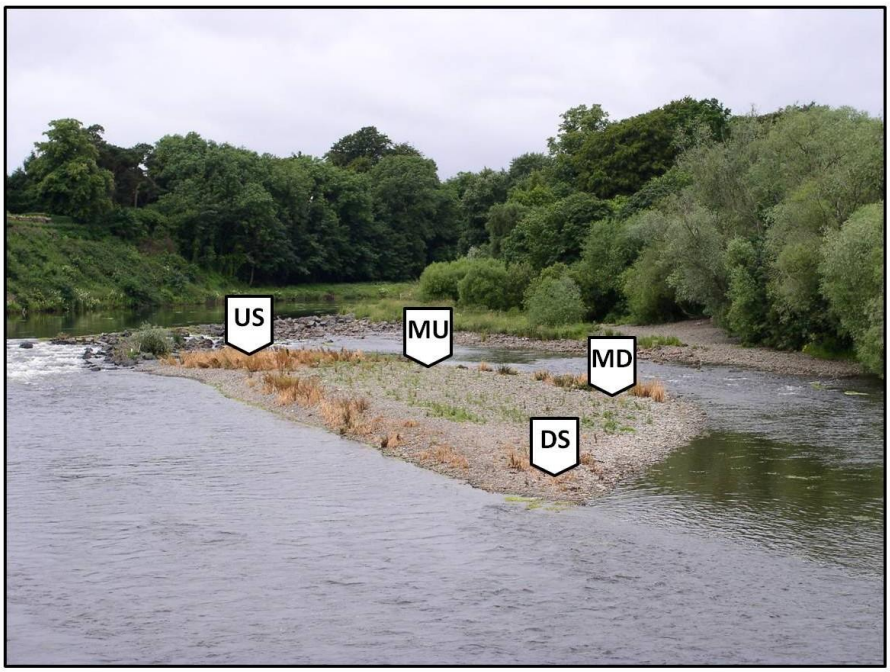

Figure 6 *An example of sub-sample location selections*
The River Tweed at Melrose (site TWM): US – upstream; MU – middle upstream; MD – middle downstream; DS – downstream

2.2.4 Digital gravelometry

In order to save time during fieldwork and to avoid the logistical problems of transporting large volumes of sediment to the laboratory it was decided to investigate the use of digital gravelometry for quantifying particle size distributions. Prior to collecting a sample the surface sediment was photographed. A standard scale, labelled with the sub-sample number, was included in the image to facilitate later processing with digital gravelometry software (Sedimetrics® Digital Gravelometer v10.0; http://www.sedimetrics.com).

The software only became available for use some time after completion of the field work. It was found that the procedure followed for recording the surface sediment was not ideal for processing

by this software. To obtain best results a quadrat of known dimensions should have been used. It was therefore necessary to determine the physical size of the area of ground represented in the photograph. The inclusion of a standard scale (a piece of whiteboard, 200 x 125 mm) in each image enabled this to be calculated.

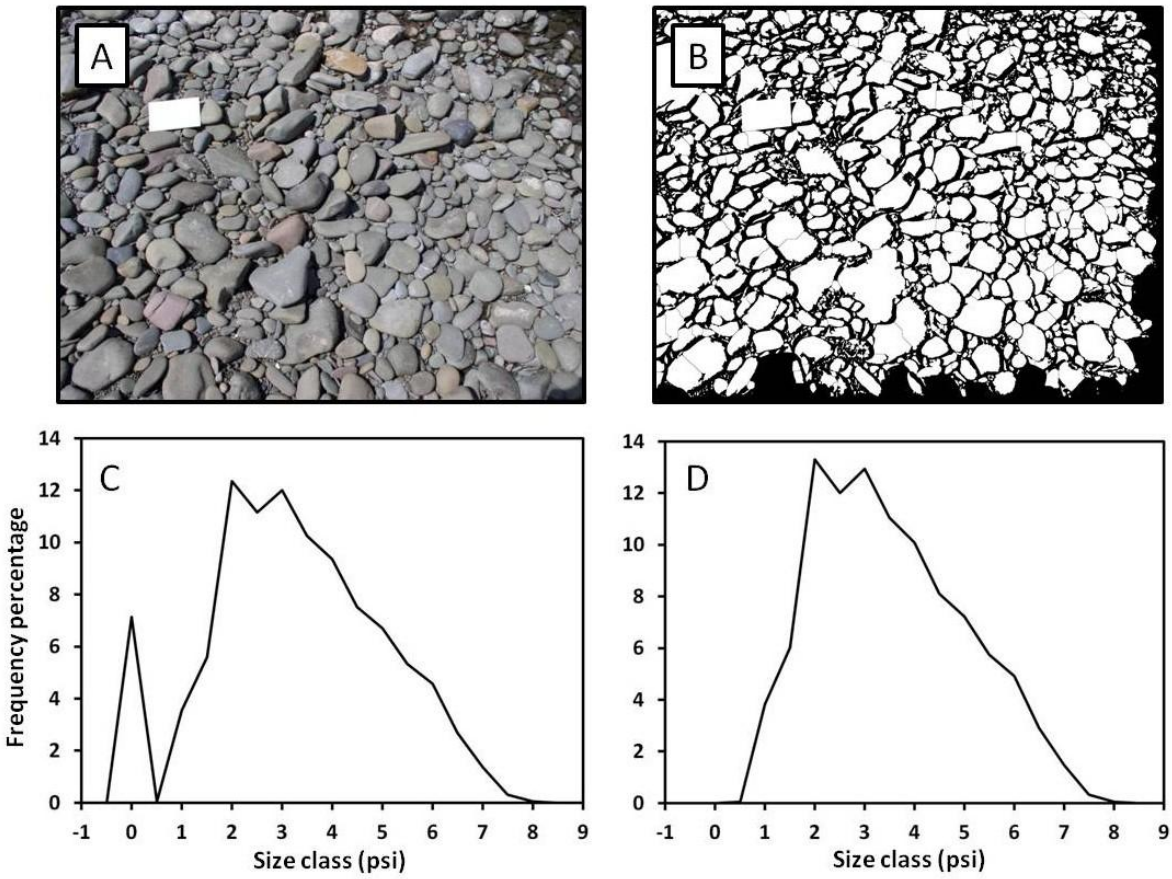

Figure 7 *Digital gravelometry processing at site TWM3*
A – original image; B – processed image; C – original histogram; D – histogram after final processing

The software rectifies the portion of the image selected for analysis (*i.e.* removal of perspective effects incorporated by not taking the image perpendicular to the ground) and rotates it if necessary. Edge detection and contrast manipulation algorithms then reduce the image to a version with individual grains in white on a black background. As the dimensions of the area selected are known the software then processes each of the grains in turn to determine the lengths of their *a* and

b axes, equivalent diameter, surface area, orientation and eccentricity (a measure of roundness). An example of an original image and the processed output image are shown in Figure 7 (A and B). One small issue with the software was that a small amount of noise (specifically any 'grains' with a dimension of one pixel along either of its axes) were always included in a size class below that of the smallest 'true' grain in the image thus producing a spike in the data (Figure 7C). The data was reprocessed in a spreadsheet to remove the extraneous noise; a single grain that most closely approximated to 200 x 125 mm (the size of the scale) was also removed. The frequency percentages of the size classes were then recalculated (Figure 7D).

2.2.5 Data collection and sample analysis

The following additional data were collected with each sample:

- A high-quality photograph of the sediment prior to excavation of the pit.
- Dissolved oxygen (DO) was measured using a Hanna HI 9142 meter. This reading was always taken first after collection of the sample to avoid potential contamination by atmospheric oxygen. Experiments were conducted to determine the extent of potential contamination and they showed that there was essentially no diffusion from the atmosphere for several minutes after sample collection.
- Water temperature values were collected immediately after the DO reading using the temperature function of a Hanna HI 8124 pH meter. This was again to avoid heating or cooling by holding the water for extended periods under different environmental conditions.
- pH was measured using a Hanna HI 8424 meter.
- Conductivity was measured using a Hanna HI 98129 Combo EC / pH meter. This also acted as a backup in case of failure of the HI 8124 pH meter.
- DO, water temperature, conductivity and pH were measured in the river water.

- Air temperature.
- Grid reference. The location of each sample was recorded to 10 m accuracy using a Magellan 2000 handheld GPS unit.
- River width (bankfull height) was measured using a Leupold RX-III handheld laser rangefinder. Four measurements were taken approximately 1 river width apart and then averaged.
- General site photographs were also taken.

The pH, conductivity and DO meters were calibrated every morning prior to fieldwork.

The following information was later determined for each site from maps:

- Site altitude.
- Source altitude.
- Distance to source.
- Catchment slope (from the source to the sample site).
- Reach slope (between the two 10 m contours upstream and downstream of the site).

The following chemical analyses were undertaken:

- Nitrate, nitrite and ammonia concentrations were determined with a Bran+Luebbe AutoAnalyser 3.
- Total dissolved phosphate was determined colorimetrically using the ascorbic acid technique: reaction with ammonium molybdate and antimony potassium tartrate in an acid medium.

- Total alkalinity was determined by titration with 0.01 M hydrochloric acid using BDH 4.5 indicator.

Invertebrates were identified to the highest taxonomic level possible. Insects were generally identified to species level. However, as a result of the large number of early instars present they were only analysed at family level. All Cladocera, harpacticoid copepods and mature female cyclopoid copepods were identified to species level. The following keys were used:

- General invertebrates – Tachet, 2006.
- Plecoptera – Hynes, 1993.
- Ephemeroptera – Elliott *et al.*, 1988.
- Trichoptera – Edington & Hildrew, 1995; Wallace *et al.*, 2003.
- Crustacea (Malacostraca) – Gledhill *et al.*, 1993.
- Crustacea (Cladocera) – Scourfield & Harding, 1966.
- Crustacea (Copepoda) – Gurney, 1931, 1932, 1933; Dussart, 1967, 1969; Einsle, 1996.

Ostracod identification was attempted using Henderson (1990); however, no positive identifications could be made as a result of the low number of mature specimens present.

2.3 Long term monitoring

Two attempts were made to set up long term monitoring sites with fixed steel piezometers that could be visited monthly to assess seasonal changes in water chemistry and composition of the hyporheos. At the first site (the River Teith downstream of Doune, NN723005) a spate after three months sampling damaged the piezometers to such a degree that it had to be abandoned. A second site was set up (Afton Water above Afton Reservoir, NS639032) at a remote upland stream where

spates were less likely to occur. However, snowfall and low temperatures over the winter of 2009-10 meant site access was so limited that this also had to be abandoned.

2.4 Sample sites

A total of 25 sites were sampled from spring to autumn 2008. The sites were selected to cover most river types present in Scotland (montane, upland, lowland, east coast draining, west coast draining) and also to cover the latitudinal gradient from south to north. A list of the sites with their abbreviations is given in Table 4, a map in Figure 8 and a summary of their chief physical characteristics in Table 5.

The sites were selected to cover a range of environmental qualities from the most natural systems available to more degraded examples. Two sites (CRE and TIG) have catchments with significant areas of commercial coniferous forestry in their upper reaches. The catchments of two sites have significant urban zones upstream of the sampling point (ALE and CAD). Five sites are in areas of upland sheep farming (ALL, BLA, DEV, EAR and TEC). The remaining 16 sites are relatively undisturbed.

In order to determine if there was a relationship between the conservation status of rivers and the underlying HZ, sites were selected from rivers that had and did not have SAC status (13 and 12 sites respectively). The sites with SAC status are listed below along with the primary reasons for their designation.

- River Bladnoch (BLA): Atlantic salmon (*Salmo salar*).

- River Spey catchment (FEB, FEF, FEU, MOR, SPD, SPK, TRU): Freshwater pearl mussel (*Margaritifera margaritifera*); Sea lamprey (*Petromyzon marinus*); Atlantic salmon (*S. salar*); Otter (*Lutra lutra*).
- River Teith (TEC, TED): Sea lamprey (*P. marinus*); Brook lamprey (*Lampetra planeri*); River lamprey (*Lampetra fluviatilis*).
- River Tweed (TWM): water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation; Atlantic salmon (*S. salar*); Otter (*L. lutra*).
- Tummel Shingle Islands (TUP): alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*).
- Inverpolly (KIN - the River Kirkaig forms the northern boundary of this area): oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and / or of the *Isoëto-Nanojuncetea*; natural dystrophic lakes and ponds; northern Atlantic wet heaths with *Erica tetralix*; blanket bogs; transition mires and quaking bogs; depressions on peat substrates of the *Rhynchosporion*; Otter (*L. lutra*).

2.4 Statistical analysis

Basic statistical calculations and optima calculations were carried out using MS Excel 2007. Analysis of variance (ANOVA), general linear models and regression were calculated using IBM SPSS 19. For ANOVA results the basic results are given in the text and a full breakdown in Appendix 5. Canonical Correspondence Analysis (CCA) was calculated using Canoco for Windows 4.5. Synthetic species accumulation curves were calculated using EstimateS 8.2.0 (Colwell, 2009).

Site	River	Location	NGR	Latitude (°N)	Longitude (°E)
ALE	Almond	Edinburgh Airport	NT156752	55.963	3.350
ALL	Allan	Bridge of Allan	NS789967	56.148	3.950
BLA	Bladnoch	Spittal	NX359577	54.888	4.558
CAD	Carron	d/s Denny	NS834816	56.014	3.870
CRE	Cree	Newton Stewart	NX415647	54.952	4.475
DEV	Devon	u/s Tillicoultry	NS939972	56.157	3.707
EAR	Earn	Dupplin	NO061188	56.353	3.519
END	Endrick	Fintry	NS615868	56.054	4.224
FEB	Feshie	Ballintean	NH843012	57.088	3.909
FEF	Feshie	Feshie Fan	NH843060	57.131	3.911
FEU	Feshie	u/s Carnachuin	NN843927	57.011	3.904
INV	Inver	Little Assynt	NC153250	58.176	5.140
KIN	Kirkaig	Inverkirkaig	NC084193	58.122	5.253
LIN	Loannan	Inchnadamph	NC247199	58.134	4.977
MOR	Abhainn Ruigh-eunachan	u/s Loch Morlich	NH983092	57.163	3.680
NEV	Nevis	Glen Nevis	NN136701	56.786	5.051
ROY	Roy	Glen Roy	NN301876	56.949	4.792
SPD	Spey	Dalnavert	NH850066	57.137	3.900
SPK	Spey	Kingussie	NN759998	57.073	4.046
TEC	Teith	Carse of Lecropt	NS761971	56.151	3.993
TED	Teith	Doune	NN769006	56.181	4.047
TIG	Water of Tig	Heronsford	NX116835	55.111	4.954
TRU	Truim	Dalwhinnie	NN640853	56.940	4.235
TUP	Tummel	d/s Pitlochry	NN962554	56.679	3.695
TWM	Tweed	Melrose	NT543345	55.603	2.724

Table 4 *Sample site locations*

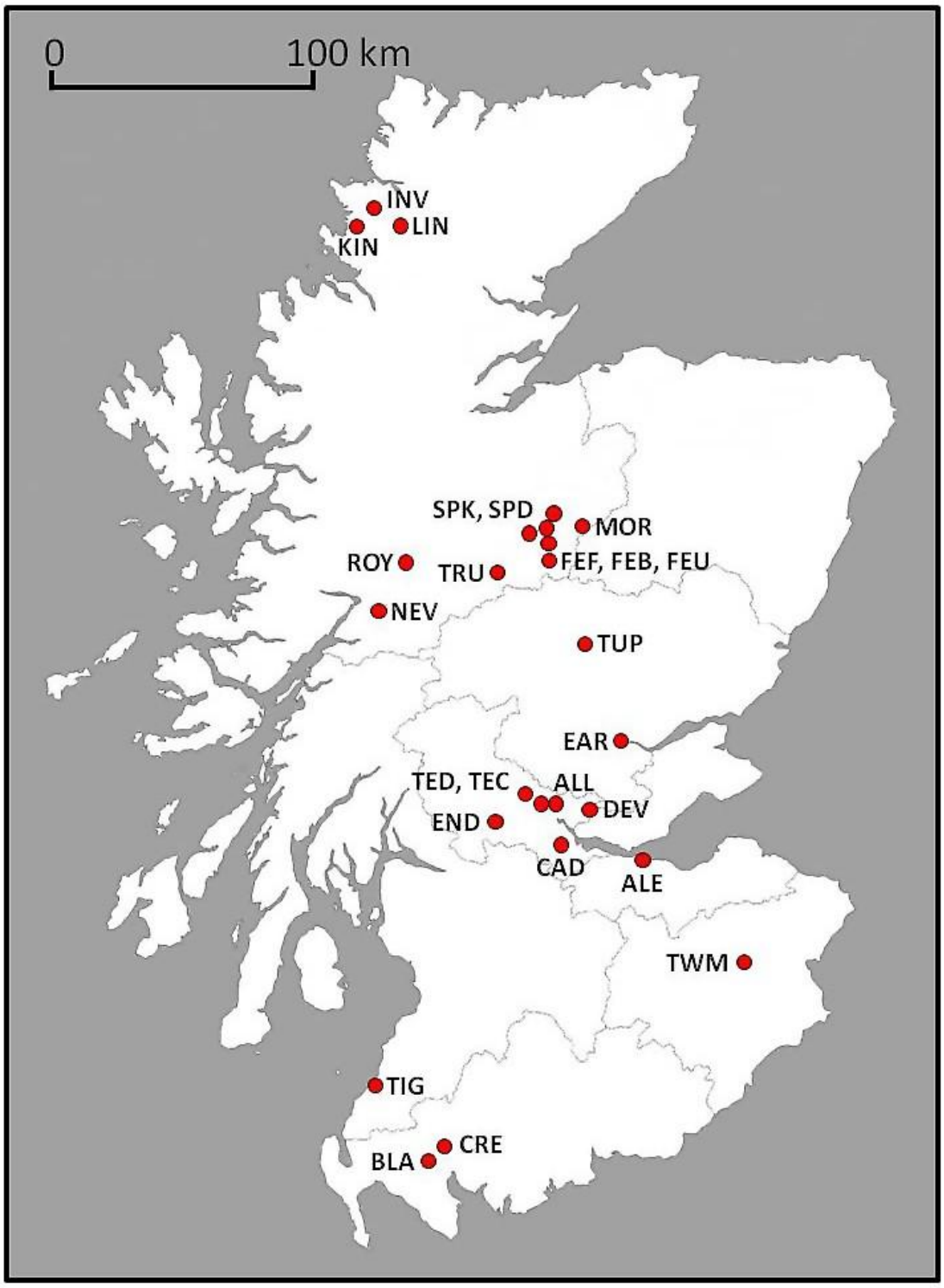

Figure 8 *Map of sample sites*

Labelled as in Table 4

3 RESULTS

3.1 Environmental variables

A summary of the main physical characteristics of the sample sites is given below (Table 5).

Site	Width (m)	Altitude (m)	Source altitude (m)	Reach slope (m km^{-1})	Catchment slope (m km^{-1})	Distance from source (km)
ALE	14.4	25	275	1.21	5.56	45.0
ALL	19.8	5	426	2.25	12.46	33.8
BLA	18.5	18	137	2.70	4.51	26.4
CAD	23.7	18	331	2.44	12.67	24.7
CRE	33.3	5	330	7.41	8.51	38.2
DEV	20.3	19	539	2.47	15.38	33.8
EAR	42.3	10	731	0.66	10.87	66.3
END	15.7	82	434	5.13	23.16	15.2
FEB	73.2	276	1135	6.40	28.97	29.7
FEF	158.1	222	1135	9.52	26.09	35.0
FEU	98.9	360	1135	8.08	38.75	20.0
INV	45.7	64	455	2.09	16.50	23.7
KIN	24.4	5	382	12.90	12.96	29.1
LIN	18.6	75	455	8.70	40.00	9.5
MOR	13.2	330	1058	10.26	97.72	7.5
NEV	46.5	29	920	6.35	48.56	18.4
ROY	33.1	169	945	9.52	40.21	19.3
SPD	56.7	208	765	1.15	9.00	61.9
SPK	26.0	222	765	0.88	11.27	48.2
TEC	47.5	5	536	0.69	8.36	63.5
TED	37.9	14	536	4.17	8.97	58.2
TIG	11.4	19	317	14.29	20.48	14.6
TRU	18.9	349	920	12.90	47.58	12.0
TUP	84.9	69	635	2.17	6.38	88.7
TWM	57.8	81	546	2.42	5.54	83.9
Min	11.4	5	137	0.66	4.51	7.5
Max	158.1	360	1135	14.29	97.72	88.7

Table 5 *Physical characteristics of the sites*

Elements of this dataset (altitude, source altitude, reach slope and distance from source) were used in the ordination technique of Jeffers (1998) to split the data into three groups for comparison

purposes (Figure 9). Three sub-equal groups were defined on the basis of PCA component 1 which is an analogue for the degree of montane influence:

Lowland (PCA1 < 0): ALE, ALL, BLA, CAD, CRE, DEV, EAR, KIN, TEC, TED

Upland (PCA1 > 0; < 1): END, INV, LIN, SPD, SPK, TIG, TUP, TWM

Montane (PCA1 > 1): FEB, FEF, FEU, MOR, NEV, ROY, TRU

The only exception to this rule was site NEV (River Nevis due south of Ben Nevis summit) for which an anomalous value is calculated using this procedure. This is a result of the peculiar geography of the catchment in that the upper portions of the mountains here are naturally without deep soils so stream sources occur at much a lower elevation than would normally be the case.

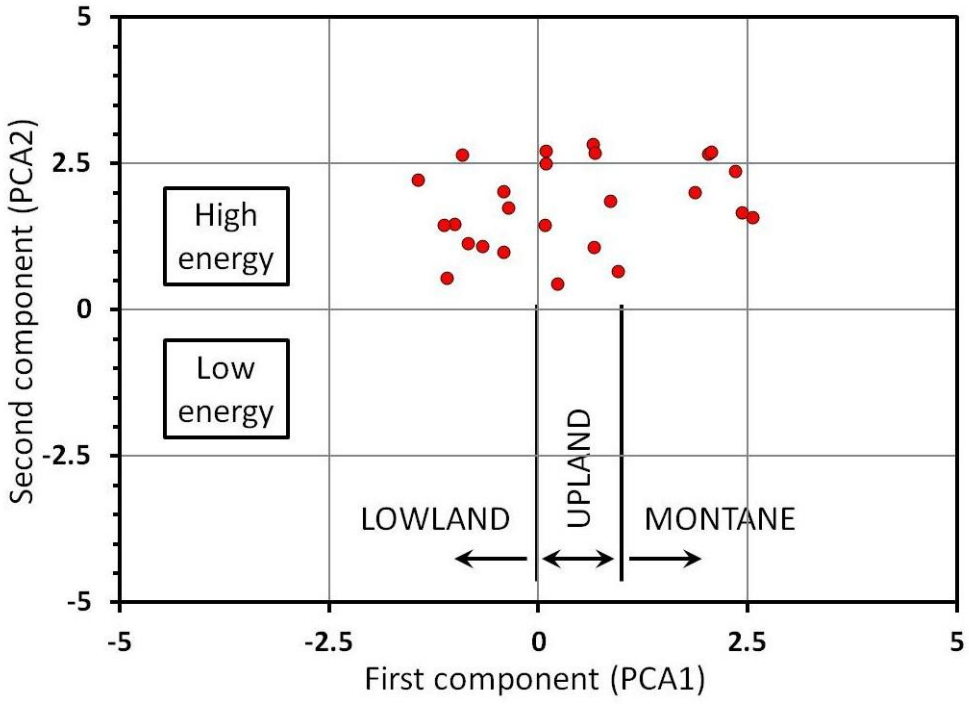

Figure 9 *Characterisation of sample sites using the Jeffers (1998) ordination procedure*

The average water temperature from pits reflected ambient weather conditions. The average temperature from the pipe sample reflected conditions form the recent past. In 12 of the 25 sites

the average water temperature in the pits was higher than that from the pipes; at in the remaining 13 it was lower.

There were significant differences in water chemistry between sites (Table 7). Conductivity, pH and total alkalinity were generally similar in all of the sub-samples at site level. Nitrite and ammonia were very variable between sub-samples and nitrate and total dissolved phosphate showed an intermediate level of within-site variability.

Graphs showing changes in water chemistry between lowland, upland and montane sites and by bar position are given in Appendix 3(B). All chemical parameters measured, exceptig nitrite, showed significant differences between the three groups (Table 6). Conductivity, pH, nitrate, total dissolved phosphate and total alkalinity increased from montane to lowland while DO and ammonia decreased.

Parameter	**F**	**P**
Conductivity	(2, 182) = 20.384	0.000
pH	(2, 182) = 3.945	0.021
DO	(2, 178) = 64.117	0.000
Nitrate	(2, 182) = 32.190	0.000
Nitrite	(2, 182) = 1.529	0.219
Ammonia	(2, 182) = 35.424	0.000
Total Alkalinity	(2, 182) = 23.501	0.000
Total dissolved phosphate	(2, 182) = 18.018	0.000

Table 6 *ANOVA results from a comparison of chemical parameters at lowland, upland and montane sites*
See Appendix 5A for full statistical results

Chemical changes along the gravel bar from the upstream end to the downstream were more subtle. Conductivity and total alkalinity both increased slightly along the profile; pH, dissolved oxygen,

nitrate and nitrite both decreased downstream. The only parameter that showed statisically significant differences between bar positions was DO (ANOVA: F (3, 177) = 6.063, P = 0.001; see Appendix 5B) where values decreased downstream with bar position.

Site	pH	Cond. (μS)	Temp. (°C)	DO (%)	Alkalinity (meq L^{-1})	Nitrate (μg L^{-1})	Nitrite (μg L^{-1})	Ammonia (μg L^{-1})	Phosphate (μg L^{-1})
ALE	7.31	864	15.7	32.1	3.07	1373	10.63	5.02	115.5
ALL	7.63	247	19.9	33.6	2.26	802	0.00	0.00	226.2
BLA	6.39	117	15.9	45.3	0.35	325	4.68	7.15	76.0
CAD	6.98	156	14.7	45.0	1.00	764	2.55	11.12	196.5
CRE	6.26	64	15.4	49.3	0.22	320	25.36	13.58	38.9
DEV	7.10	147	15.1	51.0	1.35	449	1.29	12.25	141.2
EAR	6.62	90	7.5	87.6	0.69	1203	4.79	11.24	37.0
END	6.61	78	12.0	59.5	0.68	286	56.27	23.59	45.6
FEB	6.94	25	13.1	95.0	0.24	148	6.17	49.31	21.3
FEF	6.89	27	13.8	90.3	0.23	170	8.59	36.03	28.2
FEU	6.55	18	12.0	97.0	0.21	148	4.74	60.77	13.7
INV	6.91	105	13.5	55.8	0.59	382	5.18	8.02	0.0
KIN	6.39	114	14.5	54.3	0.35	128	4.62	17.54	0.0
LIN	7.03	179	19.2	53.2	1.73	331	5.29	11.56	12.3
MOR	6.50	28	10.6	89.4	0.17	140	0.12	81.74	11.2
NEV	6.36	20	16.4	90.1	0.15	114	4.31	57.62	11.8
ROY	6.79	32	16.4	87.0	0.32	111	8.37	29.96	12.8
SPD	6.35	69	13.8	35.1	0.41	274	1.98	12.40	39.2
SPK	6.37	56	13.8	57.1	0.31	342	1.02	10.82	41.1
TEC	6.59	74	8.0	62.4	0.52	574	6.53	32.94	40.2
TED	6.83	57	17.0	64.4	0.37	393	5.90	6.94	45.0
TIG	7.15	119	13.3	61.9	0.43	330	3.59	14.52	50.6
TRU	6.64	45	9.4	83.6	0.32	170	0.23	102.60	8.7
TUP	6.91	43	15.8	55.7	0.32	442	6.64	31.75	12.2
TWM	7.48	154	15.3	56.9	1.22	1036	26.82	2.55	155.5
Min	6.26	18	7.5	32.1	0.15	111	0.00	0.00	0.00
Max	7.63	864	19.4	97.0	3.07	1373	56.27	102.60	226.2

Table 7 *Mean values for environmental variables at each site*
A more comprehensive breakdown of these data is given in Appendix 3

Variability in piezometric head between the pit and pipe showed an interesting pattern when compared with sample position on the bar (Figure 10). At the upstream end of bars the predominant

trend was for water to be downwelling. This is to be expected as river water pushing against the bed at this point will create a negative vertical hydraulic gradient. However, the samples at this location also exhibit the greatest variability, some samples even being upwelling. At progressively further downstream positions on the bar the variability decreases in a stepwise manner and the average piezometric head increases to the middle downstream position. At the downstream end the vertical gradient decreases slightly to the point where there is effectively no vertical gradient present.

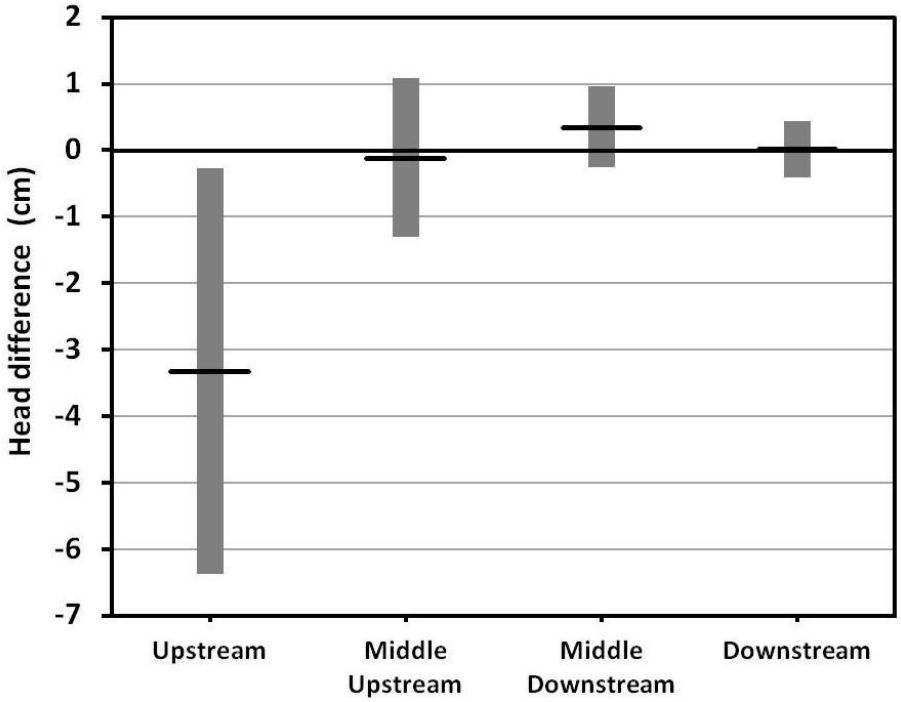

Figure 10 *Variability of piezometric head by position on gravel bar*
Mean (black line) and 95% confidence limits (grey bars)

3.2 Digital gravelometry

The software proved a useful way to rapidly characterise the nature of the sediment at a site. A total of 305,553 individual grains were measured by the software from the 100 images taken during the survey. The minimum number measured on an image was 1,462 and the maximum was 6,165. Examples of variation at a site scale are given in Figure 11.

The raw data was used to calculate percentile values for the particle size distribution (5, 10, 16, 30, 50, 70, 84, 90 and 95). Mean particle size, sorting, skewness and kurtosis were also calculated. Mean particle size varied from a minimum of 7.46 mm on the Abhainn Ruigh-eunachan above Loch Morlich (MOR) to 16.96 on the River Spey at Kingussie (SPK). One interesting observation was the variability in average eccentricity across the sites; the lowest value of 0.693 was found at the Water of Tig at Heronsford (TIG) and the highest value of 0.763 on the River Nevis (NEV). When considering the number of grains measured at these sites (8,293 and 11,298 respectively) this difference is quite remarkable.

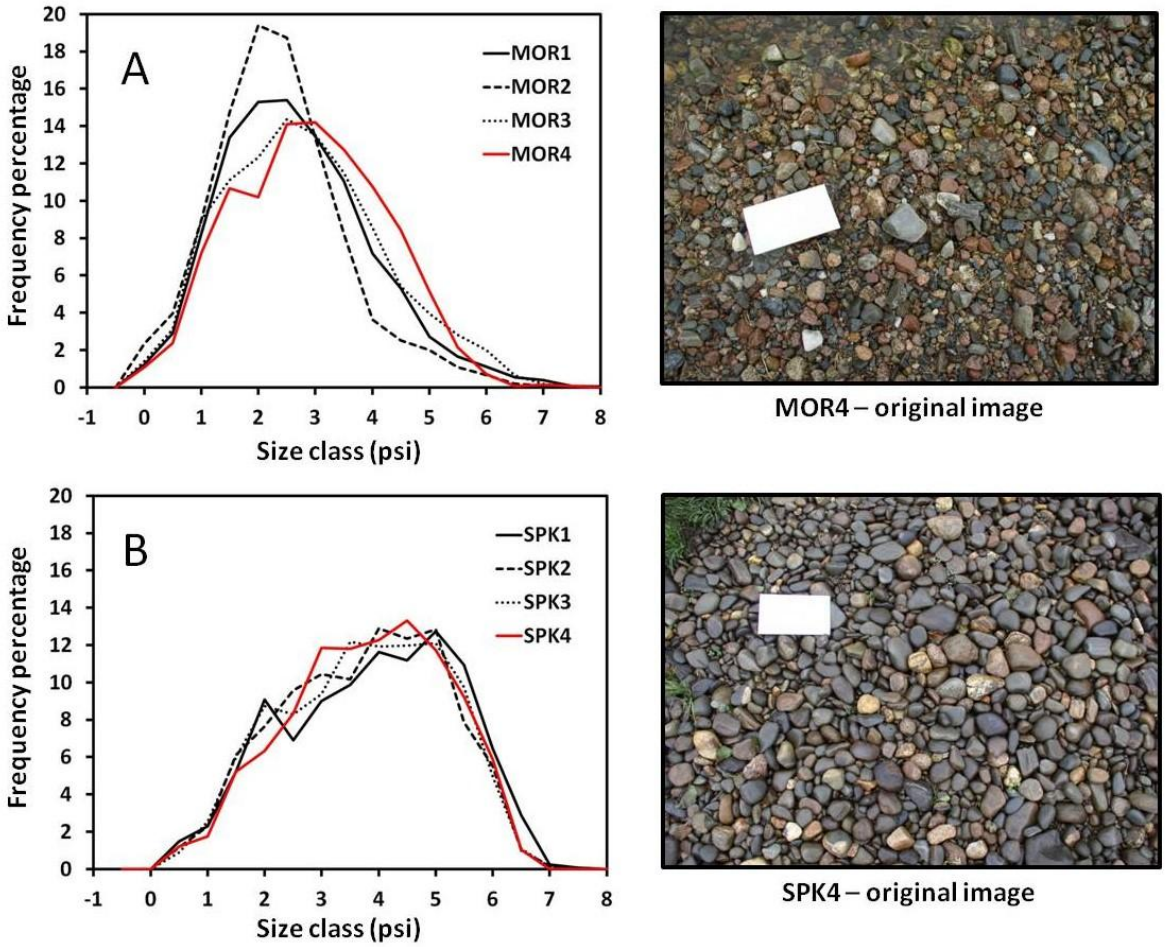

Figure 11 *Comparison of size frequency histograms from sites MOR (A) and SPK (B) Original images for the highlighted histograms are shown on the right*

The results clearly show differences in heterogeneity at a site level. As an example, Figure 11 shows the four histograms generated from sites MOR and SPK. It can clearly be seen that particle size distribution across the four locations at SPK is far more homogeneous than at MOR.

3.3 Invertebrate results

3.3.1 Group level data

A total of 10,257 invertebrates were collected during the survey, 9,453 (92.2%) from the pit samples and 804 (7.8%) from the pipes. A comparison of the key statistics from these data is given in Table 8. A more complete breakdown of the data is given in Appendix 3.

	Site total	Pits	Pipes
Maximum	658	645	148
Minimum	181	159	0
Mean	410.3	378.1	32.2
Standard deviation	161.3	150.1	35.1

Table 8 *Comparison of the total number of invertebrates present in the 25 samples*

One of the most notable features to emerge from this study has been the remarkable uniformity in the total number of invertebrates present in the shallow hyporheic zone – the maximum number found (at TED) is only 3.7 times that of the minimum (at KIN). The pipe samples were much more variable with three sites (BLA, INV and KIN) producing no invertebrates at all; the maximum number recovered was also from the Teith at Doune (148). Even though the pit and pipe samples were collected using different sampling methods and sample volumes (20 L and 10 L respectively) the disparity in these numbers suggests that the overall number of invertebrates in the hyporheic zone

in this region is greatest in shallow samples and decreases rapidly with increasing depth. Numbers also become more variable with depth.

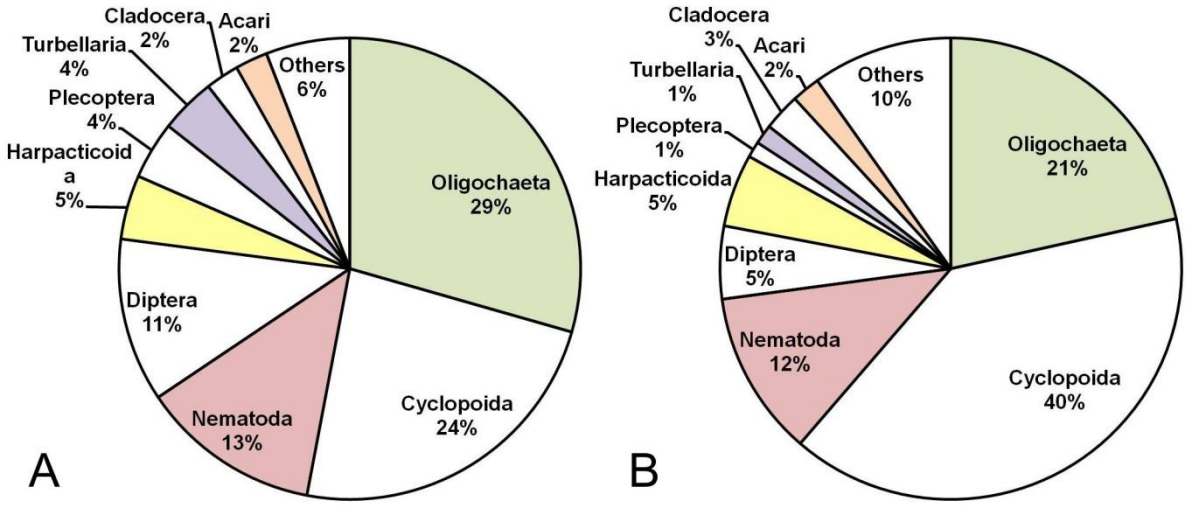

Figure 12 *Invertebrate composition of the two sample types*
A – pit samples; B – pipe samples

The faunal assemblage (Figure 12) was dominated by four major groups – Oligochaeta, cyclopoid Copepoda, Nematoda and Diptera which accounted for 77.2% of all invertebrates from pit samples and 78.0% from pipe samples. However, the relative proportions of these groups varied between the two sample types – in pits oligochaetes were the dominant group (29%) with cyclopoid copepods second (24%); pipes were dominated by cyclopoids (40%) and oligochaetes (21%). The coarse resolution composition of the faunal assemblage at a site level is illustrated in Figures 13-16. In each of these figures the sites are arranged from left to right in order of the PCA1 score generated from the Jeffers (1998) equation.

There was no statistically significant difference in the ratio of invertebrates from pit and pipe samples (Figure 13). There is, however, a tendency for the number of invertebrates recovered from the pipe samples in montane sites to be slightly higher and less variable than upland and lowland sites.

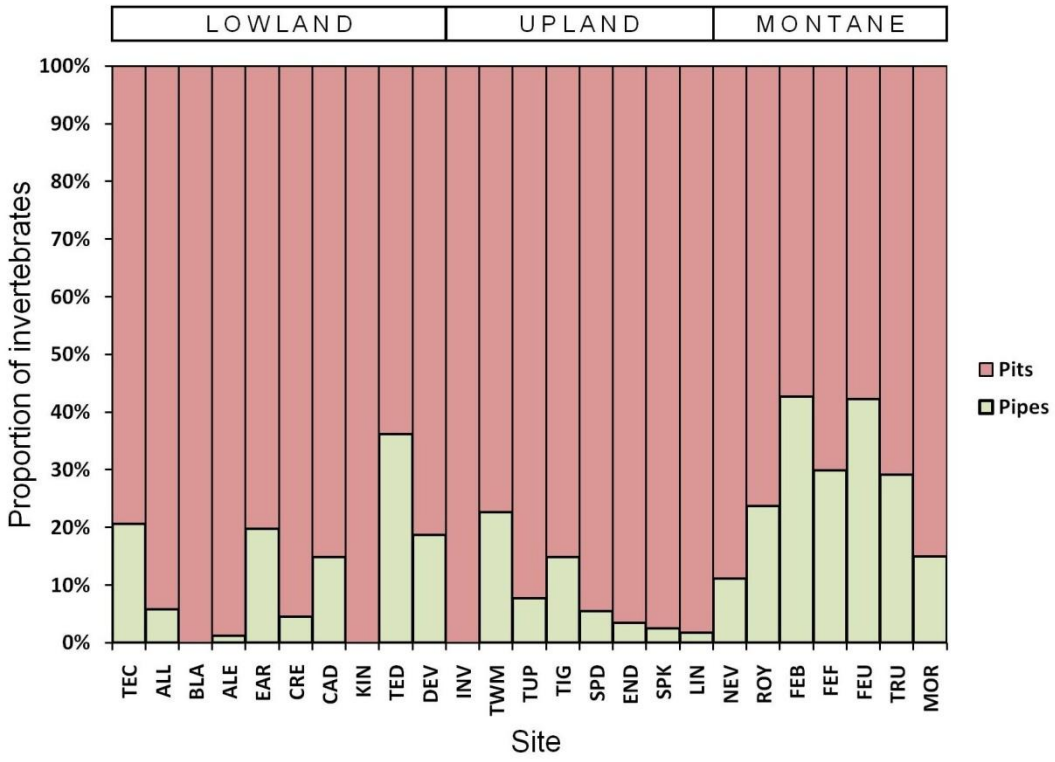

Figure 13 *Relative abundances of invertebrates in pit and pipe samples (adjusted for difference in sample volume)*

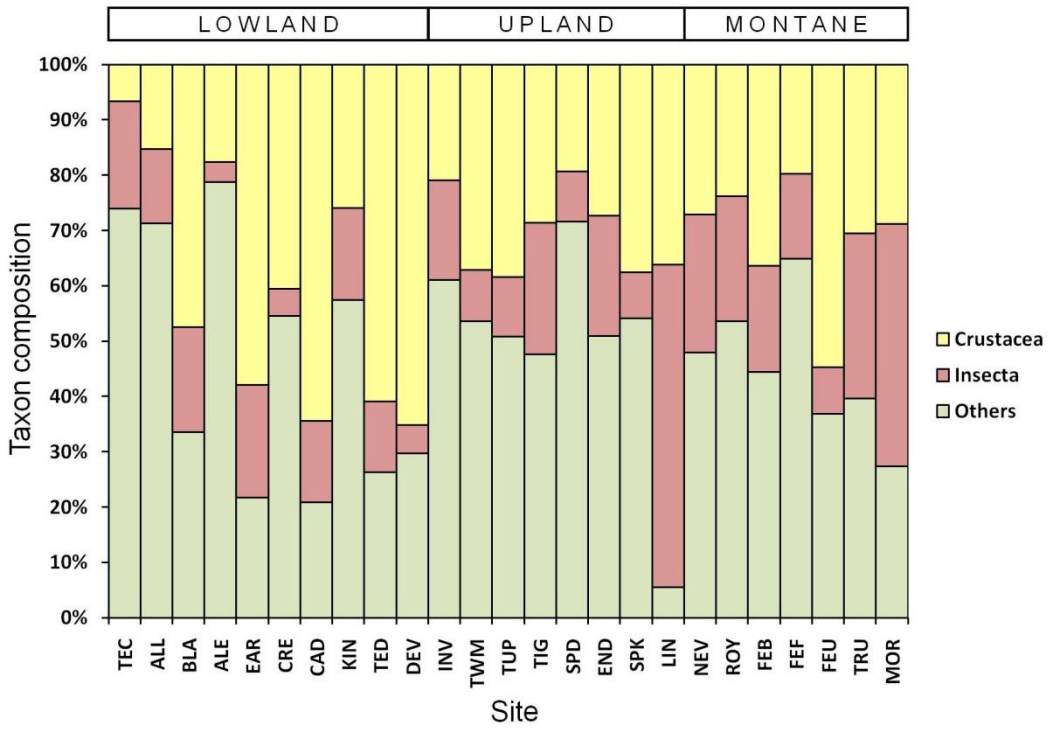

Figure 14 *Relative abundances of crustaceans, insects and other invertebrates in each sample*

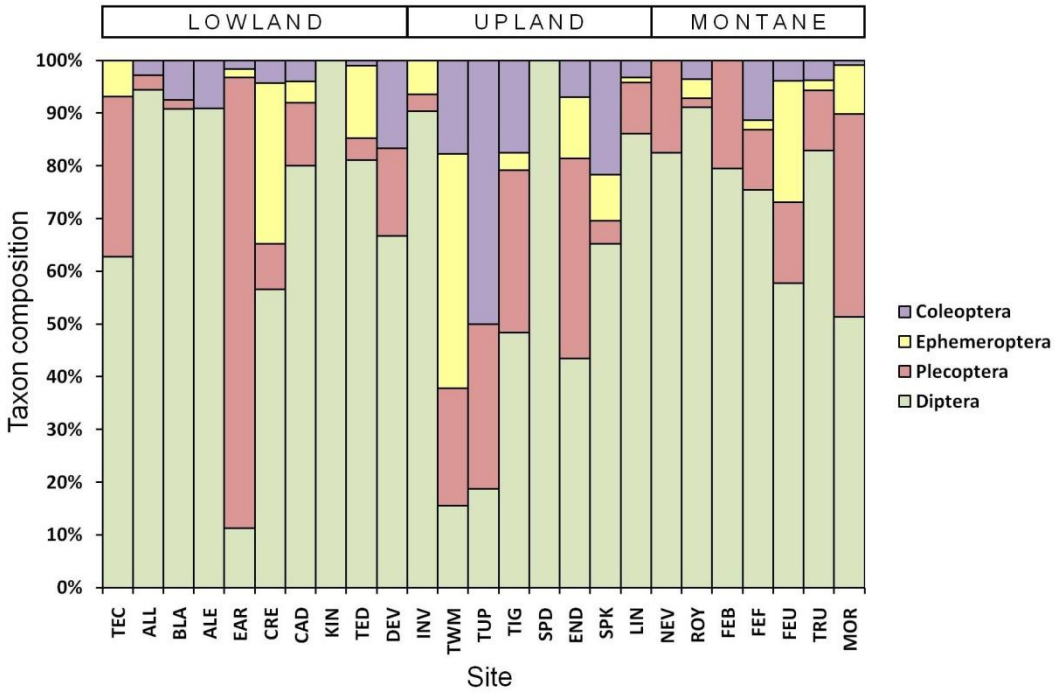

Figure 15 *Relative abundances of the four major insect groups in each sample*

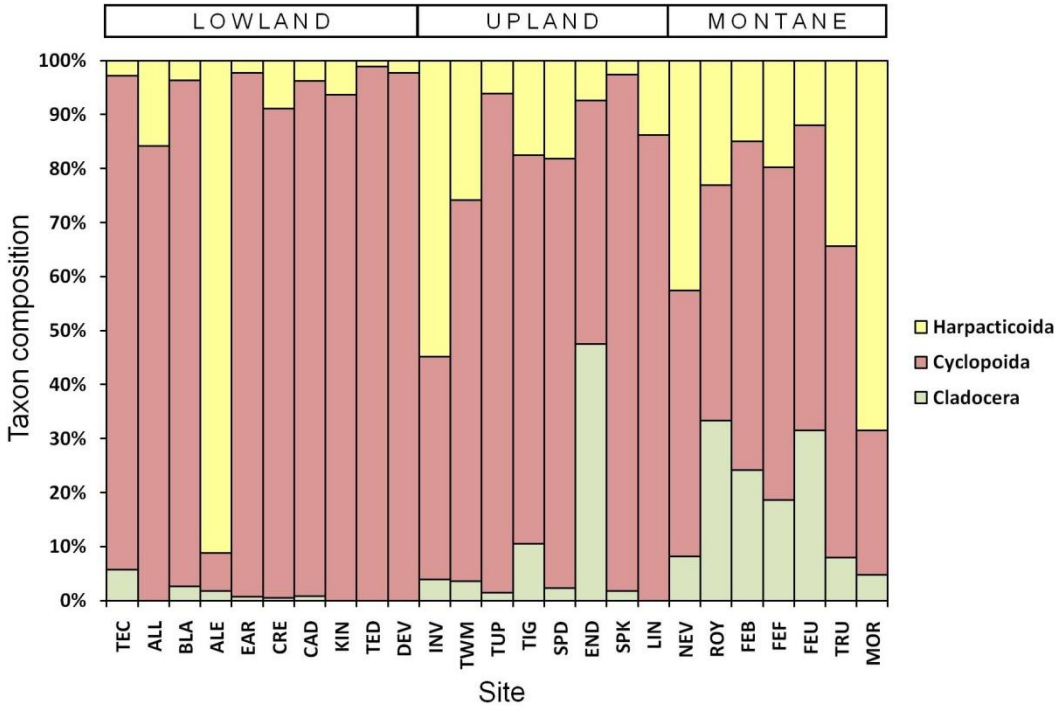

Figure 16 *Relative abundances of the three dominant microcrustacean groups in each sample*

When comparing the total number of crustaceans, insects and 'others' (worms, nematodes, flatworms, etc.) present at each site a more complex picture begins to emerge (Figure 14). In lowland sites the dominant fauna tends to be either crustaceans or 'others', with a variable, small, but fairly consistent number of insects. Upland and montane sites seem to have a more predictable fauna with approximately 30% crustaceans, 30-50% 'others' and a variable percentage of insects. There is some indication of an increasing proportion of insects with increasing PCA1 score, although this is not statistically significant.

The relative proportions of the four major insect groups (Coleoptera, Ephemeroptera, Plecoptera and Diptera) are given in Figure 15. No clear patterns are discernible in these data, although there appears to be a decrease in the proportion of Diptera in upland sites with a corresponding increase in the contribution of Coleoptera in this group.

The relative proportions of the three major groups of microcrustacea (harpacticoid copepods, cyclopoid copepods and Cladocera) are given in Figure 16. It can be seen that lowland and upland sites are dominated by cyclopoids with generally only a small contribution from the other two groups (site ALE, the Almond at Edinburgh Airport, is atypical, being the most heavily impacted of the sites studied). The proportion of harpacticoids and cladocerans tends to increase with increasing PCA1 score so that in montane sites the split is approximately 15% Cladocera, 25% harpacticoids and 60% cyclopoids.

3.3.2 Family level data

The insect families recovered during this survey represent a subset of the standard macroinvertebrate fauna recovered by traditional kick sampling. The vast majority of insects were very small early instar specimens which are missed with a standard 500 µm mesh kick net.

The Diptera were dominated by the Chironomidae (82.3%), the next largest family represented being the Ceratopogonidae (9.9%). The remaining 10 groups identified (comprising 7.8% of specimens) were patchily distributed with no group being found at more than five sites. Diptera from the pipe samples were dominated by Chironomidae with 36 specimens being recovered from 8 sites. The remaining Diptera from the pipe samples consisted of two specimens of ceratopagonids which were recovered from montane sites (MOR and FEB).

A total of 89 ephemeropteran specimens were recovered from the samples; these were from four families. Two of these, the Heptageniidae and Baetidae, were fairly widespread, occurring at 10 sites each and accounting for 43.8 and 28.1% of specimens, respectively. Caenidae were recovered from four sites; however, 16 of the 21 individuals recovered were from a single site (TWM). Four leptophlebiids were recovered from a single site (END). Of the ephemeropterans recovered 91.0% were from the pit samples; five heptageniids were recovered from pipe samples at three sites and three baetids were recovered from depth at a single site (MOR).

Plecoptera were dominated by Leuctridae (86.3%) and Nemouridae (8.7%). These were the only two families to be recovered from depth with three leuctrids being recovered from three sites and five nemourids from four sites; no site shared both these families at depth. Plecoptera were found to be the only invertebrate group suitable for assessing changes in size frequency distribution through the sampling period; other groups either occurred in too few numbers (Ephemeroptera, Trichoptera) or proved too difficult to measure (Diptera). Of the 336 specimens measured 77% belonged to the sub-2 mm size class, however, significant numbers of larger specimens were found including 5 in the 7 – 7.9 mm range (Figure 17A). The total number of specimens over 2 mm long generally declined through the sampling period (Figure 17B). This trend was reflected in the sub-2 mm class up to August, but September saw a very large increase in numbers with a slight drop again in November.

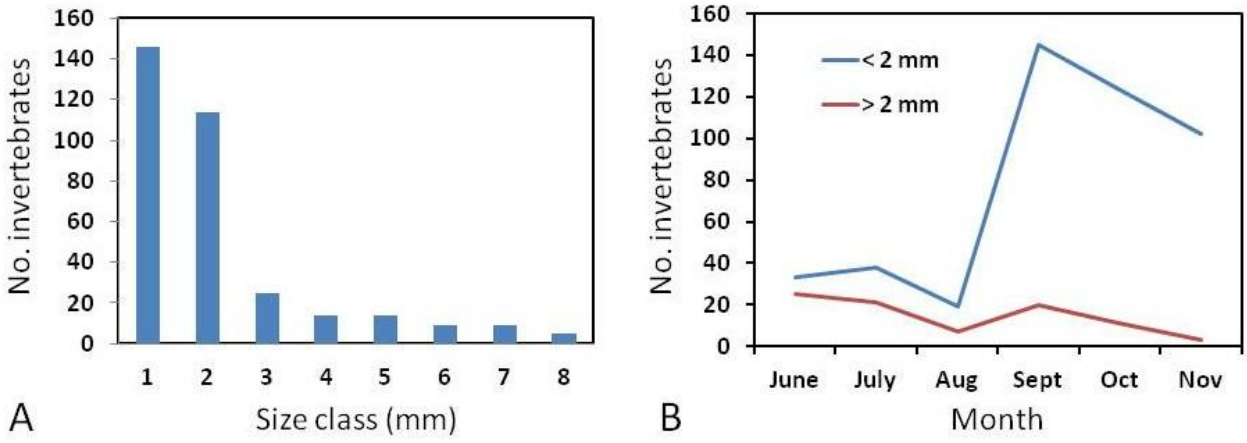

Figure 17 *Plecoptera size distribution*

A – size class distribution; B – monthly changes in numbers above and below 2 mm

Twenty Trichoptera specimens were recovered includinf representatives of seven families, the dominant one being Polycentropodidae (11 specimens). All specimens were recovered from pit samples. It must be noted, however, that a single trichopteran (a specimen of *Sericostoma personatum* (Spence *in* Kirby) approximately 10 mm long) was recovered from depth in the initial survey from the River Teith at Doune. This particular pipe sample also contained 13 Gammaridae and was notable for the high concentration of coarse particulate organic matter present within it. This could be the result either of the burial of a leafpack containing eggs and / or small larvae which continued to feed on the food resource, or could possibly be an indication of detritivores actively burrowing to reach a buried food resource.

A total of 82 Coleoptera were recovered, including representatives of 11 families. The fauna was dominated by Elmidae (64.6%) and Dryopidae (11.0%) which were found at 13 and five sites, respectively. The remaining eight families were found at one or two sites only. Three specimens were recovered from pipe samples – a single dytiscid from site FEF and a single elmid each from sites ROY and TIG.

3.3.3 Species level data

Of all the insects recovered the vast majority were too small to identify reliably beyond family or genus level. All of the insects identified beyond this level were common and widespread, the only exception being the scirtid beetle *Hydrocyphon deflexicollis* (Müller) of which a single adult was recovered from site TIG. This species is on the national Notable (B) list; it has been reported at nine sites in Scotland since 1980 (Foster, 2001).

A total of 199 amphipods were collected at 11 sites, 195 of which belonged to the non-native *Crangonyx pseudogracilis* Bousfield. The remaining four specimens were of *Gammarus pulex* (L.) and were found in a single pit at site EAR; thirty-five *C. pseudogracilis* were also recovered from this site.

A total of 220 Cladocera were recovered from 20 sites and determined to 19 species and subspecies. All specimens were from the family Chydoridae with the exception of a single bosminid (*Bosmina coregoni* Baird) from site EAR. *Alona guttata* Sars was the dominant species, with 146 specimens being found (66.4%). However, even this species was patchily distributed; it was present at a total of 10 sites with two individual pit samples at sites END and FEU accounting for 70 and 29 specimens (31.8 and 13.2% of all Cladocera) respectively. Nine species were present at a single site only. One of the more unusual discoveries given the physical habitat was the species *Eurycercus lamellatus* (Müller) at sites FEB and TRU (two and one specimens respectively). This species is usually associated with well-vegetated backwaters and lochs.

A total of 471 harpacticoid copepods were collected, these were present at every site with a minimum number of 1 (at TEC) and a maximum of 97 (at MOR). It was possible to determine 394 of these (83.7%) to species level and a total of 15 species were found. Twelve species belonged to the Canthocamptidae with a single species each present from the Ameiridae, Darcythompsoniidae and

Tachidiidae. The number of individuals found at each site ranged from 1 to 71 (average 18.9) with faunal richness between 1 and 9 species (average 3.7); the relationship between the number of individuals and the number of species was linear and statistically significant (R^2 = 0.606, F = 35.425, St.Er. = 0.964, β = 0.779, P = 0.000). The three commonest (almost cosmopolitan) species were *Bryocamptus zschokkei* (Schmeil), *Bryocamptus pygmaeus* (Sars) and *Echinocamptus praegeri* (Scourfield) which accounted for 31.2, 27.4 and 12.7% of specimens respectively; one or more of these three species were found at 23 of the 25 sites. The remaining species were more sparsely distributed with none found at more than six sites; four species were found at single localities. Ten species were recovered from the pipe samples at a total of eight sites; six of these species were found at only a single site.

During the survey 2,550 cyclopoid copepods were collected, these were present at every site. The total number per site ranged from 4 (at ALE) to 343 (at TED) with an average of 102. As the identification of males and immature females is more problematic only gravid females or pairs *in copula* were identified. A total of 307 cyclopoids could be identified to species level; two sites produced no identifiable specimens with the remainder producing between 1 and 54 identifiable specimens. A total of 11 species were identified with the number per site ranging from 0 to 8 (average 3.3). Three species were common and widespread:

Microcyclops varicans (Sars) – 102 specimens from 16 sites (33.2% of specimens);
Acanthocyclops robustus (Sars) – 74 specimens from 15 sites (24.1% of specimens);
Diacyclops bisetosus (Rehberg) – 59 specimens from 14 sites (19.2% of specimens).

Five species were recovered from pipe samples, the commonest of which was *M. varicans* which was found at eight sites and comprised 42.3% of cyclopoids from these samples.

Eleven species of copepod were collected during the pilot study (Gilvear *et al.*, 2007), two of these (the cyclopoid *Eurycyclops serrulatus* (Fischer) and the harpacticoid *Moraria brevipes* (Sars)) were not found in the present study; conversely 17 species were collected in this study that were not found in the pilot study. This large discrepancy is explained by differences in geographical locations, sampling effort and method (pit sampling, where diversity and numbers are highest, was not used in the pilot study). There is also the possibility that there was an element of misidentification in the pilot study as no *M. varicans* were recovered but nine individuals were identified as '*Paracyclops* (juv)' and could well have been this species (despite effort the specimens could not be relocated for verification).

3.3.3.1 Spatial trends in abundance and richness

Both the total number of invertebrates and invertebrate richness decreased northwards (Figure 18), however, only the decrease in total numbers was statistically significant (R^2 = 0.249, F = 7.632, St.Er. = 0.001, β = -0.499, P = 0.011). There was no significant correlation with the Jeffers (1988) PCA1 score.

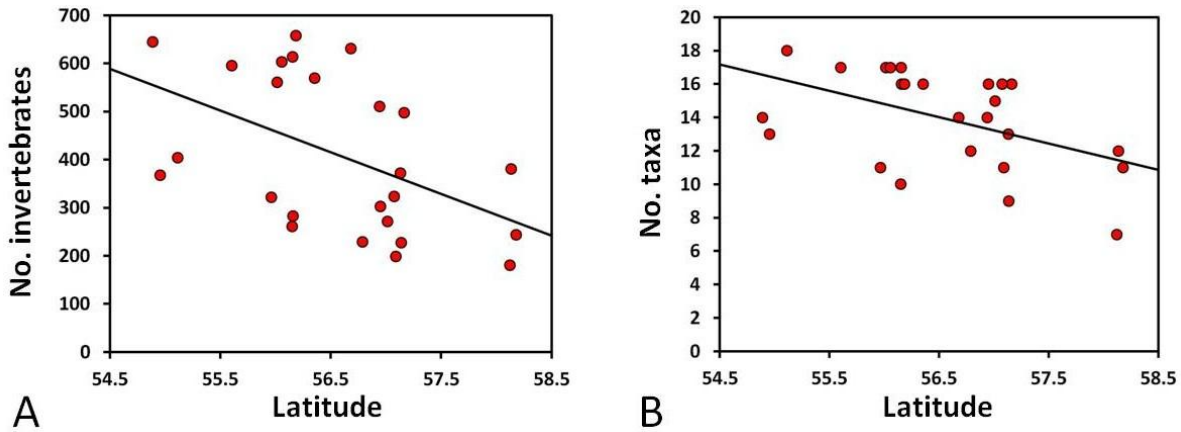

Figure 18 *Latitudinal changes*
A – number of invertebrates recovered; B – taxonomic richness

The average number of invertebrates recovered per sample increased from montane to lowland sites and the number of taxa recovered decreased slightly over the same gradient (Figure 19), however, the only statistically significant difference was for the total number of taxa recovered from montane and lowland sites (ANOVA: F (1, 66) = 9.893, P = 0.002 (see Appendix 5C)).

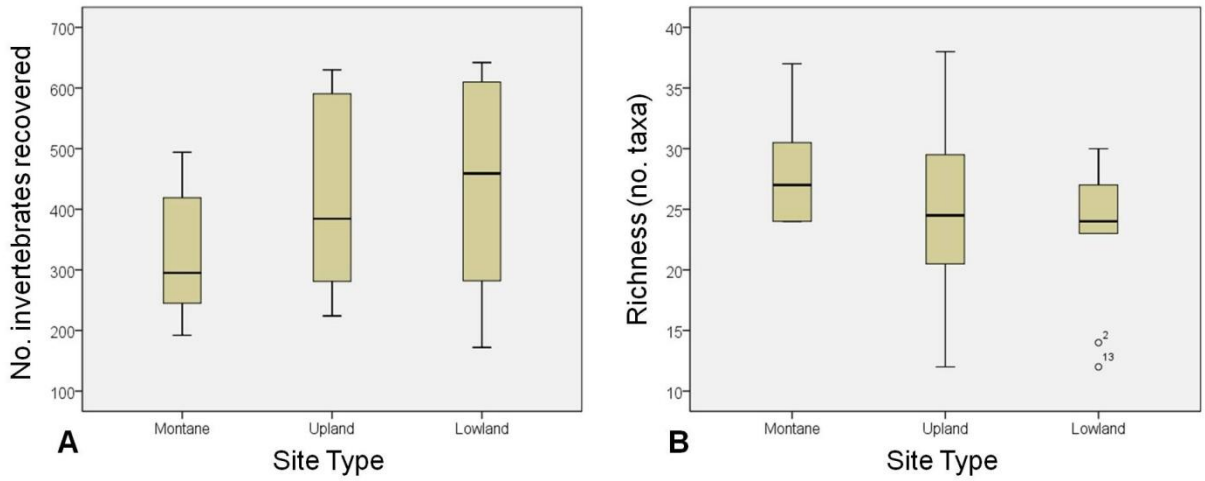

Figure 19 *Comparison of total invertebrates, taxonomic richness and site type*
A – total number of invertebrates recovered; B – invertebrate richness

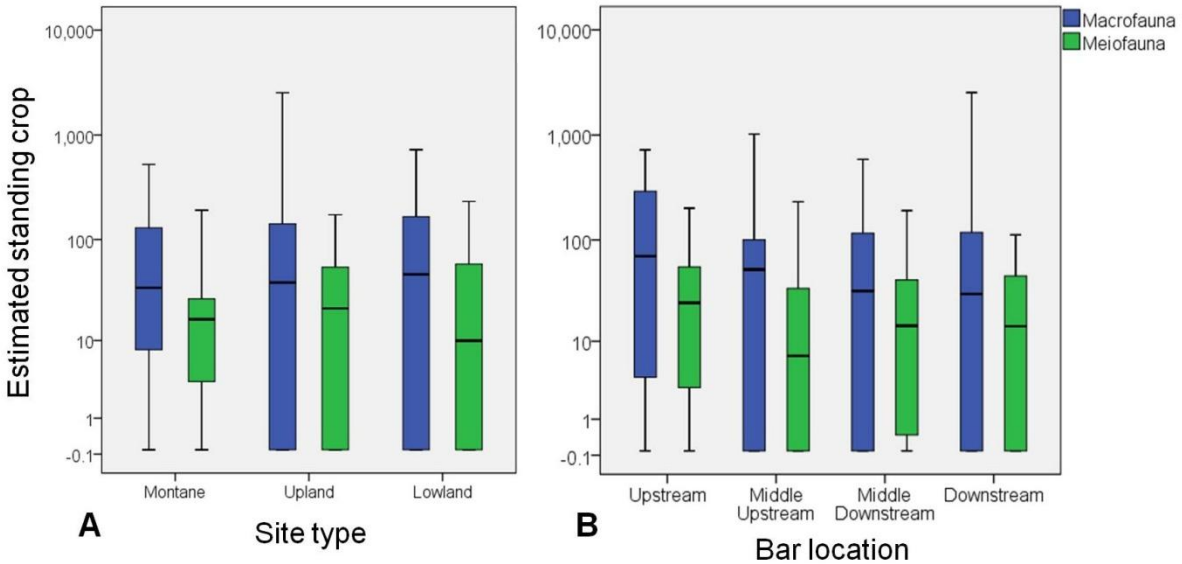

Figure 20 *Comparison of relative estimated standing crops of macro- and meiofauna*
A – site type; B – bar position

An attempt was made to assess invertebrate standing crop across sample sites. To this end each of the invertebrates was assigned a body size class of 1, 2, 4, 8 or 16 from the smallest to largest collected. These values were then multiplied by the number of invertebrates of that size class collected in each sample and summed to generate an estimated relative standing crop. These data are presented in terms of site type and bar position in Figure 20. Meiofauna constitute a consistently smaller portion of the biomass than macrofauna across all site types and sample locations. There are no statistically significant differences in the data; however, two slight trends are apparent. First, the average standing crop of macrofauna increases slightly from montane to lowland rivers; second, the standing crop of macrofauna decreases slightly from the upstream to the downstream end of a bar. Patterns in the meiofauna are less clear.

3.3.3.2 Species accumulation curves

Species accumulation curves were calculated using the EstimateS statistical package (Colwell, 2009) in order to determine how comprehensive the survey was. Figure 21 gives taxon accumulation curves for all taxa, all insects and all crustaceans. It can be seen that all three curves are still rising after the collection of 25 samples. This is a reflection of the patchy nature of the hyporheos and the presence within it of many rarely encountered species. An extrapolation to 50 sites increases the number of taxa expected (at the taxonomic resolutions used) from 100 to 120 with more new taxa being expected from the Crustacea than the Insecta.

Examining the data for the three dominant microcrustacean groups in greater detail a more complex picture emerges (Figure 22). The cyclopoid copepods were exhaustively sampled during the survey; doubling the number of sites to 50 would increase the number of species by only 7%. Harpacticoid copepods were less exhaustively collected with an expected increase of 18% with a doubling of site numbers. The Cladocera, however, were still far from completely surveyed at the end of the

sampling period – doubling the sampling effort here would be expected to increase the number of species collected by almost 30%.

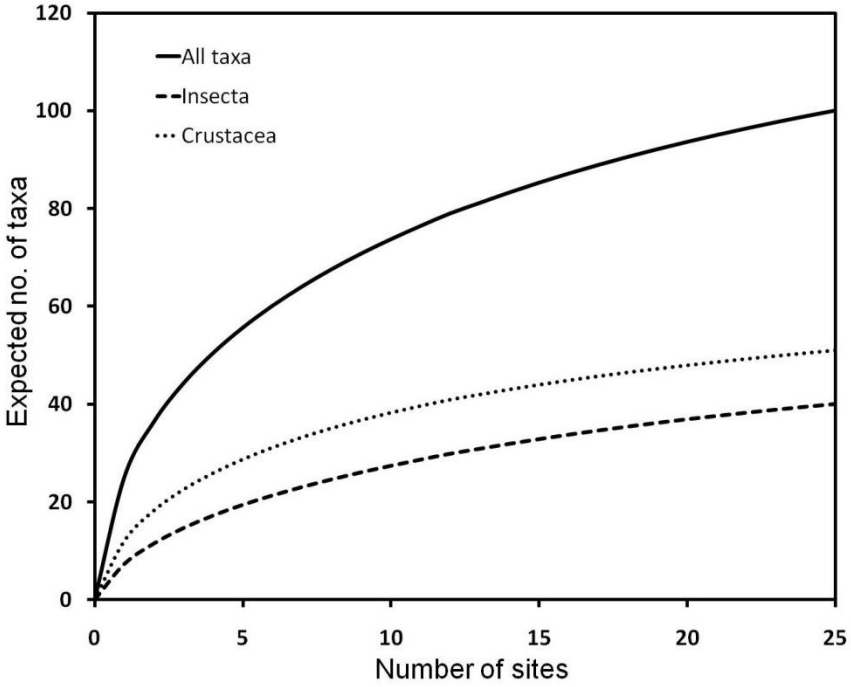

Figure 21 *Synthetic taxon accumulation curves for all taxa, Insecta and Crustacea*

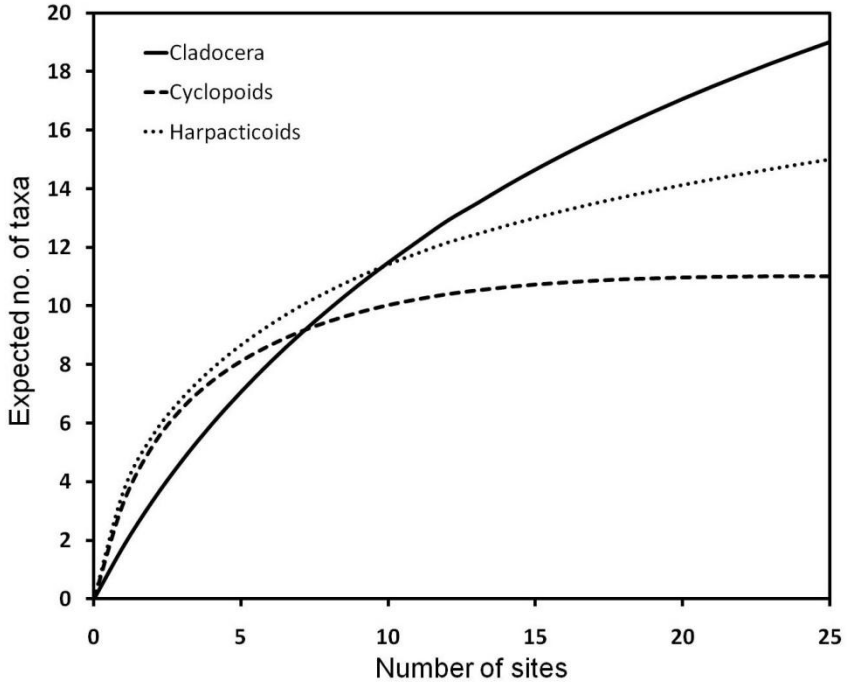

Figure 22 *Synthetic taxon accumulation curves for the three dominant microcrustacean groups*

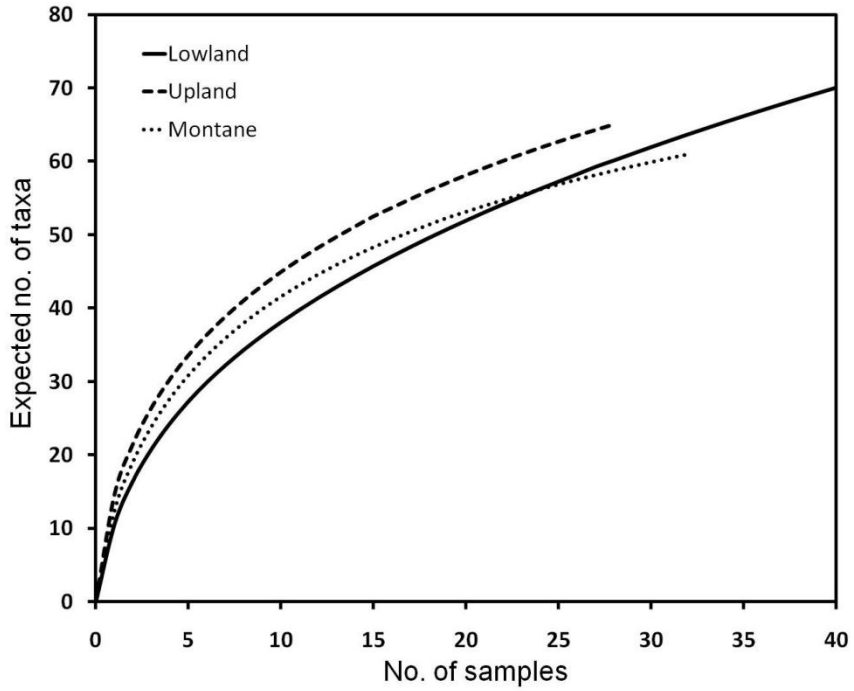

Figure 23 *Synthetic taxon accumulation curves for lowland, upland and montane sites*

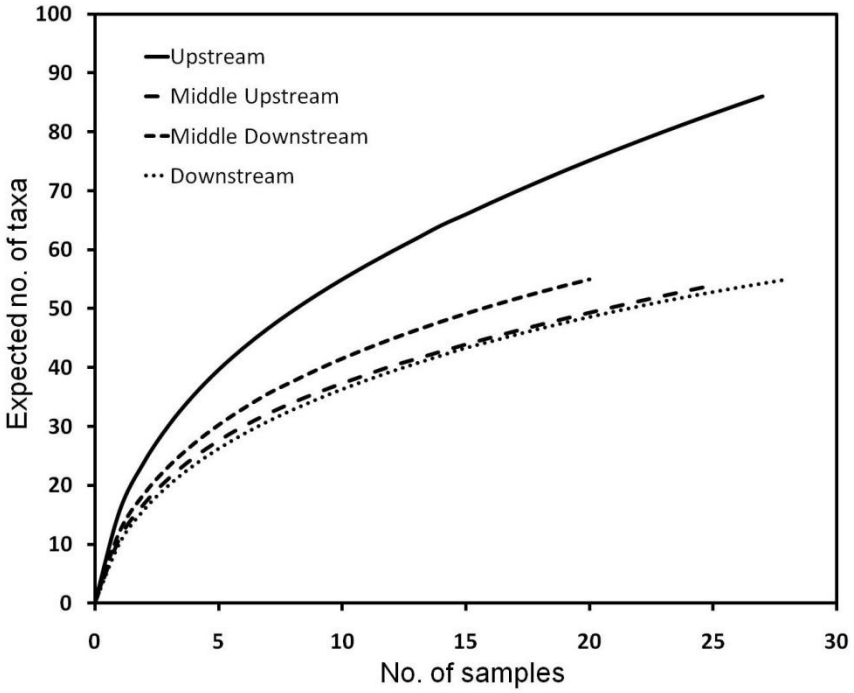

Figure 24 *Synthetic taxon accumulation curves for the four bar sampling positions*

The synthetic taxon accumulation curves created for lowland, upland and montane sites (Figure 23) are very similar. Extrapolation to 50 combined pit and pipe samples would theoretically retrieve a total of 74, 76 and 68 taxa from the three site types respectively. This suggests that the montane species pool is slightly more restricted than those of upland or lowland sites.

Repeating this procedure for bar position (Figure 24) clearly reveals the importance of the upstream end of bars compared with the middle and downstream positions. Extrapolating the sampling effort to a total of 50 would theoretically recover 95, 67, 61 and 61 taxa from the four positions in downstream order. This suggests that there is little difference in richness between the three lower positions.

3.3.3.3 SAC status

Comparison was made between rivers with and without SAC status to see if there was any difference in terms of their HZ richness (Figure 25A). Using combined pit and pipe data the mean number of taxa recovered per site was almost identical from SAC and non-SAC rivers. However, the mean number of taxa recovered from pipes was slightly higher in SAC rivers than non-SAC rivers (7.8 and 5.0 taxa per sample respectively), although this was not statistically significant. Species accumulation curves generated for the two river types (Figure 25B) were almost coincident indicating that the distribution and patchiness of species was almost identical. There were, however, slight differences in community composition.

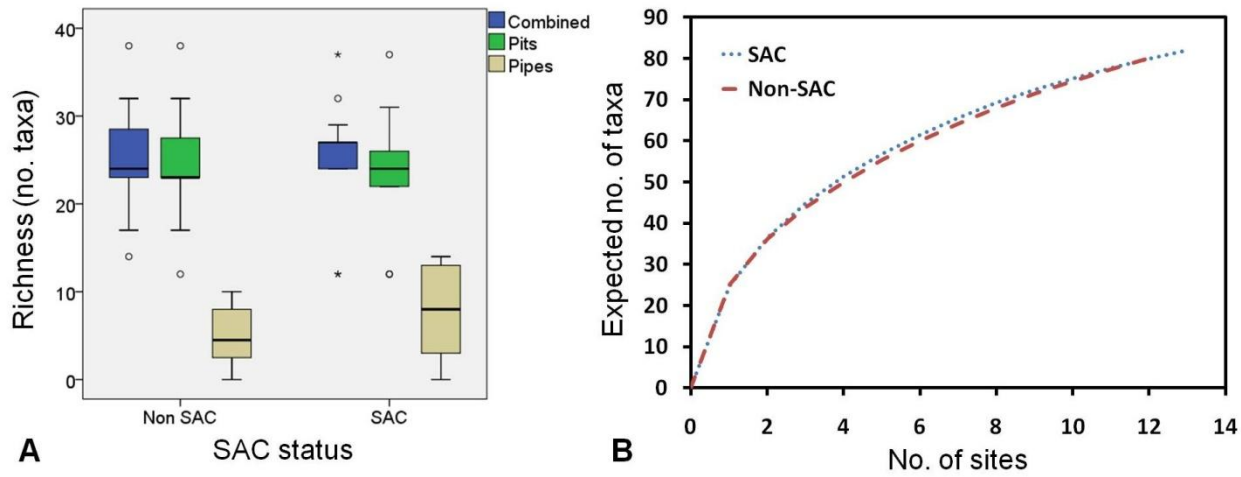

Figure 25 *Comparison of invertebrate richness in SAC and non-SAC designated rivers A – number of taxa recovered from combined samples, pits and pipes; B – synthetic species accumulation curves*

3.4 Influence of environmental parameters on community composition

The relationship between the composition of the hyporheos and environmental variables was assessed using Canonical Correspondence Analysis. In this analysis the dispersion of samples in ordination space is constrained according to the environmental data provided and samples are located such as to maximise their correlation with these data. Biplots are a useful way of visualising the relationships between samples or species, and between samples or species and environmental variables. The significance of each environmental variable in explaining the variation within the biological data was then assessed using a forward selection approach supported by random permutation of the data. For these analyses data was subdivided into pit and pipe sub-samples.

Figure 26 summarises the output of the analysis of pit samples. The principal arrangement of samples from left to right along axis 1 reflects a gradient from montane (high source altitude, low distance to source) on the left, to lowland, or downstream sites (lower source altitude, long distance to source) on the right. The major exception to this is ALE on the River Almond at Edinburgh Airport,

a site with exceptionally high conductivity compared to all other sites. This site occupies an elevated position on axis 2 and contained an unusually high relative abundance of harpacticoid copepods and oligochaetes, possibly reflecting its polluted status. The only statistically significant determinants of the composition of hyporheic invertebrates in pits (Table 9) were distance to source, conductivity and source altitude, which points to a predictable upstream-downstream change in composition, possibly with the additional influence of conductivity when values are highly elevated.

	Pits				Pipes		
	LambdaA	P	F		LambdaA	P	F
Distance to source	**0.09**	**0.002**	**1.79**	**Site altitude**	**0.28**	**0.002**	**2.63**
Conductivity	**0.09**	**0.026**	**1.72**	**Longitude**	**0.18**	**0.034**	**1.82**
Source altitude	**0.07**	**0.016**	**1.48**	**Total alkalinity**	**0.2**	**0.02**	**2.02**
Dissolved oxygen	0.06	0.084	1.3	River width	0.15	0.068	1.61
Ammonia	0.06	0.172	1.23	Nitrate	0.14	0.06	1.59
River width	0.06	0.19	1.2	Dissolved oxygen	0.14	0.06	1.61
Nitrate	0.05	0.24	1.17	Conductivity	0.11	0.25	1.26
Total alkalinity	0.05	0.352	1.07	Total dissolved P	0.1	0.306	1.17
pH	0.06	0.17	1.27	Distance to source	0.09	0.314	1.15
Total dissolved P	0.06	0.186	1.27	Temperature	0.1	0.356	1.11
Piezometric head	0.05	0.336	1.15	Nitrite	0.09	0.298	1.18
Temperature	0.05	0.31	1.14	pH	0.09	0.294	1.14
Nitrite	0.05	0.388	1.09	Latitude	0.1	0.262	1.22

Table 9 *The significance of different environmental variables as revealed by a forward selection procedure and Monte Carlo random permutation testing*
Factors significant at the P < 0.05 level in bold

A broadly similar pattern emerges for pipe samples (Figure 27), with a similar spread of sites from montane to lowland from left to right along axis 1. The only notable outliers were ALL (River Allan) and LIN (River Loannan) where higher water temperatures and alkalinity (possibly associated with

increased inputs of base-rich ground water) may have been important additional influences on the fauna. Altitude was the single most important influence on the pipe fauna, followed by longitude and alkalinity (Table 9). The importance of longitude suggests that there is a biogeographical component to the distribution of the deeper hyporheos sampled using pipes, although this may be due simply to covariation with other more proximate factors.

It is notable that the ability to explain the variation of the composition of the fauna in pipes was significantly higher than for pits, this suggests that there is a greater degree of environmental determinism and associated specialisation within the deeper hyporheic fauna when compared to the shallow hyporheic fauna which seems more likely to be composed of generalists or taxa that use widely distributed resources.

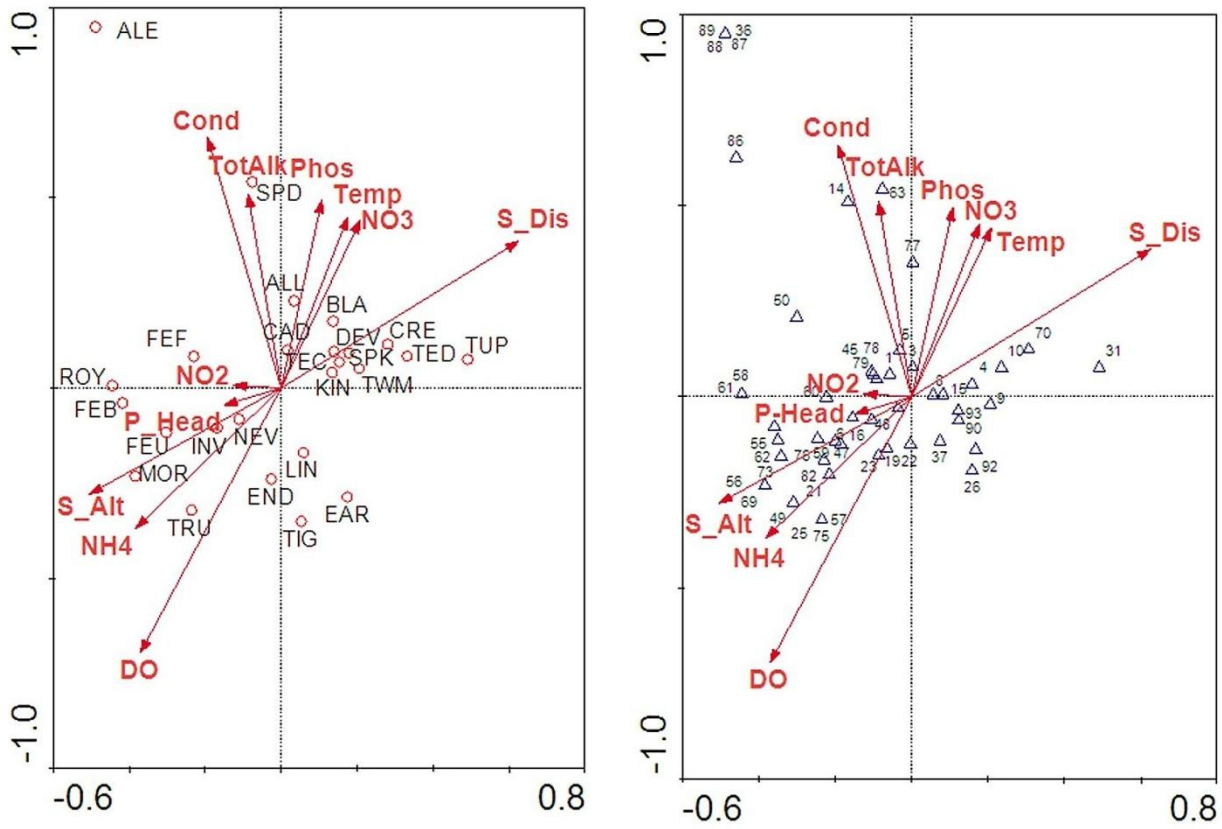

Figure 26 *Ordination of samples (left) and species (right) by Canonical Correspondence Analysis of pit samples (Species numbers as given in Appendix 2)*

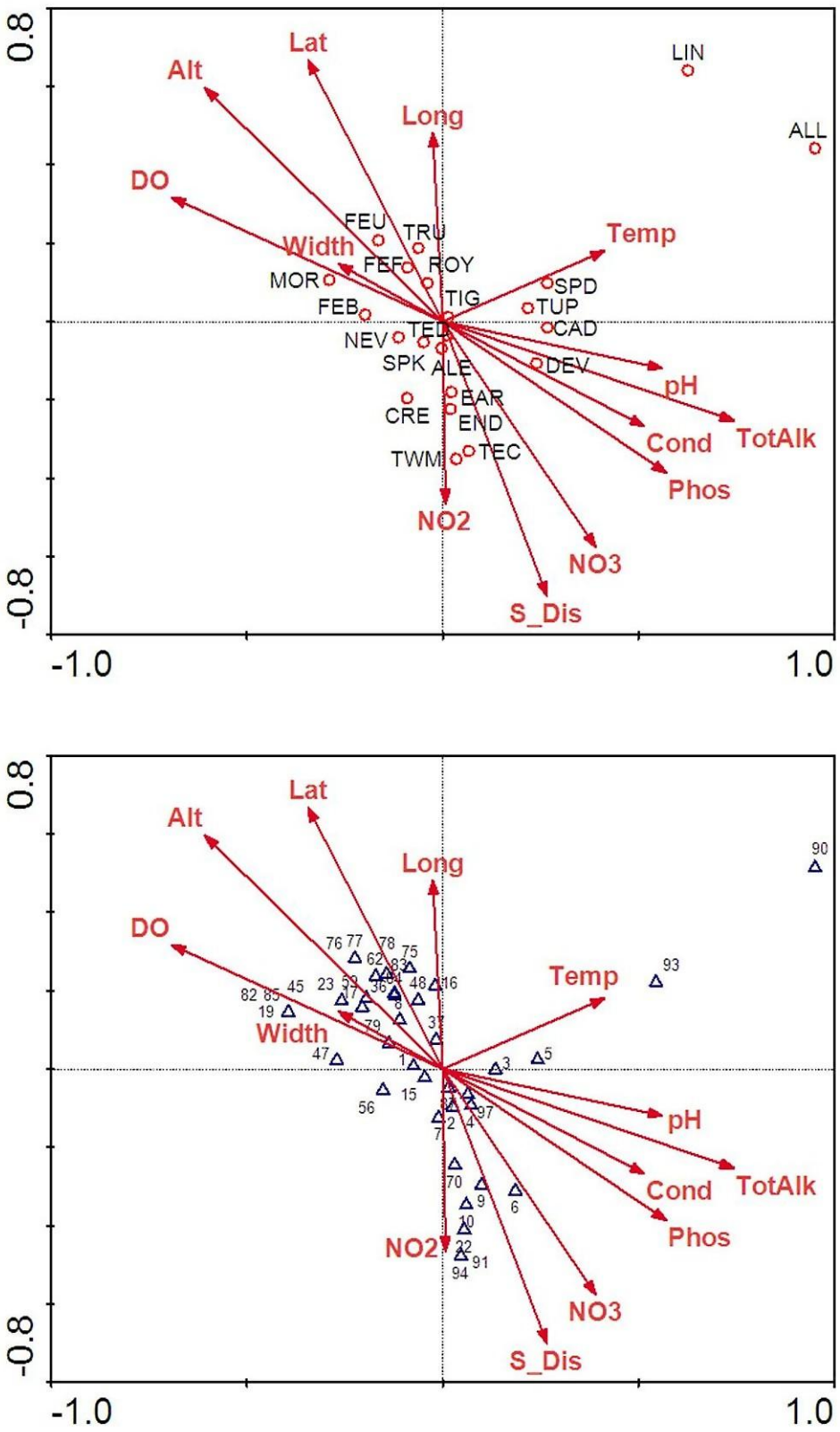

Figure 27 *Ordination of samples (above) and species (below) by Canonical Correspondence Analysis of pipe samples (Species numbers as given in Appendix 2)*

3.5 Invertebrate scoring systems and optima calculations

Invertebrate taxa included in the revised BMWP (Walley & Hawkes, 1996) and LIFE scoring systems were used to produce site scores from the macroinvertebrate component of the HZ samples (*i.e.* as if a standard kick-sweep sample had been taken). BMWP scores ranged from 7.2 at KIN to 103.4 at END with a median score of 52.4 at DEV. ASPT scores ranged from 3.60 at KIN to 7.64 at NEV with a median score of 6.08 at TUP. LIFE scores ranged from 0.00 at KIN to 2.60 at DEV with a median score of 2.00 at ALL, MOR and ROY. ASPT values generated from this data were compared with those from SEPA monitoring sites where these were available within 2 km of the sample site (a total of 15 sites); although a positive trend was present ($R^2 = 0.202$), this was not statistically significant.

In the revised BMWP scoring system invertebrates with known scores are used to create a value for the site as a whole, based on averaging or weighted averaging of the scores of the taxa that are present. The BMWP is an expert-based scoring system. However, it is also possible to generate empirical optimal values for different environmental parameters for all species following the same principle. Hill *et al.* (2000) provide an illustration of this technique based on adjusting Ellenberg indicator scores for the British flora. First it is necessary to take the weighted average of site scores to create an ASPT value for those species that currently lack a score. These data are then used in the same way to produce a weighted average (or optimal value) for each taxon for each of the environmental parameters recorded. The derived ASPT values can then be plotted against the empirical environmental optima to assess which environmental parameters have the greatest influence on the distribution of different groups of taxa. A summary of the R^2, β and P values from these correlations for insects and crustacea is given in Table 10.

	Insecta					Crustacea				
	R^2	F	St.Er.	β	P	R^2	F	St.Er.	β	P
Stream width	0.069	0.968	0.012	-0.263	0.343	0.049	1.143	0.012	0.222	0.297
Altitude	**0.660**	**25.190**	**0.001**	**0.812**	**0.000**	**0.481**	**20.405**	**0.001**	**0.694**	**0.000**
Source altitude	0.593	18.963	0.001	0.770	0.001	**0.612**	**34.748**	**0.001**	**0.783**	**0.000**
Catchment slope	**0.789**	**48.755**	**0.004**	**0.889**	**0.000**	**0.514**	**23.225**	**0.007**	**0.717**	**0.000**
Reach slope	0.486	12.302	0.039	0.697	0.004	**0.645**	**39.928**	**0.034**	**0.803**	**0.000**
Distance to source	0.509	13.491	0.007	-0.714	0.003	0.406	15.023	0.009	-0.637	0.001
Jeffers (1998) PCA1 score	**0.761**	**41.355**	**0.100**	**0.872**	**0.000**	**0.676**	**46.002**	**0.099**	**0.822**	**0.000**
Conductivity	**0.783**	**46.815**	**0.003**	**-0.885**	**0.000**	**0.470**	**19.545**	**0.001**	**-0.686**	**0.000**
Temperature	0.505	13.279	0.067	-0.711	0.003	**0.525**	**24.285**	**0.070**	**-0.724**	**0.000**
pH	0.323	6.202	0.632	-0.568	0.027	0.093	2.263	0.761	-0.305	0.147
Dissolved oxygen	**0.838**	**67.365**	**0.006**	**0.916**	**0.000**	**0.821**	**100.751**	**0.005**	**0.906**	**0.000**
Nitrate	0.414	9.183	0.001	-0.643	0.010	**0.568**	**28.871**	**0.000**	**-0.753**	**0.000**
Nitrite	0.176	2.778	0.016	-0.420	0.119	0.003	0.065	0.024	-0.054	0.802
Ammonia	**0.799**	**51.601**	**0.003**	**0.894**	**0.000**	**0.587**	**31.306**	**0.004**	**0.766**	**0.000**
Total alkalinity	0.459	11.025	0.465	-0.677	0.006	**0.438**	**17.142**	**0.218**	**-0.662**	**0.000**
Total dissolved phosphate	**0.710**	**31.857**	**0.002**	**-0.843**	**0.000**	**0.445**	**17.674**	**0.004**	**-0.667**	**0.000**

Table 10 *Regression results from comparison of recalculated ASPT scores and calculated optima for environmental parameters for insects and crustaceans*
For Insecta df = 1, 13, for Crustacea df = 1, 22. (Only taxa where > 5 invertebrates were recovered are included; values where P < 0.001 in bold, where P > 0.01 in grey)

The most notable feature of these data is the number of strong correlations between recalculated ASPT and the environmental parameters. The strongest association, both for insects and crustaceans, was with dissolved oxygen. The second, third and fourth strongest correlations for insects and crustaceans are not identical, indicating that these two groups respond differently to environmental stimuli. For crustacea the next three factors are the Jeffers (1998) score along with reach slope and source altitude, both factors involved in calculating the Jeffers score, thus indicating that the distribution of these species is secondarily driven by topographical situation. For insects the next three drivers are ammonia, catchment slope and conductivity indicating that a more diverse set of environmental stimuli determine their distribution.

It is also possible to compare the original revised BMWP values for macroinvertebrate taxa with back-calculated scores for the same taxa derived from the HZ survey data and hence assess the applicability of this scoring system to the hyporheos (Figure 28). It can be seen that the spread of revised BMWP values of taxa recovered in the survey covers almost the entire range of values used in the scoring system (from Asellidae with a score of 2.1 to Chloroperlidae with a score of 12.4). However, back-calculated hyporheic BMWP values for these taxa occupy a much narrower range (from 5.50 for Caenidae to 7.12 for Nemouridae). The relationship between the two is statistically significant (R^2 = 0.412, F = 11.897, St.Er. = 1.101, β = 0.642, P = 0.03). This implies that within the HZ high- and low-scoring macroinvertebrates have a higher probability of co-occurring than they do in benthic communities.

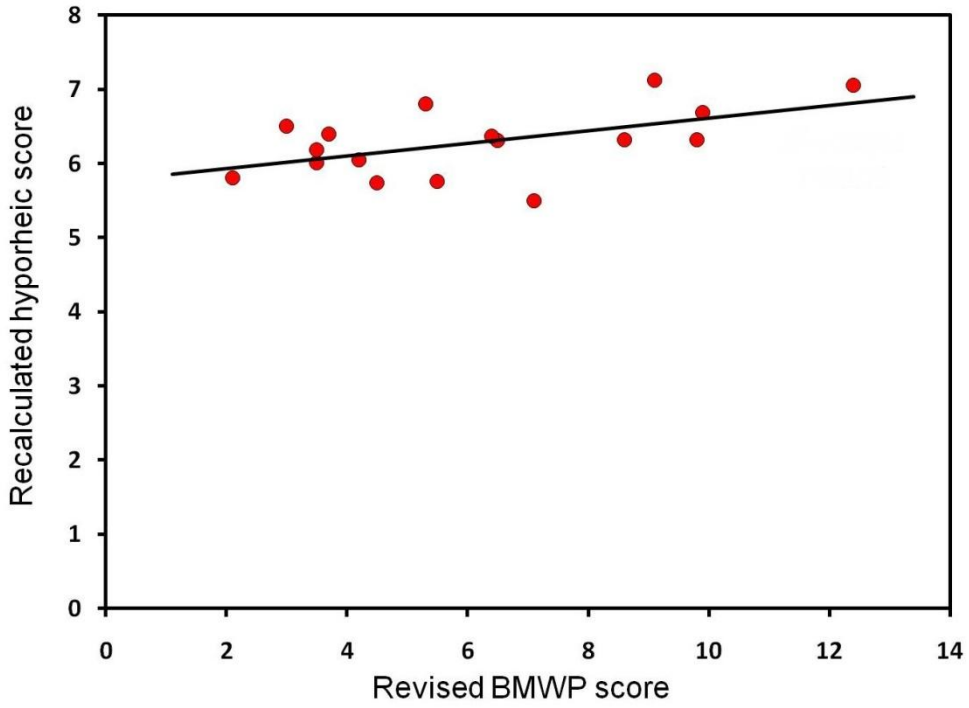

Figure 28 *Relationship between original BMWP scores and re-calculated scores for 17 macroinvertebrate taxa found in the HZ*
Only taxa where >= 4 specimens recovered plotted

4 DISCUSSION

In undertaking this work I consider that I have developed a robust technique for sampling the hyporheic fauna of coarse-grained gravel-bed rivers. The results presented here represent the first national assessment of the hyporheic fauna of Scotland and may indeed be among the first national surveys of the hyporheos on a global scale.

4.1 Environmental characteristics of the HZ

Being, by definition, a subsurface zone, the environmental characteristics of the HZ are difficult to characterize quantitatively by non-invasive techniques. The act of sampling within the zone will disturb sediment structure and the distribution of those organisms present. Many of the properties that are most desirable to quantify (*e.g.* porosity, permeability and measures of compactedness) are extremely difficult to determine directly without the application of complex, novel, or logistically complicated procedures. Consequently the collection of data from the HZ will always be a compromise that relies, to a greater or lesser extent, on analogues such as using changes in water chemistry to infer rates of metabolism within the zone (Uzarski *et al.*, 2004).

During the course of this investigation over 100 Karaman-Chappuis pits were excavated in exposed gravel bars. One of the more notable features was the degree of variability in permeability that was encountered. Two pits (at ALL and FEF) remained dry after considerable excavation (to a depth of approximately 40 cm, despite being within 2 m of the river channel and the bases being over 25 cm below the river level). On another occasion at the head of a bar on Montraw Burn in the initial survey to determine the number of replicates the throughflow in the pit was such that standing ripples were detectable on the water surface. It was never possible to make an accurate prediction

of sediment permeability prior to excavation. This had implications for the sampling procedure since, in some cases, it was necessary to take the next sample while waiting for the water in the pit to refill. In other cases it was necessary to deploy two pumps simultaneously in order to extract a usable sample containing the finer sediments and invertebrates.

Pipe insertion also proved difficult on occasions as larger cobbles and boulders present below the pit could not be seen. It was often necessary to try two or three positions around the base of the pit before the pipe could be inserted to the desired depth. In this respect the narrower gauge pipe with the central driving bar had considerable advantages over the piezometric tubes used by Gilvear *et al.* (2007) as the bar, being slightly more flexible, finds its way through the sediment more readily. The greater heterogeneity of permeability at depth is demonstrated by the number of pipes inserted that proved dry. Thus, 13 of the 100 pipes inserted produced no fluid at all, despite the fact that the pump proved capable of raising gravel up to 10 mm diameter up the length of the pipe. On several of these occasions the pipe was found to have entered clays when it was extracted, despite there being no signs of clays on the surface at the site. On one occasion it was found that, once the armoured layer had been penetrated, the pipe had entered sands and could be inserted the remainder of the way by hand.

These comments all serve to highlight the spatially heterogeneous nature of this zone with complex gradients of porosity and permeability in a three-dimensional matrix. This variability is generally not apparent on the surface as a result of the creation of an essentially uniform armoured layer by previous floods.

It was initially hoped that survey work could be undertaken away from the main channel to assess the lateral extent of the HZ, its associated fauna and physicochemical characteristics. Unfortunately this proved impractical as the sample pipe was found to be extremely difficult to insert into the

thicker sediments found here. Further research will be necessary to determine optimal techniques for sampling this region. An alternative sampling strategy could be to keep a watching brief for potential engineering work planned at or near the water table in floodplains (*e.g.* pipeline burial) and liaising with the engineers in order to obtain samples.

4.2 General attributes of the hyporheic fauna of Scottish rivers

First indications suggest that the shallow hyporheic zone is a unique biological community with a significantly different fauna compared to the overlying benthos. The fauna is truncated according to body size with higher proportions of grazers and detritivores than other functional feeding groups. Missing are higher predators (*e.g.* Perlidae), larger instars of the dominant benthic groups (e.g. Leuctridae, Baetidae, Heptageniidae) and the case-bearing Trichoptera. The fauna is dominated by oligochaetes, cyclopoid copepods, nematodes and dipterans. It should be noted that two of the dominant taxa found here that are included in traditional benthic monitoring systems (dipterans and oligochaetes) are almost always meiofaunal in size in these samples and would pass through the 1 mm mesh of a standard kick-sweep net.

On an international scale the numbers of organisms recovered in this survey are lower than those where comparable sampling has been undertaken. Table 11 summarises the total number of invertebrates recovered using the Bou-Rouch technique in published work that is sufficiently similar to permit direct comparison. The values from Boulton *et al.* (2004) are particularly interesting. Two sites, a downwelling area and a weakly upwelling anabranch of the River Rhône were sampled with individual 1 L aliquots being taken to a total of 10 L in order to assess the effect of sample volume. In the downwelling site an average of 222 invertebrates per 10 L were recovered; however, in the weakly upwelling site an average of over 7800 were recovered, highlighting the patchy nature of the

fauna. The majority of reported values are in the range from low tens to low hundreds of invertebrates per 10 L.

Of particular interest is the difference in the number of invertebrates recovered from pipes in the current work and that of the pilot survey (Gilvear *et al.*, 2007) where the average number of invertebrates recovered per sub-sample is over an order of magnitude higher (Table 11). The most obvious explanation for this difference is the sampling method used. In the pilot study standard 25 mm galvanized steel piezometers were inserted into the sediment. These pipes have a series of holes drilled into the driving point that are open to the sediment through which it is inserted; the various parts are also connected by collars which disturb the sediment to a greater extent than if they were of one-piece construction. In the present study a pipe was inserted with a metal driving bar completely filling the interior so that, when extracted, the sample would be taken from that particular level in the HZ.

Study	**Country**	**Invertebrates per sample (6-10 L)**
Boulton *et al.*, 2004	France	222 and 7831
Malard *et al.*, 2003b	Switzerland	344
Hancock, 2006	Australia	67
Fowler & Death, 2001	New Zealand	26
Kibichii, 2009	Ireland	27
Gilvear *et al.*, 2007	Scotland	118 *
This study	Scotland	9

Table 11 *Reported densities of invertebrates from studies employing the Bou-Rouch method*
** Includes contamination from the shallow hyporheos (see text)*

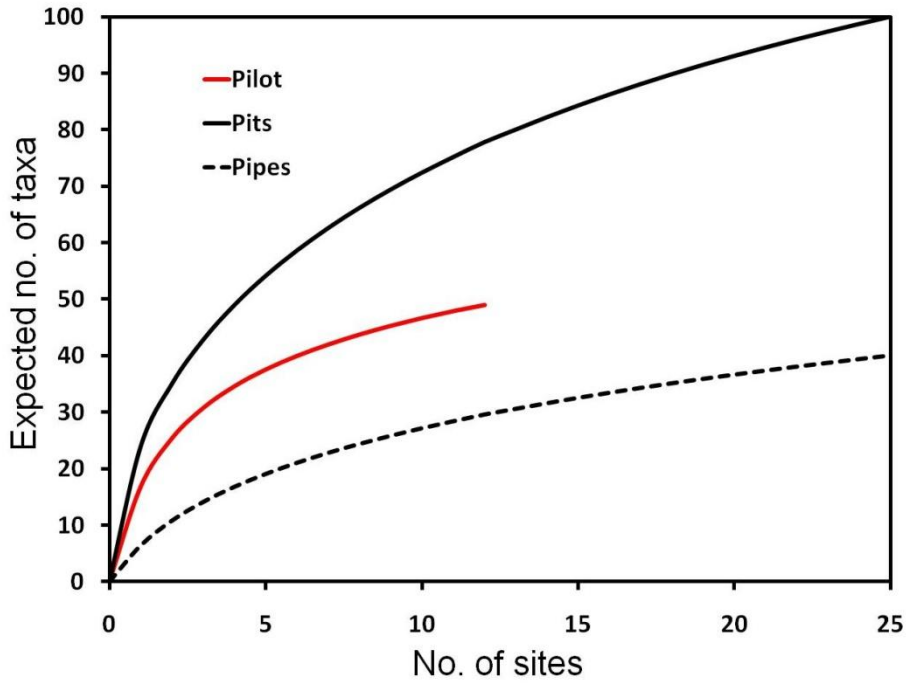

Figure 29 *Comparison of species accumulation curves from the pilot study with the pit and pipe sampling from the present work*

It is hypothesized that as the piezometers were inserted during the pilot study a portion of invertebrates in the shallow sediment through which the pipe passed were dislodged downwards, flowed in and thus became incorporated into the deep sample. To assess this possibility individual species accumulation curves were generated for pit and pipe samples from the present study and compared with the same data generated from the pilot study. In the pilot study more samples were collected per site than in the present work (six, seven or eight as compared to four). In order to make the data as comparable as possible the subsamples from the pilot study were randomly split into two groups of either three or four pipes, giving an average of 3.58 pipes per sample. In the present study the presence of 13 dry pipes out of 100 gives an average of 3.48 pipes per sample. The results of this exercise (Figure 29) are that the synthetic species accumulation curve for the pilot study sits approximately mid-way between the two curves generated for pits and pipes from the present work. The lowermost portion of the pilot study curve rises rapidly, sub-parallel with that from pit samples. From the second sample onwards the number of extra taxa generated per sample

reduces markedly, until, from five samples onwards, it becomes sub-parallel with the pit sample curve. This fits well with the hypothesis that each deep sample would become contaminated with a randomized selection of shallow HZ invertebrates during pipe insertion, thus explaining the differences in total numbers observed between studies.

At the coarsest taxonomic level the invertebrate composition of the samples from the present work are broadly comparable to those found globally (*e.g.* McElravy & Resh, 1991 – California; Boulton *et al.*, 2004 – France; Olsen & Townsend, 2005 – New Zealand). The taxa recovered included no truly subterranean (stygofaunal or stygobiont) species such as *Niphargus glenniei* (Spooner) (Knight, 2009). Recent work on this group appears to confirm that the northern edge of their distribution in the UK lies at or near the southern limit of the Devensian glaciation (Hänfling *et al.*, 2009; Robertson *et al.*, 2008).

No Bathynellidae (Crustacea: Syncarida) were recovered in this survey. The only known Scottish representative of the group, *Antrobathynella stammeri* (Jakobi), has not been found in the country since its initial discovery (Maitland, 1962). However, this species is known to inhabit the HZ of streams in the southern Lake District and has recently been recovered using Bou-Rouch methodology on the rivers Lathkill and Skirfare in northern England (Stubbington *et al.*, 2009c). An attempt to relocate this species at its Scottish site, the Altquhur Burn near Drymen (NS490868), proved unsuccessful.

It is unfortunate that the only exhaustive regional study of the ecology and distribution of the microcrustacea in Britain (Fryer, 1993) was based almost entirely on still-water habitats and the uppermost headwaters of streams (primarily boggy flushes and seepages). This survey of the Yorkshire fauna recovered numerous species not found in the present work, as would be expected due to differences in habitat and sampling technique. However, it is notable that only 10 of the 42

species of cladocerans or copepods recovered in the present work are absent from the Yorkshire list; these are:

Cladocera:	*Bosmina coregoni* Baird
	Alona rustica Scott
	Chydorus latus Sars
	Tretocephala ambigua (Lilljebourg)
Cyclopoid copepods:	*Eucyclops serrulatus* (Fischer)
Harpacticoid copepods:	*Bryocamptus vejdovskyi* Mrázek
	Epactophanes richardi Mrázek
	Horsiella brevicornis (van Douwe)
	Maraenobius vejdovskyi Mrázek
	Tachidius discipes Giesbrecht

The fact that the highest proportion of 'missed' species is in the harpacticoid copepods is to be expected as this group contains a larger proportion of species that have a preference for subsurface habitats than the other microcrustacean groups.

A comparison of the benthic Cladocera and Copepoda reported in Robertson *et al.* (1997) with the hyporheic species from the pilot study (Gilvear *et al.*, 2007) and the present work is given in Table 12. Of the 49 species recovered in the three studies, only six were not found in this work. In contrast, 22 species were found here and in the pilot study that were not recorded by Robertson *et al.* (1997). This would seem to indicate that the microcrustacean fauna is primarily to be found in the shallow HZ in the UK, although further research would be required to confirm this.

	Robertson *et al.* (1997)			Gilvear	
	Wales (n = 2)	England (n = 5)	Scotland (n = 2)	*et al.* (2007) (n = 6)	This study (n = 25)*
Cladocera					
Acroperus harpae Baird		+			I (1.50)
Alona affinis (Leydig)				+	I (1.00)
Alona costata Sars					I (1.00)
Alona guttata Sars				+	III (14.60)
Alona quadrangularis (Müller)	+	+	+		I (2.25)
Alona rectangula Sars		+	+		I (2.00)
Alona rustica Scott	+	+	+	+	I (3.67)
Alonella nana (Baird)	+	+	+		I (1.00)
Alonopsis elongata Sars	+				I (5.00)
Bosmina coregoni Baird					I (1.00)
Chydorus latus Sars					I (1.50)
Chydorus ovalis Kurz			+		I (1.00)
Chydorus piger Sars					I (4.00)
Chydorus sphaericus (Müller)	+	+	+		II (2.63)
Disparalona rostrata (Koch)		+			
Eurycercus lamellatus (Müller)					I (1.50)
Monospilus dispar Sars	+	+			
Peracantha truncata (Müller)					I (1.00)
Pleuroxus uncinatus Baird		+			
Tretocephala ambigua (Lilljebourg)				+	I (5.00)
Cyclopoida					
Acanthocyclops robustus Sars					IV (4.93)
Acanthocyclops vernalis (Fischer)	+	+	+	+	II (2.13)
Acanthocyclops viridis (Jurine)					II (4.00)
Diacyclops bisetosus (Rehberg)				+	III (4.12)
Diacyclops languidiodes (Lilljeborg)	+	+	+	+	I (1.75)
Diacyclops languidus (Sars)	+	+	+		
Diacyclops nanus (Sars)					II (1.57)
Eucyclops serrulatus (Fischer)		+	+	+	II (1.20)
Microcyclops varicans (Sars)					IV (6.38)
Paracyclops affinis (Sars)		+			I (1.33)
Paracyclops fimbriatus (Fischer)	+	+	+	+	I (1.66)
Paracyclops poppei (Rehberg)				+	I (1.00)
Harpacticoida					
Attheyella crassa (Sars)	+	+	+		I (9.00)
Bryocamptus cuspidatus (Schmeil)	+	+	+	+	II (6.00)
Bryocamptus minutus (Claus)		+	+	+	II (3.00)
Bryocamptus pygmaeus (Sars)	+	+	+		IV (6.35)
Bryocamptus vejdovskyi Mrázek					I (1.00)
Bryocamptus weberi (Kessler)	+	+	+		
Bryocamptus zschokkei (Schmeil)	+	+	+	+	V (6.15)
Canthocamptus staphylinus (Jurine)		+			I (1.75)
Echinocamptus echinatus (Mrázek)	+	+	+		I (3.25)
Echinocamptus praegeri (Scourfield)				+	IV (2.94)
Epactophanes richardi Mrázek					I (1.33)
Horsiella brevicornis (van Douwe)					II (1.67)
Maraenobius vejdovskyi Mrázek					I (1.00)
Moraria brevipes (Sars)	+	+	+	+	
Moraria varica (Graeter)		+			
Nitocra hibernica (Brady)					I (4.00)
Tachidius discipes Giesbrecht					I (1.00)

* Key: Frequency of occurrence: I – 1-5 sites; II – 6-10 sites; III – 11-15 sites; IV – 16-20 sites; V – 20-25 sites. Numbers in brackets give mean number of individuals where present.

Table 12 *Comparison of microcrustacea taxa recorded from Robertson et al. (1997), Gilvear et al. (2007) and this study*

The composition of the Cladocera (16 chydorids, one bosminid) is consistent with that reported from other hyporheic communities worldwide (*e.g.* Coe, 2001 for Washington State) which are generally dominated by Chydoridae with small contributions from one or two other families. In a French study, Boulton *et al.*, (2004) recovered 13 species of chydorid (eight of which were also recovered in this study) and three species of daphnid. As these samples were recovered from a lowland section of the Rhône it is possible that bosminids are replaced by daphnids downstream with chydorids essentially ubiquitous.

The most unusual discovery among the Cladocera was *Eurycercus lamellatus* (Müller). Fryer (1993) reports the species as being predominantly lowland in distribution in Yorkshire, its only upland (> 150 m) sites being the alkaline Fishlake and Malham Tarn. It is most usually found in larger water bodies amongst dense aquatic vegetation and detritus, although it has been also reported from rocky lake shores. The recovery of this species from the Feshie at Ballintean (altitude 276 m) and the Truim at Dalwhinnie (349 m), both montane rivers, is therefore unexpected.

Another interesting species is the copepod *Horsiella brevicornis* (van Douwe) (Figure 30) which was recovered in low numbers from six sites (FEU, INV, KIN, MOR, TRU and TWM). This minute invertebrate (0.6 mm long and 0.06 mm wide) was only ever found by accident amongst live copepods in the separate dish used to concentrate the specimens during initial sample sorting. It does not appear to have been reported from the hyporheic zone before. Dussart (1967) describes it as (in English translation) 'a species of fresh and brackish water, able to tolerate a wide range of salinity, living in or between the stems of *Typha*, *Scirpus*, *Iris* and *Sparganium*, *Zostera* and *Fucus*, also in cracks in submerged wood'. Gurney (1932) gives its British distribution as: Norfolk (Hickling Broad, Horsey Mere, Heigham Sounds, Calthorpe Broad and Barton Broad) and Sussex (Littlehampton). Elsewhere the species has been found in Germany, Poland, the former Yugoslavia,

Greece, western Siberia and Egypt. The fact that the species was found in our study from the Inver in Sutherland in the north to the Tweed in the south, and is also present in the Norfolk Broads, indicates that it is almost certainly widely distributed in the UK.

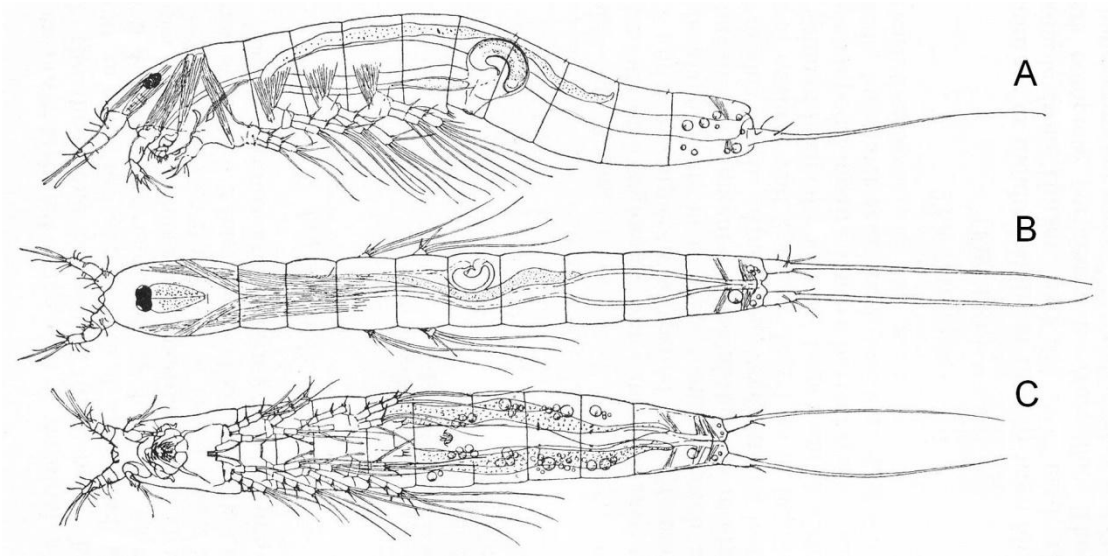

Figure 30 *Horsiella brevicornis (van Douwe)*
A – lateral; B – dorsal; C – ventral. From Gurney (1932)

One site in particular, the Almond at Edinburgh Airport (ALE), had a markedly different fauna from any other site sampled. This site was the most degraded in terms of water quality with an average conductivity of 864 μS cm^{-1} and an average nitrate concentration of 1.4 mg L^{-1} in the HZ samples. While the conductivity of the river itself (843 μS cm^{-1}) was similar to the HZ the river nitrate concentration was much higher at 7.8 mg L^{-1}. Of the 325 invertebrates recovered the majority were oligochaetes (200) and nematodes (48). The most surprising aspect of the fauna was the diversity present within the harpacticoid copepods – of the 54 animals recovered it was possible to determine 50 specimens to a total of nine species. The three next lowest site diversities in this group were all montane: FEU (eight species), MOR and TRU (seven species each). The only insects recovered from the HZ sample at ALE were chironomids (nine specimens), a single dipteran (Rhagionidae) and a single coleopteran (*Stictonectes lepidus* (Olivier)). It is possible that the reduced competition from

other invertebrate taxa at this site has allowed the development of a diverse microcrustacean fauna, although apparently not in the cyclopoid copepods of which only four specimens were recovered (three of which were able to be determined to two species). An alternative possibility is that microcrustaceans can recolonise more quickly after perturbations associated with pollution events, or are simply more tolerant of the generally adverse conditions than are insect taxa.

The appearance within the samples of a cohort of smaller Plecoptera from September onwards indicates that the zone may be an important nursery area for the earliest instars of this group. The timing of this surge of F1 and F2 instars coincides with the hatching period of many species (Elliott, 1967). It has also been observed that the earliest instars of Plecoptera and Ephemeroptera are very seldom encountered using standard kick sampling, even when specifically searching for them (Craig Macadam pers. comm.). It seems likely, therefore, that following hatching the larvae make preferential use of this habitat until they are either too large for interstitial life or they are more able to cope with the greater competition and shear stress found in the epibenthic zone.

4.3 Variability at the local (bar) scale

Much hyporheic work has centred on changes in physicochemistry and ecology along the longitudinal profile of riffles (*e.g.* Franken *et al.*, 2001; Malcolm *et al.*, 2004). This study has focused on gravel bars for two specific reasons: (a) invertebrates present within it are more likely to belong to the obligate hyporheos and (b) sampling the tail end of riffles in the largest Scottish rivers is difficult due to the presence of rapid flows and deep water. However, gravel bars, particularly when located in mid-channel, are usually found adjacent to riffles as they are also formed as a physical response to the change in gradient at that point. Indeed, at many sites gravel bars constitute the exposed section of a riffle and become part of it when inundated at higher flows. The results from this study should, therefore, be comparable with those conducted on riffles.

In an in-depth study of a single riffle in the Speed River, Ontario, Franken *et al.* (2001) studied changes in physicochemistry, bacterial density, protein content, detritus content and faunal composition of the downwelling and upwelling zones respectively at its head and tail. It was found that there were significant differences between downwelling and upwelling water in temperature, pH, redox potential, dissolved oxygen (DO) and nitrate. The composition of the taxa in the two zones was different and its distribution was correlated with depth, dissolved oxygen, detritus and protein content. The main drivers for this community therefore appear to be dissolved oxygen, dissolved nutrients and the presence of CPOM.

The water chemistry results from this study show some clear trends in water quality from upstream to downstream (Appendix 3B) although these are not statistically significant. Conductivity and total alkalinity both increase in pit and pipe samples through the bar with total dissolved phosphate increasing in pits only. Dissolved oxygen, pH, nitrate and nitrite all decreased through the bar with nitrite decreasing in pits only. This increase in total dissolved phosphate is possibly attributable to bird faeces as it was observed that waterfowl were preferentially roosting at the lower ends of bars at several sites, though why nitrate and ammonia are not similarly elevated is unexplained. In pit samples both the total number of invertebrates and invertebrate richness were highest at the upstream end of the bar and lowest at the downstream end; results from the middle of the bar were ambiguous with MD having slightly higher numbers than MU in both cases.

Decrease in dissolved oxygen through the bar has been reported widely in the literature (*e.g.* Boulton *et al.*, 1998, Franken *et al.*, 2001) and these results agree with this. The pattern of more elevated nutrients at the upstream end of a 'riffle' and lower values at the downstream end has not been reported widely in other studies where, generally, the reverse has been found (Valett *et al.*, 1990). One possible explanation is that downwelling water containing nutrients and dissolved

oxygen creates a hot spot for microbial production at the riffle head. This hypothesis is supported by the higher number of invertebrates and increased diversity present at these sites.

One important factor that was not investigated during this research was the physical compactedness of the sediment being sampled. It is suspected that the highly compacted nature of the sediment encountered at many sites and concomitant implications for porosity and permeability are a limiting factor in the development of a complex HZ. When conducting any future research I would recommend the employment of an index of compactedness against which invertebrate numbers and diversity could be tested. This could be expressed as an index of pipe insertion difficulty (*e.g.* 1 = easy, 2 = moderate, 3 = difficult, 4 = very difficult, 5 = extremely difficult) or an analogue of the total effort required (a count of the number of hammer-blows required to install the pipe) as physical measurements of this parameter would be logistically complex in the field. It is my suspicion that infiltrating water creates a local hotspot with a rich microbial and invertebrate fauna where the majority of hyporheic processes occur. The physical compactedness of the sediment along with decreasing DO then act as a filter with mostly microbial activity thereafter. This is supported by evidence from the pipe samples where, although the chemical gradients broadly reflect those from the pit samples, invertebrate numbers and taxonomic richness are not correlated.

4.4 Environmental controls on the biota of the HZ

The chemical properties of the HZ in this study exhibit a mixture of heterogeneity and homogeneity. For three parameters (conductivity, pH, and total alkalinity) values measured in the separate sub-samples were similar at the site level and reflected values in the surface water. Two parameters (nitrite and ammonia) were particularly variable at the site level and a further two (nitrate and total dissolved phosphorus) showed an intermediate level of variability. DO was generally similar among pits and pipes at a site; however, values were consistently higher in pits than in pipes. The only

statistically significant relationship was between nitrate and total dissolved phosphorus (R^2 = 0.328, F = 89.457, St.Er. = 0.324, β = 0.573, P = 0.000).

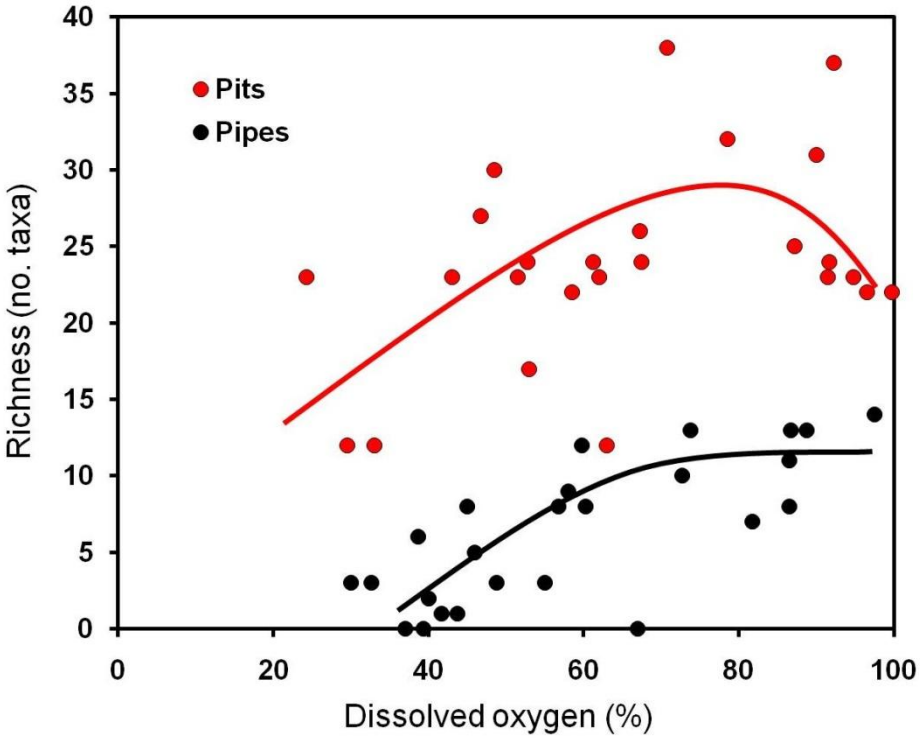

Figure 31 *Relationship between DO and taxonomic richness in pit and pipe samples*

The only significant correlations between any of the environmental parameters measured and the invertebrate fauna at sub-sample level were between dissolved oxygen and taxonomic richness (R^2 = 0.176, F = 22.389, St.Er. = 0.274, β = 0.425, P = 0.000) and dissolved oxygen and the total number of invertebrates found (R^2 = 0.027, F = 5.014, St.Er. = 0.027, β = 0.166, P = 0.026). However, the relationship at a site level is strongly non-linear (shown for the total number of taxa in Figure 31); it can be seen that the number of taxa and dissolved oxygen are positively correlated from aobout 30 to 80%, but beyond this point the number of taxa decreases notably. In pipe samples this relationship is less apparent as a result of the lower number of taxa present and the small number of samples with high DO values, but there is potentially a lessening in the number of additional taxa above 80%. This is most easily explained by increased competition between individuals at high DO

leading to elimination of less competitive taxa. At depth, the number of animals is consistently much lower and densities are therefore unlikely to reach levels where the number of taxa is reduced by competition.

The distribution of the invertebrate fauna, therefore, appears to be primarily correlated with DO. While concentrations of the main limiting nutrients (N and P) are correlated with DO, they do not appear to be main drivers of faunal community structure in the HZ. While the CCA analysis did not find DO as a significant factor explaining invertebrate community composition two correlated analogues of it (distance to source in pits and site altitude in pipes) were found to be significant.

The higher levels of ammonia found at montane sites when compared to upland and lowland sites (Appendix 3B) is an anomaly for which I currently have no explanation.

4.5 Value of the hyporheos in bioindication

The development and application of a standardized sampling technique across multiple spatial scales in this study is analogous to the establishment of sampling methods to support the assessment of the biological quality of rivers *via* the BMWP / ASPT scoring system (Hawkes, 1997). Although the range of BMWP scores generated from the collected samples is roughly comparable to those expected from standard benthic kick sampling, the back-calculated BMWP scores for taxa in the HZ are much more restricted. The BMWP scoring system was designed to highlight biological differences across a wide spectrum of river types (from high altitude torrential streams dominated by Ephemeroptera, Plecoptera and Trichoptera to sluggish, lowland rivers dominated by chironomids and oligochaetes). In contrast, the HZ, although not actually a natural part of that spectrum, is analogous to a highly truncated portion of it, with all samples being taken from essentially the same microhabitat, almost without regard to the character of the overlying river. The

range of back-calculated values (5.50 – 7.12) is therefore much narrower than the revised BMWP values for these macroinvertebrate taxa (2.1 – 12.4). Within HZ samples, therefore, it is much more likely that high BMWP scoring taxa will occur alongside low scoring taxa than would be the case in the benthos.

4.6 The contribution of the hyporheos to ecological characterisation

A comparison of SEPA kick sample data and hyporheic invertebrate data for one site (the River Cree at Newton Stewart) is given in Table 13. The basic pattern shown here is replicated at all sites where comparative data was available. A summary of this data from the 20 sites where comparable BMWP data was available is given in Table 14. Multiple kick sample results were available for each site. In order to give as accurate a picture as possible the sample chosen for comparison was the one closest in calendar date to that of the hyporheic sample in order to minimise seasonal effects.

When analysed at the same taxonomic level as kick sampling, hyporheic sampling collects a similar number of taxa (an average of 22.3 and 17.3 respectively), the number of BMWP scoring taxa collected in the hyporheic samples is much lower (8.6 per sample). This reflects the relative scarcity of benthic macroinvertebrates in the HZ, the truncated functional biodiversity of these organisms and also the dominance of meiofauna in the sample that are not collected with the standard kick net (the mesh being too coarse). It should also be noted that even if collected in a kick sample meiofauna are ignored as they do not form a part of the scoring system. When assessing the conservation criteria for a site the taxonomic richness of the fauna is one of the primary measures used for determination. It can be seen from Tables 12 and 13 that the inclusion of hyporheic organisms significantly increases the taxonomic richness at these sites with an average of 58% more taxa being recovered.

	SEPA 13/05/2005	SEPA 16/09/2005	SEPA 18/04/2006	SEPA 03/04/2007	Hyporheic 02/07/2008
Baetidae	40	40	42	127	1
Caenidae	60	10	36	31	
Ephemerellidae	30				
Heptageniidae	53	80	65	22	
Leptophlebiidae		1			
Chloroperlidae	4		9	5	
Leuctridae	9	9	9	4	1
Nemouridae		3	8	2	
Perlodidae	5	2	7	1	
Taeniopterygidae			4	7	
Hydropsychidae	27	70	41	10	
Hydroptilidae	25	2	4	2	
Lepidostomatidae	30	100	40	23	
Leptoceridae	1	1	2		
Limnephilidae	1		4		
Odontoceridae		1	2		
Polycentropodidae		2			1
Psychomyiidae	6	3	1		
Rhyacophilidae		15	3	1	
Sericostomatidae	1	4	15		
Elmidae	30	80	104	14	
Gyrinidae	1		1	1	
Hydrophilidae	2	15	4		
Scirtidae		1	4	2	
Ceratopogonidae			1		1
Chironomidae	150	80	120	20	8
Empididae		1	8		
Simuliidae	35		8	5	3
Tipulidae		2			
Gammaridae	8	8	3		
Ancylidae	2	4	2		
Sphaeriidae		1			
Hydrobiidae				1	
Lymnaeidae		30	4		
Planariidae	4	1	3	1	1
Erpobdellidae	2	8	9	1	
Glossiphoniidae	1	1			
Oligochaeta	80	60	80	27	55
Mites	15	1	15	4	29
Number of Scoring Taxa	25	29	29	21	8
BMWP Score	163	183	192	130	39
ASPT	6.5	6.3	6.6	6.19	4.88
Additional HZ taxa:					
Dryopidae					1
Scizomyzidae					1
Nematoda					18
Microturbellaria					6
Ostracoda					1
Cyclopoida					224
Harpacticoida					9
Cladocera					2

Table 13 *Comparison of SEPA kick sample data from the River Cree at Newton Stewart with hyporheic results from sample CRE*

Hyporheic sampling produced very few BMWP scoring taxa that were not present in the benthic samples analysed (an average of 2.7 taxa per sample). However, examination of the multiple datasets available showed that the majority of these taxa were present in other unpaired kick

samples from the same site. This implies that there are no BMWP taxa that are more likely to be present in the HZ than in the kick sample. When the kick sample and hyporheic sample are combined the kick sample comprises, on average, 65% of total taxa (range 43% to 77%).

Site	KS taxa	HZ taxa	Total taxa	% KS of total taxa	Total scoring HZ taxa	Shared taxa	Taxa only in KS	Taxa only in HZ	No. BMWP scoring taxa only in HZ
ALE	22	11	31	71.0	3	2	20	9	1
ALL	23	11	30	76.7	5	4	19	7	1
BLA	32	19	46	69.6	7	5	27	14	2
CAD	29	19	41	70.7	9	7	22	12	2
CRE	25	19	36	69.4	8	6	19	11	2
DEV	28	17	39	71.8	10	6	22	11	4
EAR	24	20	35	68.6	11	8	16	11	3
END	27	24	40	67.5	15	11	16	13	4
FEB	17	14	26	65.4	7	5	12	9	2
FEF	17	18	30	56.7	8	5	12	13	3
MOR	19	20	31	61.3	10	8	11	12	2
NEV	10	15	22	45.5	7	3	7	12	4
ROY	14	16	25	56.0	7	5	9	11	2
SPD	15	9	23	65.2	3	1	14	8	2
SPK	19	18	30	63.3	10	7	12	11	3
TEC	30	20	44	68.2	11	6	24	14	4
TED	29	17	40	72.5	9	6	23	11	3
TIG	26	23	38	68.4	13	11	15	12	2
TRU	12	20	28	42.9	9	4	8	16	5
TUP	28	15	37	75.7	9	6	22	9	3
Average	**22.30**	**17.25**	**33.60**	**64.8**	**8.55**	**5.80**	**16.50**	**11.30**	**2.70**

Table 14 *Comparison of the number of shared and unique taxa at 20 sites with comparable SEPA kick sampling data*
KS – Kick sample; HZ – Hyporheic zone

A more detailed breakdown of the BMWP and non-BMWP taxa recovered is given in Appendix 4. Of the additional (non-BMWP) taxa recovered using hyporheic sampling techniques the majority were either ubiquitous (*e.g.* nematodes, cyclopoid and harpacticoid copepods), or were present in very

low numbers (four extra families of Coleoptera were recovered, but these are represented by only 13 specimens from three sites). Six taxa show potential use as bioindicators (present in variable numbers from the majority, but not all, sites), these are:

- Cladocera (220 specimens from 20 sites)
- Ostracoda (115 specimens from 21 sites)
- Ceratopogonidae (148 specimens from 20 sites)
- Mites (231 specimens from 23 sites)
- Cnidaria (29 specimens from 9 sites)
- Microturbellaria (73 specimens from 15 sites)

However, little is known of their relative sensitivities to pollution and disturbance and more research would be required before they could be used for this purpose. It is also possible that these are routinely present in kick samples but either pass through the mesh or are simply too small to be observed in the normal sample sorting procedure.

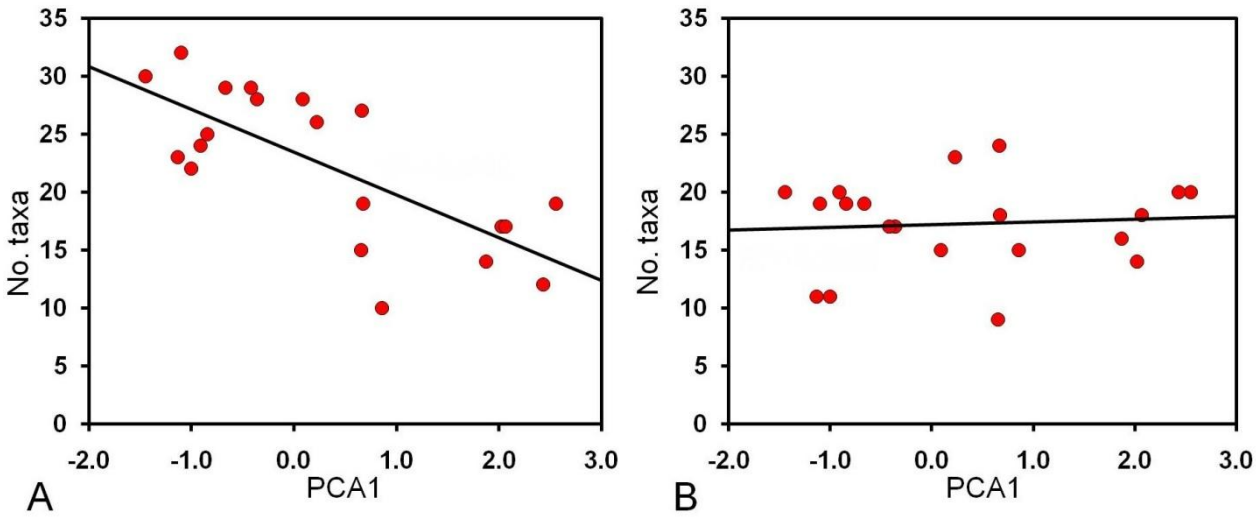

Figure 32 *Total number of taxa recovered vs. Jeffers (1998) PCA1 score*
A – kick sampling; B – hyporheic sampling

A comparison of the total number of taxa recovered by kick sampling and hyporheic sampling with the Jeffers (1998) PCA1 ordination score is given in Figure 32. For kick sampling the number of taxa recovered clearly decreases with increasing PCA1 score (greater 'montaneness'), this is statistically significant (R^2 = 0.541, F = 21.195, St.Er. = 0.032, β = -0.735, P = 0.000). As there is no clear trend in the hyporheic data; this indicates that incorporation of this sampling technique into the current stream sampling methodology would add little useful information. The implication is that HZ invertebrates are responding to environmental drivers in a fundamentally different way to that of the epibenthos.

Another fundamental consideration when assessing the incorporation of this technique into the current stream sampling method is that of time and logistics. The current epibenthos monitoring technique is rapid (approximately 30 minutes to collect and one day to analyse to species level per sample); the equipment is also inexpensive and widely available. To take a hyporheic sample with four replicates as outlined above takes between two and three hours on site and, despite the use of the low-tech option, still requires a moderate investment in specialist equipment. Another cost is in the time required to sort and analyse samples which amounted to two to three days per site (once familiar with the organisms).

4.7 The hyporheic zone and the RCC

The River Continuum Concept (RCC) (Vannote *et al.*, 1980) is one of the most widely used conceptual models to characterise pristine running water systems. It states that a river contains a continuous gradient of physical conditions from source to sea and that this gradient interacts with the biotic community to produce a concomitant gradient within it. The biotic community present at any point will be adapted to the average physical conditions prevalent at that site. The RCC is designed to be

applicable to all rivers, up to and including the Amazon, and is scaled by stream order. In a Scottish context this allows truncation of the model as the lowest section (stream orders 7-10) is not applicable.

Where rivers are very visibly continuous the hyporheic zone in this region is very much discontinuous. Even in the lowland sections of Scotland's largest rivers many river-width bedrock outcrops can be found (a famous example being Campsie Linn on the lower Tay near Stanley). Where no hyporheic linkage in the parafluvial zone or floodplain gravels is present this will result in a complete break in the hyporheic continuum. This fundamental difference to the RCC has prompted Stanford & Ward (1993) to propose the Hyporheic Corridor Concept (HCC) where the HZ is viewed as a string of beads. Individual HZ units will be present along the river continuum, each functioning as an individual unit, but responding to the river above and being influenced by upstream hyporheic units.

The results of this survey agree well with the HCC. A well-developed HZ, though shallow and relatively depauperate is present across the region. CCA analysis found that the primary factor explaining differences between communities in pits was the distance to source and in pipes was site altitude – both fundamental aspects of the RCC / HCC models. One major point that must be noted, however, is that the 25 samples collected here were taken from a relatively restricted section of the RCC model. The distance to source (as an analogue of stream order) varied from 7.5 to 88.7 km, effectively confined to the central section of the RCC model (approximately stream orders four to six). Further research in this region should be undertaken to extend this by sampling headwater streams and the lowest sections of the largest rivers to determine if these trends are continue.

4.8 Potential threats to the hyporheos

This study has not specifically focussed on the sensitivity of the hyporheos to human impact. However, based on the results and those of other studies one can conclude that the following actions may result in potential degradation of the habitat. An important review of human impacts on the HZ is given by Hancock (2002).

The primary threat to the hyporheos is the clogging of pores on the river bed due to siltation. Should this occur then the possibility of riverine water entering the HZ will be severely reduced, thus depriving the hyporheos of oxygen and fine / dissolved organic matter. The major drivers here are poor management practices relating to forestry, agriculture and the construction industry. Allied to this threat is increased eutrophication of lowland freshwaters associated with diffuse agricultural runoff which is likely to exacerbate the pressures of low DO concentrations. It should be noted that the recent increase (and almost certain future expansion) of wind farm construction projects could pose a particular threat, if best practices are not followed, as these are generally in the headwater section of rivers and thus could impact the entire downstream catchment.

Gravel extraction will obviously disrupt the HZ, particularly as the majority of invertebrates appear to inhabit the shallowest portion of it. This could also produce large quantities of silt thus affecting the zone immediately downstream. Any resulting gravel compaction will also have consequences for the zone, although this generally occurs at discrete points in the system and may thus mimic natural discontinuities in the HZ. Within SACs there is a presumption against gravel extraction to protect the associated biota of exposed and submerged gravel substrates (Sadler *et al.*, 2004; Scottish Natural Heritage, 2008).

Drought, whether natural or induced by abstraction or surface water diversion clearly has the potential to impact the hyporheic habitat. However, its effects are poorly understood and in many instances, recovery over the short term has been rapid, provided instream or headwater refugia remain intact and accessible (Boulton, 2003). Moreover, drought itself is a key element in the maintenance of characteristic biodiversity in intermittent freshwater habitats.

The potential effects of global warming on the hyporheos have been studied in two papers. Bärlocher *et al.* (2008) reported that heating a springbrook by an average of 4.3 °C reduced the number of leaf fragments significantly due to enhanced leaf litter processing; as these fragments were the primary food source for hyphomycetes, these too were predicted to decrease. Tixier *et al.* (2009) used the same experimental set-up to determine the effect on the chironomid community; it was found that composition and abundances changed markedly during both the manipulation and recovery period. An earlier experiment investigating the effects of warming on the epibenthos at the same site (Hogg & Williams, 1996) reported decreased total densities, increased larval growth rates, earlier emergence of adults, smaller sizes at maturity in a nemourid stonefly and altered sex ratios in a lepidostomatid caddisfly with a 2 - 3.5 °C rise in temperature. This indicates that the effects on the hyporheos are likely to be diverse and unpredictable. Elevated water temperatures, as anticipated in Scotland due to global climate change could therefore have the potential to significantly alter hyporheic and epibenthic communities.

The hyporheos may benefit from active reworking of the river bed which generates the three-dimensional mosaic of microhabitats upon which the hyporheos depends, maintains oxygenation and reduces silt accumulation. Allowing rivers to resume their natural meandering or braided status, as, for example, has been allowed in the Tummel Shingle Islands SSSI, should therefore be encouraged where possible.

One of the most interesting and important findings was the presence of a diverse hyporheic community at the most heavily impacted site surveyed (the Almond downstream of Edinburgh Airport). Although community composition differed markedly from all other sites the presence of nine species of harpacticoid copepod, including two species not found elsewhere, indicates that the hyporheic community in Scotland is adaptable and resilient. This bodes well for the future recovery of many of the more polluted streams that are present across the central belt.

4.9 Improvements and future directions

Several important lessons and pointers for future research have arisen from this project. These are outlined below.

The number of sub-samples per site should be increased in any future work. It was unfortunate that two of the three sample sites visited in the initial exercise to assess the optimum number of replicates happened to contain numbers of invertebrates in the pipe samples that were higher than subsequently encountered at any other site. Had it been known that the majority of sites nationally were similar to the third site (at the Tay / Tummel confluence) the number of replicates would have been increased to five, or even six. However, this would have been at the expense of the spatial coverage of the survey. For pure invertebrate surveys I would suggest that the collection of pipe samples be abandoned as they produced so few invertebrates and no additional taxa.

As the fauna has been found to be predominantly shallow I suggest that a standard BMWP kicksweep sample be taken in conjunction with the hyporheic sample.

Little is known of the composition of meiofauna in the epibenthic community and how this relates to that in the HZ below and plankton communities above (*e.g.* in backwaters or the drift community).

Surveys specifically targeting all three habitats together would provide valuable extra data on this subject. It is also suggested that a few standard kick-sweep samples be investigated to determine the presence of meiofauna within them. Once the bulk of the macroinvertebrates and debris have been removed a careful study of the remaining fine detritus under a stereomicroscope may produce some, possibly even many, of the groups recovered in this study (see Robertson *et al.*, 1997).

Further work should be undertaken to assess longitudinal gradients in individual rivers and to extend the survey into the more fine-grained lowland ends of the major rivers (*e.g.* Dee and Tay), and even potentially the estuarine environment (see Williams, 2003). An assessment should also be made of the feasibility of sampling in floodplain terraces away from the river. A watching brief should be maintained for floodplain engineering work that may be planned in these areas (*e.g.* the construction of bridge piers in the floodplain) so that samples can be extracted for analysis.

Further work should be undertaken into the effects of hydrodynamic disturbance (*e.g.* gravel compaction or extraction) on the fauna of the HZ. Thus, reaches that are targeted by farmers for gravel removal due to alleged bed accretion could be assessed in relation to adjacent undisturbed sections.

Further sampling should be undertaken in more polluted sites to determine the precise impacts this may have on the hyporheos. The picture is complex for hyporheic organisms as demonstrated on the Almond at Edinburgh Airport where the loss of invertebrates from the benthic fauna may have facilitated an expansion and diversification of harpacticoid copepods by removal of competition for resources.

Meiofaunal surveys should be undertaken in the karstic regions of Scotland to search for stygofaunal taxa, many of which have restricted distributions and may be under threat from

increased cave exploration or groundwater abstraction. This work could be undertaken in association with the Grampian Speleological Group who have a particular interest in exploring the caves of this region.

A particularly useful study would be an inventory of the invertebrates from boreholes in Scotland as there appears to have been no research whatsoever on the stygofauna of the region. Where abstraction is from alluvial aquifers there is the potential for the creation of a large active hyporheic zone as a result of draw-through of large volumes of hyporheic water from the river. An example of this could be the Spey wellfield (see Mackie-Dawson *et al.*, 1988); this could be a priority site for investigation. Samples from this habitat are rapid to collect, require only the use of a plankton net and are relatively easy to process as a result of the cleaner samples and possibly more limited nature of the fauna.

Discussions should be undertaken at a European level (under the auspices of the CEN) in order to establish standardised sampling protocols for assessment of the hyporheos at an international level. While the PASCALIS project has defined a standardised methodology for groundwater sampling (Gibert, 2001) and proposed refinements after its conclusion (Dole-Olivier *et al.*, 2009) there is no current standard for the hyporheic zone and, as highlighted by the findings of this study, much food for thought.

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Appendix 1 Preliminary survey results

A – Afton Water / Montraw Burn (NS639032 and NS639035)

	Pits							Pipes						
	A1	A2	A3	M1	M2	M3	M4	A1	A2	A3	M1	M2	M3	M4
Cyclopoida	19	24	6		3	4	1				41	44	7	37
Harpacticoida	20	15	5			7	38				50	10	2	
Ostracoda	2	1	6	1	1	3	1				1			2
Cladocera	4	78	3		2						8	4	3	7
Gammaridae														
Asellidae														
Plecoptera	12	20	33		17	5	11				4	1		
Ephemeroptera	4	3	22	2	1	8	2				1			4
Trichoptera		4	4											
Coleoptera			1		2		2					2		
Diptera	18	26	24	2		16	4				3	2	5	2
Collembola						1								
Oligochaeta	16	94	43	5	21	110	52				109	2	11	7
Tricladia			5			2	12						2	
Nematoda	6	4	3		14	71	6				39	1	34	2
Acari	1	2	1		2	5	1				10	1	33	3
Hydrozoa														
Number of groups:	10	11	13	4	9	11	11	0	0	0	10	9	8	8
Number of individuals:	102	271	156	10	63	232	130	0	0	0	266	67	97	64

B – River Teith (NN729006)

	Pits								Pipes							
	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H
Cyclopoida	3	12	12	4	18	27	8	20	24	56	2	68	81		2	26
Harpacticoida	20	14	2		41		15		4	4		17	1			
Ostracoda	1	1			7	3		1				1	8			
Cladocera		1	1				1									1
Gammaridae	5	48		2	4	5	1		6	8	1	4	47		7	
Asellidae	1	3	1	1	1					4		4	5			
Plecoptera		3		1			1		1						1	
Ephemeroptera		2			1								1			
Trichoptera		1											1			
Coleoptera								1							1	
Diptera	19	17	2	3	21	2	2	6	4		1	8	2		1	
Collembola																
Oligochaeta	5	12	16	5	5	4	6		3	6		11	11			
Tricladia	3		3		6		1	1	1				1			
Nematoda	19	71	9	3	86	54	19	22	4	9	1	8	13	4	2	1
Acari	13	7	3		10		7	6					3			
Hydrozoa					1	1										
Number of groups:	10	13	9	7	12	7	10	7	8	6	4	8	12	1	6	3
Number of individuals:	89	192	49	19	201	96	61	57	47	87	5	121	174	4	14	28

Appendix 1 Preliminary survey results (contd.)

C – River Tummel / Tay confluence (NN979510)

	Pits							Pipes						
	A	B	C	D	E	F	G	A	B	C	D	E	F	G
Cyclopoida	10	11	1	1	34	7	247	1						1
Harpacticoida	16	107	3	21	6	10	3	1	1			2		
Ostracoda	1	1				2	94							
Cladocera	1	2			7									
Gammaridae				6			6							
Asellidae				1			10							
Plecoptera					2									
Ephemeroptera														
Trichoptera														
Coleoptera				1	1									
Diptera	1	8		1	2	1	91							
Collembola	1		2		1									
Oligochaeta	94	126	3	89	35	40	20	1	1	6	4			1
Tricladia	3	2	3	9	6	11	1			4				
Nematoda	223	19	52	3	4	38	84	3			1			
Acari	1	11			1		30							1
Hydrozoa				2										
Number of groups:	10	9	6	10	11	7	10	4	2	2	2	1	0	3
Number of individuals:	351	287	64	134	99	109	586	6	2	10	5	2	0	3

Appendix 2 Invertebrate site totals

A) Insecta (Ephemeroptera, Plecoptera, Trichoptera and Homoptera)

[The number in the first column is the taxon reference number as used in the CCA analysis (Figures 26 and 27)]

No.			ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM	**Total**
17	Ephemeroptera	Heptageniidae				7			1	7		1	3			1	3					3	12		1			**39**
18		Leptophlebiidae								4																		**4**
19		Baetidae					1		1	1						2	11		2		2	2		2	1			**25**
20		Caenidae								2				2								1					16	**21**
		Total				**7**	**1**		**2**	**14**		**1**	**3**	**2**		**3**	**14**		**2**		**2**	**6**	**12**	**2**	**2**		**16**	
21	Plecoptera	Nemouridae								1	1		1				8	1						3				**15**
22		Leuctridae		1	2	2	1	2	94	43	5	6				17	62	4	1		1	23	3	17	11	4	9	**308**
23		Chloroperlidae								4	1		1	1			12	3				4	1	2	2			**31**
24		Perlodidae							1									1										**2**
25		Perlidae																							1			**1**
		Total		**1**	**2**	**2**	**1**	**2**	**95**	**48**	**7**	**6**	**2**	**1**		**17**	**82**	**9**	**1**		**1**	**27**	**4**	**22**	**14**	**4**	**9**	
26	Trichoptera	Hydropsychidae								1														2		1		**4**
27		Hydroptilidae										1																**1**
28		Glossosomatidae																				1						**1**
29		Polycentropodidae				1	1	1	1				1			1	1		1		1			2				**11**
30		Psychomyiidae														1												**1**
31		Brachycentridae																								1		**1**
32		Sericosomatidae														1												**1**
		Total				**1**	**1**	**1**	**1**	**1**		**1**	**1**			**3**	**1**		**1**		**1**	**1**		**4**		**2**		
12	Homoptera	Alydidae																						2				**2**

Appendix 2 Invertebrate site totals (contd.)

B) Insecta (Coleoptera and Diptera)

[The number in the first column is the taxon reference number as used in the CCA analysis (Figures 26 and 27)]

No.			ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM	**Total**
33	Coleoptera	Carabidae										1																**1**
34		Chrysomelidae																					1					**1**
35		Dryopidae		1			1			4											2					1		**9**
36		Dytiscidae	1									1																**2**
37		Elmidae				1		1	2	5		1				7	1		1		3			10	6	8	7	**53**
38		Helophoridae			3																			3				**6**
39		Halpidae						1																				**1**
40		Hydrophylidae			1																							**1**
41		Hydraenidae			4																						1	**5**
42		Sciaridae																						1				**1**
43		Staphylinidae										1												1				**2**
		Total	**1**	**1**	**8**	**1**	**1**	**2**	**2**	**9**		**4**				**7**	**1**		**1**		**5**		**1**	**15**	**6**	**9**	**8**	
44	Diptera	Athericidae			1																				4			**5**
45		Ceratopog. (Ceratopogoninae)		31		4	1			3	5	3	7		1	1	7	12	7	14	1	1	1					**99**
46		Ceratopog. (Dasyheleninae)			7				1							1									34			**43**
47		Ceratopog. (Leptoconopinae)									1	1		1		1	2											**6**
48		Chironomidae	9	1	92	4	8	6	11	44	17	28	6	25	19	171	78	31	39	3	7	62	55	37	63	2	5	**823**
49		Empididae															1								1			**2**
50		Rhagionidae	1									3													1			**5**
51		Scizomyzidae			1	1	1																					**3**
52		Simulidae					3					2																**5**
53		Syrphidae				1																						**1**
54		Tipulidae (Limoniini)						1		1										1	2	1						**6**
55		Tipulidae (Hexatomini)									1		1															**2**
		Total	**10**	**32**	**101**	**10**	**13**	**7**	**12**	**48**	**24**	**37**	**14**	**26**	**20**	**174**	**88**	**43**	**46**	**18**	**10**	**64**	**56**	**37**	**103**	**2**	**5**	

Appendix 2 Invertebrate site totals (contd.)

C) Cladocera

[The number in the first column is the taxon reference number as used in the CCA analysis (Figures 26 and 27)]

No.		ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM	**Total**
	Cladocera:																										
56	*Acroperus harpae*															1	2										**3**
57	*Alona affinis*																							1			**1**
58	*Alona costata*																	1									**1**
59	*Alona guttata*				1				71	5	7	34	2			2		13						6		5	**146**
60	*Alona quadrangularis*	1								2		1											5				**9**
61	*Alona rectangularis*																	2									**2**
62	*Alona rustica*											6						4						1			**11**
63	*Alonella nana*																		1								**1**
64	*Tretocephala ambiga*											5															**5**
65	*Alonopsis elongata*									5																	**5**
66	*Bosminia coregoni*							1																			**1**
67	*Chydorus latus*					1																	2				**3**
68	*Chydorus ovalis*								1												1		1				**3**
69	*Chydorus piger*															4											**4**
70	*Chydorus sphaericus*			3		1		1																	3		**8**
71	*Chydorus sphaericus coelatus*			5							1																**6**
72	*Chydorus sphaericus leonardi*										2						1			1			3				**7**
73	*Eurycercus lamellatus*									2														1			**3**
74	*Paracantha truncata*										1																**1**
	Total	**1**		**8**	**1**	**2**		**2**	**72**	**14**	**11**	**46**	**2**			**7**	**3**	**20**	**1**	**1**	**1**		**11**	**9**	**3**	**5**	

Appendix 2 Invertebrate site totals (contd.)

D) Copepoda (Calanoida and Harpacticoida)

[The number in the first column is the taxon reference number as used in the CCA analysis (Figures 26 and 27)]

No.		ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM	**Total**
11	Calanoida sp. indet.							2																			**2**
	Harpacticoida:																										
16	Sp. indet	2		2	1	1		2	2	3	3	1	3		1	26	2	1				1	11	7	1	5	**75**
75	*Atheyella crassa*									1		3	17			6	8	1									**36**
76	*Bryocamptus cuspidatus*	5				3						1		2					4	5						1	**21**
77	*Bryocamptus minutus*	23	1	1	6	3		2	5	1	1	1			2	30	8					2	1	6		15	**108**
78	*Bryocamptus pygmaeus*	7	5	6	6	2	3			5	4	5	5		2	24	5	9	4			2	3	11	6	9	**123**
79	*Bryocamptus zschokkei*				1																						**1**
80	*Bryocamptus vejdovskyi*	1															2							1		3	**7**
81	*Canthocomptus staphylinus*				2							1				6								4			**13**
82	*Echinocamptus echinatus*	1		2	1		1		5		2	1	1		13	3	1	5			1		5	1	6	1	**50**
83	*Echinocamptus praegeri*							2			1	1															**4**
84	*Epactophanes richardi*											1	2	1		1								1		1	**7**
85	*Horsiella brevicornis*	7														1											**8**
86	*Nitocra hibernica*	1																				1					**2**
87	*Tachidius discipes*	1																									**1**
88	*Maraenobiotus vejdovskyi*	4																									**4**
89	Species A																							9			**9**
	Total (to species level)	**50**	**6**	**9**	**16**	**8**	**4**	**4**	**10**	**7**	**8**	**14**	**25**	**3**	**17**	**71**	**24**	**15**	**8**	**5**	**1**	**5**	**9**	**33**	**12**	**30**	

Appendix 2 Invertebrate site totals (contd.)

E) Copepoda (Cyclopoida), remaining crustacean groups and other invertebrates

[The number in the first column is the taxon reference number as used in the CCA analysis (Figures 26 and 27)]

No.		ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM	**Total**
	Cyclopoida:																										
15	Sp. indet.	1	22	267	112	207	146	230	52	34	31	60	20	39	94	29	22	24	35	93	22	320	62	75	169	77	**2243**
90	*Acanthocyclops robustus*		4	8	4	3	1	17	1	1					4	1				6		2	4	4	14		**74**
91	*Acanthocyclops vernalis*				1	1	2	2							3			2		1						5	**17**
92	*Acanthocyclops viridis*								5													2	5	1	7		**20**
93	*Diacyclops bisetosus*		1	4	1	13	1	13	6			1		2	1		1			3		11		1			**59**
94	*Diacyclops languidoides*						4																1		1	1	**7**
95	*Diacyclops nanus*									1			1	1						2		3	1		2		**11**
96	*Eucyclops serrulatus*			1																	2		1	1	1		**6**
97	*Microcyclops varicans*	2		2	47		3	1	9			2			16		2			3	2	4	2	4	2	1	**102**
98	*Paracyclops affinis*	1						2															1				**4**
99	*Paracyclops fimbriatus*													2									2	1			**5**
100	*Paracyclops poppei*				1																	1					**2**
	Total (to species level)	**3**	**5**	**15**	**54**	**17**	**11**	**35**	**21**	**2**		**3**	**1**	**5**	**24**	**1**	**3**	**2**		**15**	**4**	**23**	**17**	**12**	**27**	**7**	
7	Ostracoda		3	4	33	1	7	2	4		1	5			1	3	4	3	1	6	2	1	4	13	7	10	**115**
10	Isopoda						1	1													1	2				2	**7**
	Gammaroida:																										
9	Gammaridae							4																			**4**
9	Crangonyctidae			1	20		13	35	1											3	3	16	6		27	70	**195**
8	Acari	1		4	6	29	2	1	3	6	12	1	13	1	6	5	2	15		3	27	8	8	10	51	17	**231**
14	Tardigrada	1																			1						**2**
13	Gastropoda																						7				**7**
6	Cnidaria				7		2		1							3	2	10				1		2		1	**29**
5	Microturbellaria	5		7	11		1		3	1		4				1		3	10	8	9	3	5			2	**73**
4	Turbellaria		15	25	8	5	5		10	1			1		2	2	15	6		3	11	2	4	1	254	9	**379**
1	Oligochaetes (white mottles)	3	30	43	155		42	92	91	33	69	45	46	22		14	39	79	22	51	285	18	95	46	22	66	**1442**
2	Oligochaetes (others)	197	107	64	85	55	17	25	155	19	33	51	35	14	5	116	45	52	32	71	31	36	52	137	16	100	**1516**
3	Nematoda	48	37	77	29	18	19	11	44	41	153	16	67	68	14	4	11	14	97	42	114	133	28	24	24	153	**1286**

Appendix 3(A) Chemical results – tables

A) Conductivity (µS cm^{-1})

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	883	348	162	173	78	183	96	90	31	33	20	140	228	253	36	21	51	107	392	246	71	148	48	51	162
Min	841	238	102	131	57	136	88	73	20	25	17	94	76	140	24	19	29	44	40	54	56	104	43	36	150
Mean	858.3	264.0	116.9	154.3	67.7	151.9	91.1	79.4	25.3	27.9	18.3	106.8	135.3	186.4	27.9	20.4	34.3	66.4	98.3	95.3	58.9	124.6	44.5	44.9	155.5
River	843	232	103	148	55	136	87	82	21	25	18	88	93	139	24	21	29	43	42	73	55	102	48	38	160

B) Dissolved oxygen (%)

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	44	42	76	73	74	81	93	93	102	105	100	67	85	89	102	107	103	47	89	84	82	90	106	94	96
Min	23	27	29	31	34	26	78	17	80	72	93	42	37	28	70	71	48	28	35	36	45	30	69	38	37
Mean	32.1	32.8	43.6	44.3	47.3	50.5	87.0	57.7	93.1	90.0	97.0	55.8	54.3	53.2	88.4	88.3	82.1	36.6	58.1	59.4	62.0	57.9	83.0	53.6	60.9
River	198	118	100	95	96	116	94	98	104	113	106	112	111	102	98	119	116	97	114	95	109	95	101	96	102

C) pH

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	7.62	7.73	6.71	7.16	6.48	7.29	6.85	6.76	7.40	7.25	7.09	7.11	7.14	7.13	6.79	6.84	7.11	6.67	6.64	6.68	6.91	7.44	6.93	7.39	7.82
Min	7.08	7.48	6.14	6.82	6.12	6.88	6.13	6.46	6.50	6.27	6.03	6.82	5.79	6.87	6.12	5.96	6.20	6.11	6.16	6.44	6.68	6.75	6.55	6.61	7.10
Mean	7.31	7.61	6.37	6.98	6.26	7.09	6.65	6.61	6.93	6.89	6.54	6.91	6.39	7.03	6.46	6.31	6.72	6.36	6.38	6.57	6.81	7.12	6.63	6.89	7.44
River	7.31	7.56	7.35	7.34	6.66	7.53	6.51	6.39	6.95	7.58	6.75	7.04	7.38	6.71	6.77	6.59	7.45	6.87	6.75	7.02	7.02	7.89	6.88	7.53	7.85

Appendix 3(A) Chemical results – tables (contd.)

D) Nitrate (µg L^{-1})

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	1837	1619	497	1247	779	747	1291	679	242	497	329	511	276	478	180	353	227	638	654	821	447	745	203	909	1773
Min	759	264	109	302	20	115	1138	162	62	72	118	250	47	192	99	34	24	98	179	92	357	73	129	93	489
Mean	1384	802	330	767	345	450	1207	343	154	179	153	387	133	336	141	119	120	276	343	580	398	333	170	449	1063
River	7821	894	240	1083	131	709	1245	394	59	68	122	118	57	147	108	35	43	172	131	628	209	387	132	117	1295

E) Nitrite (µg L^{-1})

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	29.4	0	6.1	8.5	162.4	1.7	7.9	312.8	33.5	52.0	32.1	25.3	27.7	18.4	0.7	11.0	42.0	4.5	1.8	13.5	8.4	4.3	0.6	38.3	144.4
Min	4.3	0	3.5	1.4	2.0	0.3	3.7	1.2	1.5	0.5	0.1	0	0	1.7	0	1.8	3.1	1.0	0.1	1.9	3.8	2.9	0	1.7	6.3
Mean	10.6	0	4.7	2.6	25.4	1.3	4.8	56.3	6.2	8.6	4.7	5.2	4.6	5.3	0.1	4.3	8.4	2.0	1.0	6.5	5.9	3.6	0.2	6.6	26.8
River	7.3	0	5.8	2.2	2.6	0.5	10.1	0.9	1.1	0.5	0.4	0	0.2	43.2	0	5.5	3.1	1.5	1.6	10.4	5.4	3.9	1.1	2.2	7.0

F) Ammonia (µg L^{-1})

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	22.0	0	22.6	21.6	20.1	18.4	27.5	138.4	87.6	144.9	179.8	24.4	93.6	17.4	253.5	166.8	152.0	41.9	48.6	68.6	22.1	19.7	194.2	141.6	12.9
Min	0	0	0	4.6	10.4	5.0	4.5	0	16.1	8.4	0	0	0	4.7	20.8	13.0	0	0	0	16.6	0	9.5	43.7	8.0	0
Mean	5.0	0	7.2	11.1	13.6	12.3	11.2	23.6	49.3	36.0	60.8	8.0	17.5	11.6	81.7	57.6	30.0	12.4	10.8	32.9	6.9	14.5	102.6	31.7	2.5
River	3.2	0	21.4	6.4	13.7	9.0	12.1	0	25.8	93.0	61.2	15.0	3.1	29.3	17.4	12.4	34.1	5.6	0	14.0	4.6	20.5	126.3	22.0	0

Appendix 3(A) Chemical results – tables (contd.)

G) Total dissolved phosphate (µg L^{-1})

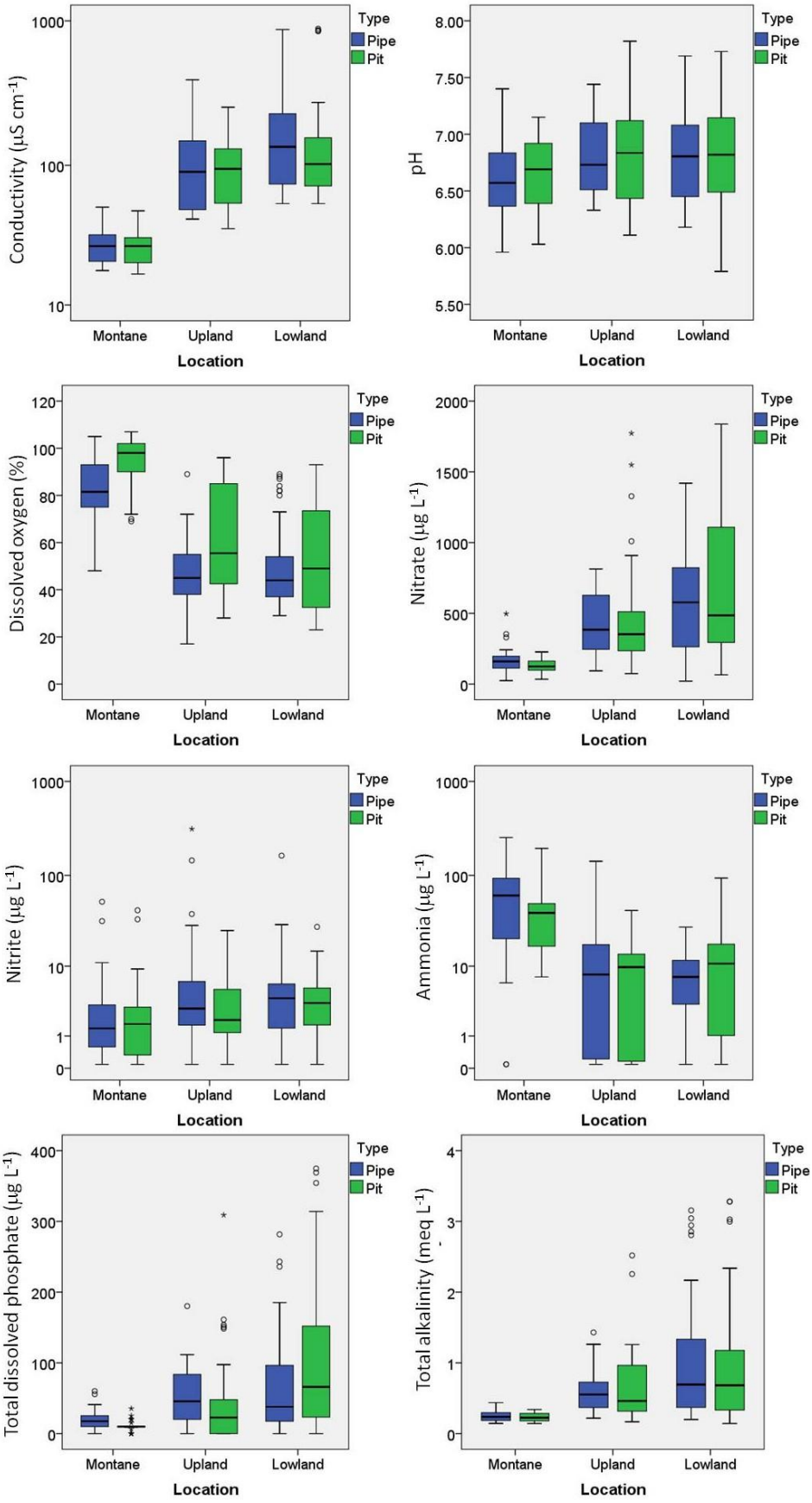
	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	368.9	374.7	153.4	281.7	236.0	153.4	59.0	96.2	60.1	55.7	30.0	0	0	30.7	20.0	40.9	20.5	86.1	111.4	80.4	126.9	150.8	20.0	97.5	309.0
Min	0	70.9	30.7	96.4	0	112.5	17.6	15.2	10.0	15.2	0	0	0	0	0	0	0	15.2	7.6	20.1	0	20.1	0	0	83.7
Mean	115.5	226.2	76.0	196.5	38.9	141.2	37.0	45.6	21.3	28.2	13.7	0	0	12.3	11.2	11.8	12.8	39.2	41.1	40.2	45.0	50.3	8.7	12.2	155.5
River	452.3	546.9	20.5	198.0	0	177.2	30.2	25.3	10.0	15.2	10.0	0	0	20.5	10.0	0	10.2	35.4	0	20.1	5.1	20.1	10.0	0	299.5

H) Total alkalinity (meq L^{-1})

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	FEU	INV	KIN	LIN	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP	TWM
Max	3.28	2.81	0.63	1.14	0.27	1.76	0.72	0.72	0.26	0.26	0.23	0.73	0.43	2.52	0.19	0.16	0.44	0.48	0.59	1.02	0.47	0.74	0.35	0.36	1.26
Min	2.86	2.00	0.25	0.88	0.14	1.07	0.68	0.65	0.21	0.22	0.20	0.50	0.20	1.21	0.15	0.14	0.28	0.37	0.22	0.34	0.33	0.17	0.29	0.26	1.13
Mean	3.07	2.26	0.35	1.00	0.22	1.35	0.69	0.68	0.24	0.23	0.21	0.59	0.35	1.73	0.17	0.15	0.32	0.41	0.31	0.52	0.37	0.43	0.32	0.32	1.21
River	3.20	2.05	0.27	0.92	0.17	1.09	0.66	0.75	0.21	0.24	0.22	0.49	0.48	1.30	0.16	0.17	0.29	0.28	0.26	0.45	0.34	0.18	0.34	0.26	1.24

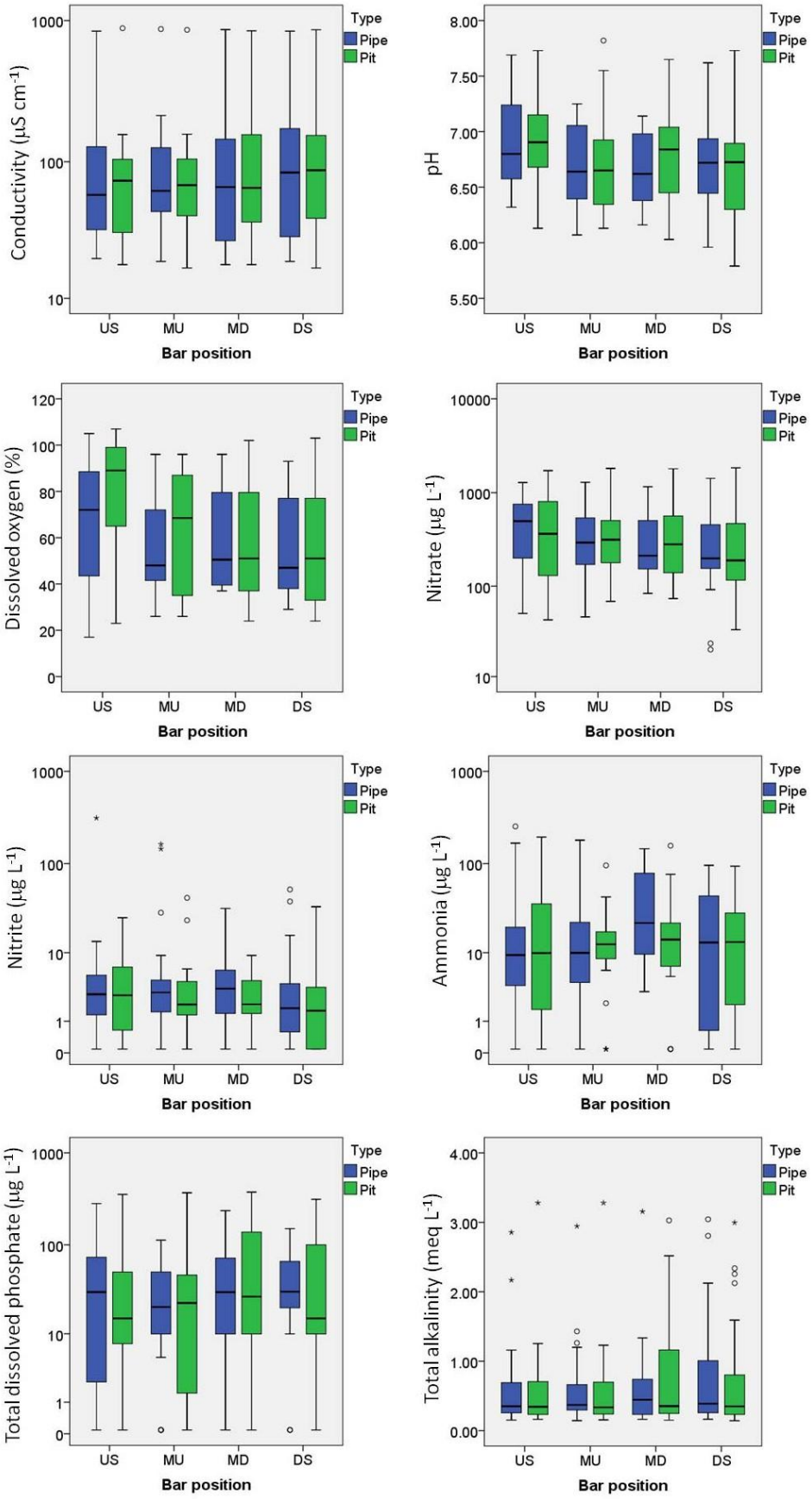
Appendix 3(B) Chemical results – graphs

Variation in chemical parameters at montane, upland and lowland sites.



Appendix 3(B) Chemical results – graphs (contd.)

Variation in chemical parameters with bar position and sample type.



Appendix 4 BMWP and non-BMWP taxa

A list of the family-level taxa belonging to BMWP and non-BMWP scoring groups

	ALE	ALL	BLA	CAD	CRE	DEV	EAR	END	FEB	FEF	MOR	NEV	ROY	SPD	SPK	TEC	TED	TIG	TRU	TUP
BMWP scoring taxa:																				
Asellidae						1	1									1	2			
Gammaridae							4													
Crangonyctidae			1	20		13	35	1							3	3	16	6		27
Hydrobiidae																		7		
Baetidae					1		1	1			11		2		2	2		2	1	
Caenidae								2								1				
Heptageniidae				7			1	7		1	3					3	12		1	
Leptophlebiidae								4												
Chloroperlidae								4	1		12	3				4	1	2	2	
Leuctridae		1	2	2	1	2	94	43	5	6	62	4	1		1	23	3	17	11	4
Nemouridae								1	1		8	1						3		
Perlidae																			1	
Perlodidae							1					1								
Brachycentridae																				1
Glossosomatidae																1				
Hydropsychidae								1										2		1
Hydroptilidae										1										
Polycentropodidae				1	1	1	1				1		1		1			2		
Psychomyiidae																				
Sericostomatidae																				
Chrysomelidae																	1			
Dryopidae		1			1			4							2					1
Dytiscidae	1									1										
Elmidae				1		1	2	5		1	1		1		3			10	6	8
Haliplidae						1														
Hydrophilidae			1																	
Scirtidae																		1		
Chironomidae	9	1	92	4	8	6	11	44	17	28	78	31	39	3	7	62	55	37	63	2
Simulidae					3					2										
Syrphidae				1																
Tipulidae						1		1	1					1	2	1				
Dendrocoelidae			5																	
Planariidae		15	20	8	5	5		10	1		2	15	6		3	11	2	4	1	254
Oligochaeta	200	137	107	240	55	59	117	246	52	102	130	84	131	54	122	316	54	147	183	38
Non- BMWP scoring taxa:																				
Cyclopoida	51	28	276	128	215	150	234	62	41	39	100	46	39	43	98	23	325	71	108	181
Harpacticoida	7	5	17	55	18	11	37	23	5	3	27	5	3		15	4	24	28	19	28
Calanoida							2													
Cladocera	1	0	8	1	2		2	72	14	11	7	3	20	1	1	1		11	9	3
Ostracoda		3	4	33	1	7	2	4		1	3	4	3	1	6	2	1	4	13	7
Homoptera																		2		
Carabidae										1										
Helophoridae			3															3		
Hydraenidae			4																	
Staphylinidae										1								1		
Athericidae			1																4	
Ceratopogonidae		31	7	4	1		1	3	6	4	9	12	7	14	1	1	1		34	
Empididae											1								1	
Rhagionidae	1									3									1	
Scizomyzidae			1	1	1															
Mites	1		4	6	29	2	1	3	6	12	5	2	15		3	27	8	8	10	51
Tardigrada	1															1				
Cnidaria				7		2		1			3	2	10				1		2	
Microturbellaria	5		7	11		1		3	1		1		3	10	8	9	3	5		
Nematoda	48	37	77	29	18	19	11	44	41	153	4	11	14	97	42	114	133	28	24	24

Appendix 5 ANOVA results

(1) Conductivity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	977778.342[a]	2	488889.171	20.384	.000
Intercept	2276461.051	1	2276461.051	94.917	.000
Type	977778.342	2	488889.171	20.384	.000
Error	4365020.437	182	23983.629		
Total	8033023.000	185			
Corrected Total	5342798.778	184			

a. R Squared = .183 (Adjusted R Squared = .174)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	202.890	18.126	11.193	.000	167.127	238.654
[Type=1]	-174.545	27.652	-6.312	.000	-229.104	-119.986
[Type=2]	-98.697	27.373	-3.606	.000	-152.708	-44.687
[Type=3]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

(2) pH

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.305[a]	2	.653	3.945	.021
Intercept	8315.123	1	8315.123	50263.512	.000
Type	1.305	2	.653	3.945	.021
Error	30.108	182	.165		
Total	8492.366	185			
Corrected Total	31.414	184			

a. R Squared = .042 (Adjusted R Squared = .031)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	6.803	.048	142.916	.000	6.709	6.897
[Type=1]	-.168	.073	-2.318	.022	-.312	-.025
[Type=2]	.030	.072	.423	.673	-.111	.172
[Type=3]	0[a]	.	.	.	.	.

(3) DO

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45608.802[a]	2	22804.401	64.117	.000
Intercept	742656.908	1	742656.908	2088.050	.000
Type	45608.802	2	22804.401	64.117	.000
Error	63309.275	178	355.670		
Total	824412.000	181			
Corrected Total	108918.077	180			

a. R Squared = .419 (Adjusted R Squared = .412)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	52.014	2.207	23.564	.000	47.658	56.370
[Type=1]	36.163	3.442	10.507	.000	29.371	42.955
[Type=2]	2.127	3.333	.638	.524	-4.452	8.705
[Type=3]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

(4) Nitrate

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8111772.260[a]	2	4055886.130	32.190	.000
Intercept	31828961.341	1	31828961.341	252.615	.000
Type	8111772.260	2	4055886.130	32.190	.000
Error	22931587.520	182	125997.734		
Total	67112514.263	185			
Corrected Total	31043359.780	184			

a. R Squared = .261 (Adjusted R Squared = .253)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	655.626	41.545	15.781	.000	573.654	737.598
[Type=1]	-508.290	63.379	-8.020	.000	-633.341	-383.238
[Type=2]	-204.346	62.741	-3.257	.001	-328.140	-80.552
[Type=3]	0[a]	.	.	.	.	.

(5) Nitrite

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2456.826[a]	2	1228.413	1.529	.219
Intercept	12367.505	1	12367.505	15.397	.000
Type	2456.826	2	1228.413	1.529	.219
Error	146187.937	182	803.230		
Total	160925.054	185			
Corrected Total	148644.763	184			

a. R Squared = .017 (Adjusted R Squared = .006)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	6.698	3.317	2.019	.045	.154	13.243
[Type=1]	-2.122	5.060	-.419	.675	-12.107	7.862
[Type=2]	6.751	5.009	1.348	.179	-3.134	16.635
[Type=3]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

(6) Ammonia

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	85462.153[a]	2	42731.076	35.424	.000
Intercept	152650.500	1	152650.500	126.546	.000
Type	85462.153	2	42731.076	35.424	.000
Error	219544.575	182	1206.289		
Total	441364.616	185			
Corrected Total	305006.727	184			

a. R Squared = .280 (Adjusted R Squared = .272)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	11.991	4.065	2.950	.004	3.970	20.011
[Type=1]	48.156	6.201	7.765	.000	35.920	60.392
[Type=2]	2.732	6.139	.445	.657	-9.381	14.845
[Type=3]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

(7) Total Alkalinity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.440[a]	2	9.720	23.501	.000
Intercept	74.818	1	74.818	180.891	.000
Type	19.440	2	9.720	23.501	.000
Error	75.277	182	.414		
Total	179.864	185			
Corrected Total	94.717	184			

a. R Squared = .205 (Adjusted R Squared = .197)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	1.023	.075	13.593	.000	.875	1.172
[Type=1]	-.787	.115	-6.853	.000	-1.013	-.560
[Type=2]	-.360	.114	-3.165	.002	-.584	-.135
[Type=3]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

(8) Total dissolved phosphate

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	179047.236[a]	2	89523.618	18.018	.000
Intercept	477926.900	1	477926.900	96.191	.000
Type	179047.236	2	89523.618	18.018	.000
Error	904272.486	182	4968.530		
Total	1642391.903	185			
Corrected Total	1083319.722	184			

a. R Squared = .165 (Adjusted R Squared = .156)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	89.991	8.250	10.908	.000	73.713	106.269
[Type=1]	-74.829	12.586	-5.946	.000	-99.662	-49.996
[Type=2]	-41.452	12.459	-3.327	.001	-66.035	-16.870
[Type=3]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

Appendix 5B: DO and Bar Position

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10149.586[a]	3	3383.195	6.063	.001
Intercept	694708.189	1	694708.189	1244.965	.000
BarLoc	10149.586	3	3383.195	6.063	.001
Error	98768.491	177	558.014		
Total	824412.000	181			
Corrected Total	108918.077	180			

a. R Squared = .093 (Adjusted R Squared = .078)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	56.580	3.341	16.937	.000	49.987	63.173
[BarLoc=1]	18.420	4.749	3.879	.000	9.049	27.791
[BarLoc=2]	3.186	4.799	.664	.508	-6.285	12.657
[BarLoc=3]	2.477	5.206	.476	.635	-7.797	12.751
[BarLoc=4]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

Appendix 5C: Total taxa compared between lowland and montane sites

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	267.782[a]	1	267.782	9.893	.002
Intercept	10444.664	1	10444.664	385.875	.000
Type	267.782	1	267.782	9.893	.002
Error	1786.454	66	27.067		
Total	12234.000	68			
Corrected Total	2054.235	67			

a. R Squared = .130 (Adjusted R Squared = .117)

Parameter Estimates

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	10.575	.823	12.855	.000	8.933	12.217
[Type=1]	4.032	1.282	3.145	.002	1.473	6.592
[Type=3]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.