# Habitat complexity and species diversity in rivers

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***For my dad and nana***

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# SUMMARY

1. Rivers are highly structured ecosystems in which habitat complexity and heterogeneity are postulated to affect species diversity and abundance. However, the exact links between habitat structure and organisms such as macro-invertebrates remain poorly understood.

2. Field surveys at the patch and reach scale were combined with a field experiment in the Rivers Wye and Usk, Wales, to: i) quantify variations in habitat complexity and heterogeneity among habitats and river sections, ii) assess their influence on macro-invertebrates, iii) separate the ecological effects of confounding physical factors and iv) determine whether habitat type and heterogeneity promote nestedness of assemblages.

3. At the patch scale, surface complexity but not habitat heterogeneity increased macro-invertebrate diversity, richness and abundance independently of surface area, but only when habitat type was ignored. Surface complexity and heterogeneity were minor determinants of variations in macro-invertebrates among habitat types.

4. A field experiment involving baskets containing cobbles, pebbles, gravel or 50:50 mixtures revealed that flow type explained significant variations in macro-invertebrate richness, abundance and composition, and appeared to filter organisms based on their body size. Surface complexity and interstitial volume had no ecological effect, implying that differences in assemblages among mineral habitats may result from flow-related effects.

5. Macro-invertebrate assemblages occurring in some mineral habitats, typically with lower macro-invertebrate diversity, richness, abundance and trait diversity, appeared to be nested sub-sets of those occurring in some organic habitats. Nested assemblages had reduced trait diversity and altered trait representation. River sections containing fewer habitats supported assemblages of lower abundance, which appeared to be weakly nested sub-sets of those in heterogeneous sections.

6. Habitat complexity and heterogeneity are concluded to have consistently weak effects on macro-invertebrates at the scales studied. In contrast, habitat type and flow type affected macro-invertebrate assemblage composition, structure, and traits, with important implications for river management and conservation.

## CONTENTS

# Chapter 1 General Introduction

## 1.1. Introduction

The habitat is a central concept in ecology, and may be considered as one of its few unifying theories (Block and Brennan, 1993; Mitchell, 2005). Variations among habitats are implicated in the distribution and diversity of species at local, regional and global scales (MacArthur, 1965; MacArthur and Wilson, 1967; Shmida and Wilson, 1985). For individual species, habitat influences traits such as life-history strategy (Southwood, 1977). At the community level, variations in habitat complexity, area and heterogeneity are important determinants of species co-existence, community structure and ecological functioning (e.g. MacArthur and Wilson 1967; Godbold *et al*., 2010).

The ecological significance of habitat structure has been documented for grasslands, woodlands, deserts, rivers, lakes, rocky shores and coral reefs, with studies reporting positive relationships between species diversity and components of physical structure for birds, mammals, amphibians, reptiles, fish and invertebrates (e.g. MacArthur, 1965; Bell *et al*., 1991; Tews *et al*., 2004). In particular, rivers are highly structured ecosystems in which there is long-standing recognition of the importance of physical habitat structure in the distribution and abundance of organisms (Percival and Whitehead, 1929, 1930; Whitehead, 1935; Jones, 1949). Over 100 years of research has shown how different assemblages are associated with riffles and pools, variations in substrate size and the presence of vegetation (Hynes, 1970). This structure is nested within a hierarchy of spatial scales, occurring from the surface of individual substrate particles up to entire river catchments (Vaughan *et al*., 2009). Within this hierarchy, rivers are mosaics of physical habitat, with heterogeneity varying both laterally, longitudinally and in relation to stream size (Tockner and Ward, 1999; Ward *et al*., 2002). Ecological theory on the importance of physical structure in influencing river organisms is also well-developed, suggesting that the biological and ecological traits of species are determined by variations in disturbance frequency and intensity among habitats creating spatial refugia (Townsend and Hildrew, 1994).

Rivers are however arguably the most simplified ecosystem as a result of widespread human influences, with direct and indirect changes to river habitats resulting from activities such as channel engineering, water abstraction and flow regulation (Malmqvist and Rundle, 2002). Whilst river management and conservation have typically focused on water quality issues, improvements in water quality in recent decades have highlighted the effects of changes in physical habitat on river biodiversity (Vaughan *et al.*, 2009). Habitat structure is also commonly a central focus of river restoration schemes, many of which are based on a 'building-block' approach since this is perceived as being more manageable than attempting to influence individual species or factors such as natural disturbance regime (Petersen *et al.*, 1992; Harper *et al.*, 1995; Palmer *et al.*, 2010). In Europe, these changes have been driven by legislative requirements which emphasise the ecological importance of physical structure in rivers. In particular, the Water Framework Directive (2000/60/EC) requires the implementation of Programmes of Measures to protect or restore 'good ecological status' at all scales from the reach to entire river basins (European Commission, 2000). Furthermore, the Directive explicitly recognises the importance of hydromorphology (European Commission, 2000).

Central to this habitat-based approach to river management and conservation is the widespread assumption that greater habitat heterogeneity supports greater biodiversity (Harper and Everard, 1998; Palmer *et al.*, 2010). Direct evidence to support this assumption remains scarce, whilst studies investigating the complexity of individual habitats have reported mixed effects of different structural features and on different aspects of assemblage composition and structure. These findings are exemplified by macro-invertebrates (e.g. Downes *et al.*, 1995), a widespread, abundant and highly diverse group of organisms which have been the focus of a large number of studies of habitat structure in rivers (e.g. Hynes, 1970). Such studies have involved a variety of approaches including field surveys, manipulative in-stream experiments and laboratory flumes. Clear understanding of the importance of habitat structure is however limited because of confounding variation in other physical factors among habitats. One such major factor is flow, which influences macro-invertebrates as well as the distribution of habitats themselves. More complex habitats also tend to have a greater surface area and may therefore support a greater number of species due to a species-area effect (e.g. Coleman *et al.*, 1982). Despite this, studies combining field surveys and in-stream experiments to separate these effects are surprisingly few (e.g. Robson and Barmuta, 1998).

## 1.2. Aims and thesis structure

The gaps and uncertainties in existing knowledge identified above created the context for this thesis. Using a combination of field surveys and a field experiment, I aimed to: i) quantify variations in habitat complexity and heterogeneity among habitats and river sections, ii) assess their influence on macro-invertebrates, iii) identify, and where possible separate, the ecological effects of confounding physical factors, and iv) determine whether habitat type and heterogeneity affect the spatial organisation of assemblages. The specific hypotheses tested were:

1. At the patch scale, greater surface complexity and surrounding habitat heterogeneity increase macro-invertebrate diversity, richness and abundance and alter assemblage composition and body size (Chapters 3 and 4).

2. Any effects of surface complexity on macro-invertebrates are not simply a result of increased surface area (Chapters 3 and 4).

3. Habitat structure is a major determinant of variations in macro-invertebrates among mineral habitats (Chapter 3).

4. Variation among river habitats in other conditions - in this case flow type - may transcend the effects of habitat structure (Chapter 4).

5. Mineral habitats support macro-invertebrate assemblages of lower taxonomic diversity, richness, abundance and trait diversity than organic habitats. The assemblages in mineral habitats are nested sub-sets of those in organic habitats (Chapter 5).

6. At the reach scale, homogeneous river sections support macro-invertebrate assemblages of lower taxonomic diversity, richness, abundance and trait

diversity than heterogeneous sections. The assemblages in homogeneous sections are nested sub-sets of those in heterogeneous sections (Chapter 5).

Although tested specifically in rivers, each of these hypotheses is intended to have broader relevance to ecology more generally. In this respect, rivers are amenable to testing hypotheses about habitat structure because they are so are highly structured across a range of spatial scales. These aims are presented as specific hypotheses in the chapters in which they are investigated. All chapters are self-contained with their own references and are intended to be developed as papers for publication.

## 1.3. Study area

All the work for this thesis was carried out in the upland catchments of the Rivers Wye and Usk, mid-Wales. The Wye and Usk catchments provide a suitable location to assess the effects of habitat structure on macro-invertebrates for several reasons. Firstly, tributaries in the middle of the Wye catchment, where the work for this thesis took place, drain Devonian Old Red Sandstone or marls (Jarvie *et al.*, 2003). Similarly, the geology of the Usk catchment is predominantly Silurian and Devonian Old Red Sandstone and mudstones, so there is little variation in underlying geology among locations. Urbanisation has a minimal impact in both catchments, with dominant land use a mixture of rough- and semi-improved pasture, although the Usk catchment is susceptible to sedimentation with localised effects on macro-invertebrates (Larsen *et al.*, 2009). Tributaries in the middle of the Wye catchment are relatively un-polluted and calcareous (50-250 mg $CaCO_3$ $l^{-1}$), contrasting the low pH of those in the acidified uplands and the moderately elevated biochemical oxygen demand (BOD) and nutrient concentrations of those in the lowlands (Ormerod and Edwards, 1987; Jarvie *et al.*, 2003; Clews and Ormerod, 2009). Water quality in the Usk catchment is also relatively uniform, with BOD and nutrient concentrations similar to the middle Wye catchment (Environment Agency, 2007, 2009). Other confounding factors were therefore minimised and should not obscure possible effects of habitat structure.

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# Chapter 2 Effects of habitat structure on river macro-invertebrates - a review

## 2.1. Abstract

1. From the early 20th Century onwards, evidence has revealed how river habitat structure is a major influence on macro-invertebrates at local, catchment and regional scales. Structure has two distinct components, complexity (the abundance of structural features) and heterogeneity (the composition and arrangement of different structural features). Structure is also nested hierarchically, with small scale features determined by processes occurring at larger spatial scales. This review evaluates: i) the hierarchical arrangement of physical structure in rivers, ii) differences in macro-invertebrate assemblages among habitats, iii) the role of habitat complexity and heterogeneity in macro-invertebrate distribution and abundance, iv) evidence for underlying mechanisms and v) current challenges and future research needs.

2. Habitats such as differently sized substrates or macrophytes of contrasting morphology typically vary in macro-invertebrate diversity, abundance and composition. The reported effects of habitat complexity on macro-invertebrate assemblages have however been mixed. Macro-invertebrate diversity, richness and abundance sometimes increase with complexity, whilst effects on composition have been less well studied. Understanding of these relationships and their underlying mechanisms are variable among habitats. Direct evidence for the widespread assumption that habitat heterogeneity enhances macro-invertebrate diversity is also scarce despite being widely applied in river restoration.

3. A major obstacle to understanding the importance of habitat structure in rivers, as well as other ecosystems, is the use of many inconsistently defined terms. Furthermore, habitat complexity is difficult to quantify, which may account for inconsistencies in relationships between habitat structure and macro-invertebrates. Finally, most studies have been limited to a single, small spatial scale, whilst few have considered whether the effects of complexity are independent of surface area. Improved measurement of complexity, assessments of scale-dependent effects and investigations to separate the

effects of habitat structure from other physical variations in rivers would all provide an important advancement.

## 2.2. Introduction

Evidence of the importance of physical habitat structure in the distribution and abundance of macro-invertebrates is amongst the oldest in river ecology (Percival and Whitehead, 1929, 1930; Whitehead, 1935; Jones, 1949). Early studies recognised that different assemblages occurred on contrasting types of river bed and that the preferences of individual species were related to their biological requirements (Percival and Whitehead, 1929; Jones, 1949). Hynes (1970) reviewed 50 years of research showing how different assemblages are associated with riffles and pools, variation in substrate size and the presence of vegetation. Since then, habitat structure has been investigated at local, catchment and regional scales, and has frequently been cited as one of the main factors influencing macro-invertebrate biodiversity (e.g. Vinson and Hawkins, 1998; Ward and Tockner, 2001; Clarke *et al.*, 2008; Heino, 2009). There are many possible mechanisms which are not mutually exclusive. Greater physical structure may increase the range of niches available for different species or reduce the likelihood of competitive exclusion (Shmida and Wilson, 1985; Townsend and Hildrew, 1994). More structured habitats might also support a greater abundance or range of resources (e.g. Gawne and Lake, 1995), or ameliorate the effects of disturbance and predation through the provision of refugia (e.g. Lancaster, 2000). However, structurally complex habitats also tend to have a greater surface area which may increase species richness through a species-area effect, for example passively by increasing the number of individuals (Coleman *et al.*, 1982).

Two distinct components of habitat structure are generally distinguished, both of which depend on the scale of measurement: complexity and heterogeneity (McCoy and Bell, 1991). Heterogeneity encompasses both the composition (number and relative abundance) and configuration (spatial arrangement) of different structural features (e.g. habitat patches or types) (Li and Reynolds, 1995). In contrast, complexity has been defined as the total abundance of structural features (e.g. crevices on the surface of a substrate particle), with more complex habitats having greater numbers of structural features within a fixed area (McCoy and Bell, 1991). However, river ecologists recognised early on that these terms are often used interchangeably, and this is a problem which still exists today (Erman and Erman, 1984; Palmer *et al.*, 2010). Problems in defining habitat structure have hindered general understanding of its ecological importance and the underlying mechanisms, and these are discussed further below. Overall, this review aims to evaluate: i) the hierarchical arrangement of physical structure in rivers, ii) differences in macro-invertebrate assemblages among habitats, iii) the role of habitat complexity and heterogeneity in macro-invertebrate distribution and abundance, iv) evidence for underlying mechanisms and v) current challenges and future research needs.

## 2.3. The hierarchical arrangement of physical structure in rivers

Physical structure in rivers occurs not only within river reaches, but at all spatial scales from the surface roughness of individual substrate particles up to and including entire catchments. This structure is nested hierarchically, with small scale structure determined by progressively less frequent processes occurring at larger spatial scales (Townsend and Hildrew, 1994). These ideas are conceptualised in the process-based framework of Frissell *et al*. (1986), in which microhabitats are nested within pool-riffle systems, pool-riffle systems within reaches, reaches within segments and segments within streams. Whilst rivers were initially considered as one-dimensional continua, for example the River Continuum Concept (Vannote *et al*., 1980), the integration of ecological, geomorphological and hydrological understanding has resulted in a development of these paradigms into those recognising rivers as dynamic mosaics arranged hierarchically within the wider landscape (Wiens, 2002; Poole, 2010).

River systems are hierarchically branching structures, and this geometry has long been of interest to geomorphologists (Grant *et al*., 2007; Brown *et al*., 2011). Many such Dendritic Ecological Networks are fractal in nature, exhibiting self-similarity across scales (Rodríguez-Iturbe and Rinaldo, 1997; Grant *et al*., 2007). As the fractal dimension of a structural feature increases, it fills the space it occupies more completely, providing a quantitative measure of complexity. Horton’s (1945) classic Laws of Network Composition are themselves scaling relationships and are recognised as describing a fractal structure (Tarboton, 1996; Rodríguez-Iturbe and Rinaldo, 1997). Since river networks drain the entirety of their river basins it might be expected that their fractal dimension should approach 2 (Rodríguez-Iturbe and Rinaldo, 1997). Studies using the dividers method have generally confirmed this hypothesis, with estimates of fractal dimension based in the range 1.65-2 (Tarboton *et al*., 1988; Crave and Davy, 1997; De Bartolo *et al*., 2006), although lower values have also been reported (Veltri *et al*., 1996). For example, an estimation of the fractal dimension of six river networks in Southern Italy extracted from 1: 25 000 maps gave values of 1.7-1.9 (De Bartolo *et al*., 2006), whilst two river networks in French Brittany had a fractal dimension of 1.65 and 1.75 based on 1: 100 000 maps (Crave and Davy, 1997).

Furthermore, the structure of river networks appears to be clustered at particular scales. River networks throughout the United States generally exhibited two distinct regions of scaling (Tarboton *et al*., 1988). The first region, between 10 and 1000 m, had a fractal dimension of ~1.05 and resolved the sinuosity of smaller streams, whilst a second region, between 1000 and 10 000 m, had a fractal dimension of almost 2 reflecting the branching characteristic of river networks. The existence of two distinct regions of scaling is not unique to rivers and does not exclude them from the fractal concept, but instead may facilitate the identification of ecologically relevant scales of habitat structure (Mandelbrot, 1977; Burrough, 1981).

Since river systems are hierarchically organised, the diversity and distribution of macro-invertebrate assemblages are expected to be organised in a similar manner (Ward *et al*., 1999; Parsons *et al*., 2003). Although numerous studies have examined the relative effect of environmental variables at different spatial scales, few have adopted a truly hierarchical approach (Parsons and Thoms, 2007). Such studies suggest that patterns in macro-invertebrate distribution are arranged hierarchically in response to physical structure occurring at multiple scales (e.g. Parsons *et al*., 2003; Parsons and Thoms, 2007). Large regions and catchments, and local reaches appear to be the important levels of organisation for physical-biological associations (Parsons and Thoms, 2007), corresponding to a hierarchy of environmental trait filters on the regional species pool, through which only species with the necessary trait characteristics are able to pass (Poff, 1997). The occurrence of organisms within a particular habitat therefore not only reflects the bottom-up influence of local habitat structure but also the top-down constraints of catchment scale factors (Malmqvist, 2002; Parsons and Thoms, 2007). Management or restoration of physical structure at a particular point in the hierarchy therefore requires consideration of the larger scale context (Roni *et al*., 2008; Palmer *et al*., 2010).

## 2.4. Differences in macro-invertebrate assemblages among habitat types

Early investigations recognised the importance of distinguishing different types of river habitat and their macro-invertebrate assemblages, although the selection of habitats was largely subjective (e.g. Percival and Whitehead, 1929). Habitat inventories have figured prominently in subsequent studies, with the development of robust multivariate statistical techniques such as TWINSPAN and Detrended Correspondence Analysis (DCA) over the last 30 years providing important insights into these characteristic assemblages (e.g. Wright *et al*., 1984; Palmer *et al*., 1991; Armitage *et al*., 1995; Harper *et al*., 1995). One approach defines ‘mesohabitats’ as medium (approximately 1 m) scale habitats which may be visually identified from the bank, introducing a scalar dimension to the term biotope (Armitage *et al*., 1995). The classification of mesohabitats in rivers in Southern England using DCA identified eight mesohabitats ('*Ranunculus* fast', '*Ranunculus* slow', 'Silt', '*Nasturtium*', '*Phragmites*', 'Sand', 'Gravel fast' and 'Gravel slow’) supporting distinct macro-invertebrate assemblages and indicator species were subsequently identified (Armitage *et al*., 1995; Pardo and Armitage, 1997). Furthermore, many of these assemblages remain relatively distinct throughout the year (Armitage *et al*., 1995; Pardo and Armitage, 1997). A parallel approach developed by Harper *et al*. (1992) focuses on the ‘functional habitats’ of rivers, groups of ‘potential habitats’ which support characteristic macro-invertebrate assemblages and together represent the habitat structure and biodiversity of the river channel (Harper and Everard, 1998). Sixteen functional habitats were identified on the River Welland in the East Midlands based on TWINSPAN, with the similarity of functional habitats on a neighbouring river of contrasting water chemistry and macro-invertebrate species indicating that this list was broadly applicable (Harper *et al*., 1992).

There are several advantages to the habitat-level approach to river management and conservation. Habitat types are visually distinct and rapidly identifiable from the bank (Armitage *et al*., 1995). They are also associated with macro-invertebrate assemblages that remain relatively consistent temporally (Armitage *et al*., 1995; Pardo and Armitage, 1997) and among locations (Harper *et al*., 1992). Studies have also shown that there are agreements between the habitats supporting distinct assemblages in Europe and elsewhere (Buffagni *et al*., 2000; Storey and Lynas, 2007). The habitat-level approach may provide a predictable link between the influence of geomorphological and hydrological processes on river habitat occurrence and macro-invertebrate communities (Harper and Everard, 1998; Harper *et al.*, 2000). The occurrence of functional habitats has been shown to be associated with flow biotopes and predictable based on depth, velocity and Froude number (Newson *et al.*, 1998; Kemp *et al.*, 1999, 2000). Habitat inventory approaches now have a central role in river survey, management and restoration. In the United Kingdom, they form an integral part of the Environment Agency's River Habitat Survey, a standard method of surveying the entire river channel, together with the riparian zone and floodplain, at the reach scale (Raven *et al.*, 1997; Harper and Everard, 1998). The assumption is that if the characteristic macro-invertebrate assemblages of different habitat types are known, habitat inventories can provide a surrogate for biodiversity and a means of assessing the effects of habitat modification (Harper *et al.*, 1992; Armitage *et al.*, 1995; Harper and Everard, 1998). Many restoration strategies also rely on a 'building-block' approach to enhancing habitat heterogeneity, in which different habitats are progressively added to a river channel as though they were units resembling building blocks (Petersen *et al.*, 1992). This approach is perceived as being more manageable than attempting to influence individual species or factors such as natural disturbance regime (Harper *et al.*, 1995; Palmer *et al.*, 2010).

There are however inevitably differences between the habitat types considered to be important to macro-invertebrates (Palmer *et al.*, 1991; Harper *et al.*, 1992; Armitage *et al.*, 1995; Brunke *et al.*, 2002). There are also overlaps in the macro-invertebrate assemblages occurring in even relatively distinct habitat types (Palmer *et al.*, 1991; Harper *et al.*, 1992; Armitage *et al.*, 1995; Pardo and Armitage, 1997). Habitat boundaries do not represent physical barriers and in reality may be indistinct, with gradients of substrate size and assemblage composition existing between mineral habitats (Pardo and Armitage, 1997; Armitage and Cannan, 2000). Most species are capable of movement among habitats through a combination of active upstream dispersal and downstream drift, and may occupy different habitats at different stages of their life cycle (Hynes, 1970; Hanquet *et al.*, 2004). Species occurrence is also influenced by the availability of local colonisation sources, reflecting patch dynamics at the reach scale (Pringle *et al.*, 1988; Townsend, 1989), as well as processes at larger scales (see above). Furthermore, macro-invertebrate assemblages may reflect seasonal fluctuations in the physical distinctiveness of habitats and the distribution of habitat patches themselves (Pardo and Armitage, 1997; Armitage and Cannan, 2000). Even within the same river, the distinctiveness of assemblages may vary longitudinally, with macro-invertebrate assemblages in the Buffalo River in South Africa associated with habitats in middle and lower reaches but not in the headwaters (Palmer *et al*., 1991). This lack of distinctiveness in upland streams may reflect the dominance of mixtures of substrate sizes, the absence of marginal depositional areas dominated by vegetation or the occurrence of small habitat patches in which physical conditions are highly variable (Palmer *et al*., 1991; Pardo and Armitage, 1997).

## 2.5. Habitat complexity

### 2.5.1. Substrate

Substrate is one of the most important factors determining variations in macro-invertebrate assemblages within and among rivers, with many species restricted to certain substrate types or showing distinct preferences for them (Hynes, 1970). Surface complexity and substrate size are among the most important characteristics and have been most commonly investigated (e.g. Vinson and Hawkins, 1998). Substrate heterogeneity is discussed later.

#### ***Complexity of the surface of individual substrate particles***

One important characteristic at the scale of individual organisms is the surface roughness of individual substrate particles, which depends largely on the geological nature of the substrate (Sanson *et al*., 1995; Boyero, 2003a). Colonisation experiments involving trays of natural substrates have reported greater total abundances on rough substrates (sandstone) compared to smooth (quartzite), although there was no effect on richness or composition (Erman and Erman, 1984). Rough artificial substrates can support greater densities and richness than smooth ones, with several individual taxa found in larger numbers on rough tiles (Clifford *et al*., 1989, 1992; Way *et al*., 1995). These artificial surfaces may not however realistically mimic the texture of natural substrates (Clifford *et al*., 1989; Downes *et al*. 2000a). To overcome this problem, Sanson *et al*. (1995) quantified the surface roughness of natural stones and Downes *et al*. (1998; 2000a, 2000b) subsequently manipulated the roughness of paving bricks within the natural range observed. Rough bricks were colonised by more species (Downes *et al*., 1998; 2000a, 2000b), and in some instances greater abundances (Downes *et al*., 1998), than smooth ones. In the same experiment, natural substrates consisting of rough-textured granodiorites and crystal-rich volcanic rocks supported more individuals and greater rarefied species richness than smooth-textured crystal-poor volcanic rocks, siltstones and sandstones (Downes *et al*., 2000a). Quantification of the roughness of stones in a previous field survey demonstrated that when stones were categorised based on their surface roughness, rough stones supported a greater species richness and abundance than smooth ones, although categories were also associated with the volume of cracks, pits and shelves (Downes *et al*., 1995). Mean cross-sectional areas of cavities less than 0.36 mm and 0.36-0.68 mm, which provided continuous measures of roughness, were also not related to richness or abundance (Downes *et al*., 1995).

The effects of surface roughness on macro-invertebrates are however likely to be complex and depend on factors such as colonisation time and other environmental conditions. Whilst rough cobbles in experimental substrate patches supported greater richness and abundance after two hours compared to smooth cobbles, no effect was observed for richness after one week and the opposite effect occurred for abundance (Boyero, 2003a). Smooth tiles may also be preferentially colonised by species such as heptageniid mayflies in response to near-bed hydraulic conditions or the ability to attach to the surface (Clifford *et al*., 1989, 1992; Boyero, 2003a).

Pits, grooves and crevices on the surface of substrate particles also provide surface complexity at a larger scale. Evidence for the effect of these structural features on macro-invertebrates comes mainly from experimental studies manipulating artificial substrates. Paving bricks with large crevices similar to those on natural stones supported greater species richness and densities of individuals in a stony, upland stream (Downes *et al*., 1998). Furthermore, the richness and abundance of species colonising artificial paving bricks of identical surface area significantly increased with the number of grooves, although there was no significant difference in the abundance of organisms on bricks with no grooves and the lowest number of grooves (Douglas and Lake, 1994). In a laboratory flume, the total density of macro-invertebrates was 2.3 times greater on grooved concrete blocks than smooth ones (Way *et al*., 1995). Macro-invertebrates may actively seek out crevices, with significantly higher proportions of baetids and hydropsychids observed in large crevices than on the equivalent area on top of creviced bricks (Downes *et al*., 1998).

Other studies have however provided contrasting results. Natural stones covered in a greater proportion of pits did not support greater densities of two species of hydropsychid larvae, whilst a subsequent colonisation experiment showed that only one of these species occurred at a greater density on pitted bricks (Downes and Jordan, 1993). The presence and number of pits also had no effect on diversity, total density or the individual density of other species (Downes and Jordan, 1993). Natural stones with a greater volume of cracks, pits and shelves or mean cross-sectional areas of cavities less than 3.6 mm also did not support a greater richness or abundance of species (Downes *et al*., 1995). Furthermore, the response of individual species to pits, grooves and crevices varies among studies. Whilst Douglas and Lake (1994) reported that the abundance of *Agapetus monticolus* was three times greater on grooved than ungrooved artificial paving bricks of equivalent surface area, in a subsequent experiment the presence of grooves had no significant effect on colonisation by *A. monticolus*, with equal densities of individuals occurring on the sides of bricks as within grooves (Gawne and Lake, 1995). Clay tiles to which pits or crevices were added did not support greater macro-invertebrate densities than plain, sand-blasted tiles without pits or crevices (Robson and Barmuta, 1998).

Current studies suggest that the effects of the surface complexity of individual substrate particles are attributed to a number of factors. In a laboratory flume, the distribution of organisms reflected the ability to cling to the substrate in their preferred flow conditions, with *Baetis rhodani* only able to persist in fast current velocities on rough substrates (Lancaster and Mole, 1999). Grooves, pits and crevices provide flow refugia as well as more favourable flow conditions for activities such as net construction by species such as polycentropodids and hydropsychids (Downes and Jordan, 1993; Way *et al*., 1995). Surface complexity may also increase the provision of epilithon, which provides food for scrapers such as *A. monticolus*. Natural stones and experimental bricks of greater surface roughness, as well as grooved bricks, have been shown to attain higher abundances of algae than smooth ones, with evidence that only scrapers were more abundant in experimental patches with rough cobbles after one week of colonisation (Gawne and Lake, 1995; Sanson *et al.*, 1995; Boyero, 2003a). Effects are however likely to be complex, with other studies reporting lower algal abundances on natural and artificial substrates with large crevices than those without or that surface roughness has no effect on algal colonisation (Downes *et al.*, 1998; Robson and Barmuta, 1998). The effect of complexity on epilithon abundance may also vary temporally (Gawne and Lake, 1995).

Furthermore, several studies investigating surface complexity at this scale have not considered the independent effects of surface area, or adjusted species richness and abundance for the increase in surface area on complex substrates (e.g. Lancaster and Mole, 1999; Downes *et al.*, 2000a). Despite evidence that macro-invertebrate richness and abundance may be correlated with surface area (e.g. Downes *et al.*, 1995; Boyero, 2003a; cf. Erman and Erman, 1984), current studies suggest the effects of complexity provided by grooves may independently influence macro-invertebrates (Douglas and Lake, 1994; Downes *et al.*, 2000a). Measurement of the possible increase in surface area with surface roughness has generally not been attempted even when it has been considered for other structural features (e.g. Downes *et al.*, 1998, 2000a). Despite this, rough surfaces still supported more species than smooth ones when richness was rarefied by the smallest number of individuals observed (Downes *et al.*, 1998; 2000a) and greater densities of individuals when abundances were adjusted for increased surface area (Clifford *et al.*, 1989).

### ***Substrate size***

Some of the earliest published studies reported that greater macro-invertebrate richness occurred on large substrates associated with riffles compared to the fine substrates typically occurring in pools (Percival and Whitehead, 1929, 1930; Hynes, 1970). Subsequent studies have confirmed these results, with clay, silt and sand supporting distinct assemblages typically of lower richness and abundance in a number of rivers (Harper *et al.*, 1992, 1995; Cogerino *et al.*, 1995; Pardo and Armitage, 1997). Fine substrates are often dominated by *Sphaerium*, *Pisidium*, *Limnaea* and *Gammarus*, whilst larger substrates typically support diverse assemblages including mayflies such as *Rhithrogena semicolorata* and *Baetis rhodani* (Percival and Whitehead, 1930; Hynes, 1970). Substrate selection by some species is reasonably distinct, with no significant variation in the preferences of macro-invertebrates colonising four substrate size classes along an altitudinal gradient in an alpine stream (Allan, 1975). Experimental studies have also shown that substrates of increasing size from sand to cobbles support greater species richness and abundance (Allan, 1975; Hart, 1978; Williams and Mundie, 1978; Erman and Erman, 1984; Bourassa and Morin, 1995). Boulders typically support lower densities (Bourassa and Morin, 1995) whilst bedrock, especially within pools, supports species-poor macro-invertebrate assemblages (Brown and Brussock, 1991; Harper *et al.*, 1995).

Larger stones often have a greater surface area, which typically increases species richness (Hart, 1978; Clements, 1987; Douglas and Lake, 1994). In Hart’s (1978) study, larger stones supported lower species richness per unit area than small ones, indicating that the greater species richness observed on large stones was attributed to their greater surface area. Whilst some studies showing positive relationships between substrate size and macro-invertebrate richness and abundance have not considered surface area (Williams and Mundie, 1978; Erman and Erman, 1984; Bourassa and Morin, 1995), other studies have reported weak relationships with surface area indicating other mechanisms are also involved (Minshall and Minshall, 1977; Reice, 1980; Heino and Korsu, 2008). For example, passive sampling was rejected as an explanation for the species-area relationship reported by Douglas and Lake (1994), with groups of small stones harbouring fewer species than large stones of the same surface area. Larger substrates are less likely to be disturbed by high flows, and this greater stability may also facilitate the development of epilithon (Douglas and Lake, 1994). Greater interstitial volume in large and medium substrates is also likely to be important providing they do not become clogged with fine sediment (e.g. Richards and Bacon, 1994; Bo *et al.*, 2007), with experimental studies showing that gravel and cobbles containing interstitial spaces supported a greater richness and abundance than cement-embedded substrates with few interstices (Flecker and Allan, 1984). Interstices provide habitat for macro-invertebrates and may act as refugia from disturbance (Williams and Hynes, 1974; Harper *et al.*, 1995; Dole-Olivier *et al.*, 1997; Robertson and Wood, 2010), although the provision of refugia from predation may be less important (Flecker and Allan, 1984). The accumulation of detritus in interstices has also been shown to determine colonisation patterns among substrates in other studies (see below).

The relationship between substrate size and macro-invertebrate assemblages is however not a simple one, with some studies reporting that small substrates are colonised by a greater number of species and total abundance than larger ones (Minshall and Minshall, 1977; Wise and Molles, 1979). Other studies have found greater abundances on intermediate sized substrates, often attributed to their greater accumulation of detritus (Rabeni and Minshall, 1977; Williams and Mundie, 1978; Reice, 1980). Some species may show no distinct preference for particular sized substrates whilst, as discussed previously, others may exhibit changes in substrate preferences with season and life stage (Williams and Mundie, 1978; Williams, 1980; Hanquet *et al.*, 2004). Relationships between substrate size and macro-invertebrate assemblages are also likely to be confounded by complex interactions with flow conditions, which play a primary role in determining macro-invertebrate assemblages as well as the distribution of differently sized substrates themselves (Allan, 1995). This co-variation in flow and substrate size in the field is observed most obviously among riffles and pools (Hynes, 1970; Minshall and Minshall, 1977).

### *Complexity of river bed surfaces*

Few studies have investigated surface complexity at scales greater than individual substrate particles, which can be described as the complexity of the river bed surface. Measurement typically involves taking profiles of the river bed using a profiler such as the one used by Gore (1978), however most studies have recorded variation in substrate height as a measure of substrate roughness or heterogeneity although not strictly included in the definition of complexity or heterogeneity given here (e.g. Gore, 1978; Statzner *et al.*, 1988; Schmid-Araya, 1998; Tockner and Ward, 1999; Crosa and Buffagni, 2002; Muotka and Laasonen, 2002; Muotka *et al.*, 2002; Brooks *et al.*, 2005; Lepori *et al.*, 2005; Muotka and Syrjänen, 2007).

An alternative approach is to calculate the fractal dimension of the river bed surface (Robson, 1995 cited in Robson and Chester, 1999; Schmid, 2000; Robson *et al*. 2002). Studies have reported that for 1-2 m transects, bedrock riffles have a fractal dimension of around 1.01 and are significantly less complex than boulder-cobble riffles, which have values of approximately 1.1 (Robson, 1995 cited in Robson and Barmuta, 1998). For 10 cm transects, however, bedrock and boulder-cobble riffles had fractal dimensions of 1.02 and 1.029 respectively, and were indistinguishable (Robson, 1995 cited in Robson and Chester, 1999). In a further study, the fractal dimension of cobble, gravel and bedrock riffles was measured along transects of approximately 1-10 m using rolling balls with a circumference of one-tenth of the transect length (Robson *et al.*, 2002). Three groups varied significantly in complexity: bedrock at all extents and gravel at 2-6.8 m which had the lowest values, gravel at the 1.2 m scale and cobbles at 4.25-7 m which had greater values, and cobbles at 1.2-2 m which were the most complex (Robson *et al.*, 2002). Whilst all values for bedrock were below 1.03, fractal dimension varied significantly across spatial scales for other habitat types and peaked at the 1.2-2 m scale (Robson *et al.*, 2002). In other words, bedrock consistently provides a simple, uniform habitat for macro-invertebrates. In contrast, cobbles and gravel appear to provide the greatest complexity at 1.2-2 m, corresponding to the fact that the greatest number of substrate particles is encountered at this scale, whilst at smaller and larger scales, surfaces are more uniform relative to transect length.

The effect of surface complexity on macro-invertebrates at scales between the surface of individual substrate particles and 10 m remains unclear. Structurally complex boulder-cobble riffles in the Mountain River, Tasmania, supported greater species richness than bedrock riffles in three seasons (Robson and Chester, 1999). However, differences in smaller scale ‘microhabitats’ among riffle types accounted for a greater amount of the variance than differences in ‘riffle-scale’ structure (Robson and Chester, 1999). Furthermore, comparisons of species richness between patches of bare rock and patches of filamentous algae, the only ‘microhabitats’ which occurred in both riffle types, revealed that neither supported greater species richness in spring or winter (Robson and Chester, 1999). A previous experiment investigating the effects of two scales of habitat structure in Mountain River reported similar results, with riffle-scale structure affecting the colonisation rate of clay tiles by two slow-moving grazers but not overall species density or the density of fast- or slow-moving grazers (Robson and Barmuta, 1998). Conversely, Taniguchi and Tokeshi (2004) manipulated the number and size of cavities on experimental plates of equal upper surface area to create five levels of complexity measured using fractal dimension. Fractal dimension was significantly correlated with taxon richness and density in all seasons except for density in spring (Taniguchi and Tokeshi, 2004).

Studies investigating the mechanisms for these effects are even scarcer. Most interest has focused on the provision of food resources, however relationships remain unclear. For example, whilst more complex experimental plates accumulated more fine particulate organic matter, ‘chlorophyll a’ concentrations weakly declined with complexity whilst coarse particulate organic matter showed no relationship (Taniguchi and Tokeshi, 2004). Differences in macro-invertebrate richness and density between resource pre-conditioned and control plates were largely insignificant (Taniguchi and Tokeshi, 2004), however the applicability of these results is limited by the lack of significant differences in resources between treatments. In another study, complex boulder-cobble riffles supported greater algal abundances than simple bedrock riffles in winter but not in summer because they created a refuge from macro-invertebrate grazing (Robson, 1996). Effects were however complex, and may also have been due to greater fish predation pressure in boulder-cobble riffles in winter weakening the interaction between macro-invertebrate grazers and algae (Robson, 1996). Despite this, evidence suggests that possible effects of the complexity of river bed surfaces may be independent of variations in surface area, with rarefaction indicating that the greater species richness in boulder-cobble riffles compared to bedrock riffles was not determined by random placement (Robson and Chester, 1999).

### 2.5.2. Macrophytes

Macrophyte stands have been the focus of long-standing interest, with studies in European rivers showing that they support greater macro-invertebrate densities and species richness than adjacent mineral substrates (e.g. Percival and Whitehead, 1929; Wright *et al*., 1992; Buffagni *et al*., 2000; Harrison, 2000). Different macrophyte species typically support different macro-invertebrate assemblages, varying in abundance and species composition (e.g. Feldman, 2001). Such differences are often attributed to differences in morphology, although growth habit is also important (Harper *et al*., 1995).

Macrophytes with dissected leaves generally support greater abundances of macro-invertebrates than those with simple morphologies (Krecker, 1939; Rooke, 1986; Humphries, 1996; Taniguchi *et al*., 2003). For example, macro-invertebrate abundances were greatest on *Myriophyllum*, with the greatest degree of leaf and stem dissectedness, in a lowland Tasmanian river (Humphries, 1996). Fewer studies have investigated differences in macro-invertebrate species richness, although these have typically shown that complex natural and artificial plants also support a greater number of species (Rooke, 1986; Taniguchi *et al*., 2003; Warfe and Barmuta, 2006). Species richness was higher on structurally complex *Ranunculus yezoensis* than on simple *Sparganium emersum*, as well as on complex artificial plants compared to simples ones of the same surface area, across a range of patch sizes in a Japanese stream (Taniguchi *et al*., 2003).

Despite this, few studies have quantified the structural complexity provided by macrophytes in rivers. Warfe *et al*. (2008) distinguished between the structure provided by three macrophyte analogues imitating the structurally complex *Myriophyllum variifolium*, less complex *Triglochin procera* and simple *Eleocharis sphacelata* using nine indices including the size and frequency of interstitial spaces and fractal dimension. Both macro-invertebrate abundance and rarefied species richness were greatest on the *Myriophyllum* analogue, and were strongly related to the average refuge space from predation and the fractal dimension at 5 x magnification (Warfe *et al*., 2008). Fractal dimension varied also among macrophytes in a Pampean stream in the order *Egeria densa* < *Stuckenia striata* < *Elodea ernstae* < *Ceratophyllum demersum* and was positively related to macro-invertebrate abundance but not rarefied richness or diversity (Ferreiro *et al*., 2011).

Numerous studies have reported the importance of macrophytes in providing refugia (e.g. Heck and Crowder, 1991; Harrison *et al*., 2005), a direct food resource for shredders and miners as well as periphyton and detritus (e.g. Sand-Jensen and Madsen, 1989), and oviposition and emergence sites (e.g. Harrison, 2000), as well as modifying physico-chemical conditions and near-bed velocities (e.g. Sand-Jensen, 1998; Champion and Tanner, 2000). Specifically, more complex macrophytes may be colonised by a greater abundance of periphyton and epiphytic algae and consequently greater abundances of scrapers (Rook, 1984; Warfe and Barmuta, 2006), although other studies have shown contrasting results (Taniguchi *et al*., 2003) or that effects vary among macrophyte species (Ferreiro *et al*., 2011). Macrophytes with finely-dissected leaves have also been shown to accumulate more detritus, and support proportionally more gatherers, in some studies (Rooke, 1984) but not others (Taniguchi *et al*., 2003). Structurally complex macrophytes may also provide more prey refuges, with the effectiveness of both pygmy perch (*Nannoperca australis*) and the predatory damselfly *Ischnura heterosticta tasmanica* significantly reduced in a structurally complex *Myriophyllum* imitation compared to the structurally simpler *Triglochin* and *Eleocharis* in a laboratory experiment (Warfe and Barmuta, 2004). Furthermore, the use of *Myriophyllum* by damselfly larvae in response to the presence of pygmy perch meant they captured fewer prey (Warfe and Barmuta, 2004). Results, however, vary among studies, with the influence of pygmy perch on macro-invertebrate grazers in a subsequent field experiment greatest on a complex *Myriophyllum* imitation (Warfe and Barmuta, 2006). The importance of macrophyte complexity *per se* also remains elusive. Whilst Ferreiro *et al*. (2011) reported that fractal dimension was independent of plant surface area, surface area was correlated with both the fractal dimension at 5 x magnification and the average refuge space from predation measured by Warfe *et al*. (2008).

Studies have revealed inconsistencies in the relationship between macro-invertebrates and macrophyte morphology. For example, Taniguchi *et al*. (2003) reported that more structurally complex artificial, but not natural, plants supported greater abundances of macro-invertebrates. Growth habit may also interact with morphology to produce complex effects on macro-invertebrates. For example, significantly greater densities of phytophilous macro-invertebrates occurred on *Vallisneria americana,* which has structurally simpler leaves but occupies the full water column, compared to *Trapa natans,* which has sparse finely dissected leaves and is largely emergent (Feldman, 2001). The importance of complexity may also be overridden by other environmental factors, with structurally simpler *Triglochin procera* and *Eleocharis sphacelata* supporting greater macro-invertebrate richness than *Myriophyllum* in a lowland Tasmanian river due to the lower likelihood of being exposure by low water levels (Humphries, 1996).

### 2.5.3. Moss and macro-algae

Greater abundances of macro-invertebrates also typically occur on mosses and macro-algae than adjacent mineral substrates (e.g. Percival and Whitehead, 1929, 1930; Brusven *et al*., 1990; Suren, 1991a; Cattaneo *et al*., 2004; Stream Bryophyte Group, 1999). They have also been shown to support distinct macro-invertebrate communities, typically of increased species richness, compared to other substrates in a range of stream types (Suren 1991a; Harper *et al*., 1992; Stream Bryophyte Group, 1999). For example, the algae *Cladophora* and *Enteromorpha* as well as the moss *Fontinalis* had distinct macro-invertebrate assemblages compared to mineral substrates and macrophytes in lowland streams in the East Midlands (Harper *et al*., 1992). Macro-invertebrate abundance and species richness typically increase with the biomass of moss (e.g. Suren, 1993; Clenaghan *et al*., 1998; Chantha *et al*., 2000; Heino and Korsu, 2008), although increased richness may simply be attributed to the greater number of individuals passively increasing species richness (Heino and Korsu, 2008).

Whilst there are no known studies investigating the effects of the structural complexity of moss and macro-algae, bedrock crevices containing moss and small patches of filamentous algae both supported greater species richness than bare rock or the underside of cobbles in boulder-cobble and bedrock riffles in the Mountain River (Robson and Chester, 1999). Furthermore, differences in these ‘microhabitats’ accounted for a greater amount of the variance than differences among riffle types (Robson and Chester, 1999). In a previous experiment, addition of artificial moss to sandblasted clay tiles increased species density, as well as the densities of fast- and slow- moving grazers, over plain tiles and those to which pits or crevices had been added (Robson and Barmuta, 1998).

Groups of stones covered in greater amounts of filamentous macro-algae have been shown to support different macro-invertebrate communities of greater species richness (Downes *et al*., 1995), although species richness and total abundance as well as algal cover itself were related to stone surface area. Overall, algal-covered bricks also supported greater richness and abundance than those from which algae were removed (Downes *et al*., 1998). However, algal abundances were also affected by other structural features and therefore their effects were confounded (Downes *et al*., 1998). A subsequent experiment to assess their independent effects on natural and artificial substrates showed that macro-algae independently increased the abundance and rarefied richness of colonising macro-invertebrates (Downes *et al*., 2000a).

Mosses are considered to influence macro-invertebrates through a number of mechanisms (Stream Bryophyte Group, 1999). The presence of moss may provide refugia from the effects of predation and high flows (Brusven *et al*., 1990; Suren, 1991a; Stream Bryophyte Group, 1999). Mosses also provide a surface for the growth of periphyton, whilst the reduction of water velocities results in the accumulation of large quantities of fine sediment and detritus (Glime and Clemons, 1972; Suren, 1991a; Suren, 1992; Cattaneo *et al*., 2004). For example, *Fontinalis dalecarlica* supported significantly greater 'chlorophyll a' concentrations, and a greater frequency and abundance of grazers than gravel in a small Québec stream (Cattaneo *et al*., 2004). Abundances of the majority of species on artificial bryophytes were related to periphyton or detrital biomass whilst 'stem' density had little effect, suggesting that shelter was less important than the provision of food resources (Suren and Winterbourn, 1992). Whilst a few species associated with moss feed on it directly, such as some *Nemoura*, most feed almost exclusively on accumulated periphyton and detritus (Hynes, 1970; Suren and Winterbourn, 1991; Harper *et al*., 1995). Experimental studies comparing colonisation of natural and artificial mosses confirm this, showing that imitations support broadly similar assemblage composition (Glime and Clemons, 1972; Suren, 1991b). Macro-algae such as *Cladophora* are also generally more important as a substrate for epiphytic algae that are subsequently grazed by macro-invertebrates than as a direct food resource (Harper *et al*., 1995). Whilst the majority of studies have not assessed whether the presence of moss and macro-algae are simply attributed to an increase in surface area (Robson and Barmuta, 1998; Robson and Chester, 1999; Downes *et al*., 1995), stones and experimental bricks with macro-algae still supported more species than those without when richness was rarefied by the smallest number of individuals observed (Downes *et al*., 1998; 2000b).

### 2.5.4. Woody debris, leaf litter and tree roots

Several reviews have considered the importance of woody debris in rivers (e.g. Gregory *et al*., 2003) and its influence on macro-invertebrate assemblages (e.g. Hoffman and Hering, 2000; Benke and Wallace, 2003). Woody debris may support a larger biomass and diversity of macro-invertebrates than other habitats, despite typically occupying a small proportion of the river channel (O'Connor, 1991; Benke and Wallace, 2003). Small scale additions of woody debris are now a common goal of river restoration schemes (e.g. Lester and Boulton, 2008), with an increasing number of experimental studies investigating the ecological effects (e.g. Hilderbrand *et al*., 1997; Lemly and Hilderbrand, 2000; Spänoff *et al*., 2006; Lester *et al*., 2007; Entrekin *et al*., 2008).

Despite increasing recognition of the importance of woody debris, few studies have investigated the effect of their variable surface complexity provided by interstitial spaces and furrows. Current research shows that the diversity and density of macro-invertebrate communities is significantly related to surface complexity, which is dependent on the degree of wood decay (O'Connor, 1991; Phillips, 2003; cf. Warmke and Hering, 2000). Individual species may also show a preference for wood with surface furrows and avoid wood with a smooth surface (Warmke and Hering, 2000). To assess this experimentally, O'Connor (1991) added rows of shallow grooves to blocks of red gum. Grooving increased species richness as well as evenness and the abundance of small particle feeders compared to ungrooved blocks of the same surface area (O'Connor, 1991). Complexity provided by the number of sticks or branches has also been shown to have a significant effect on macro-invertebrates (Scealy *et al*., 2007; Schnieder and Winemiller, 2008). Scealy *et al*. (2007) compared the macro-invertebrates colonising groups of *Eucalyptus* sticks and branches to those colonising single trunk logs of a similar surface area. After 30 days, groups of sticks and branches supported a greater number of species and individuals, as well as altered assemblage composition (Scealy *et al*., 2007). Other studies however have found contrasting results. Whilst wood hardness and in-stream pre-conditioning had an effect on the densities of some taxa, rough wood where more than 75% of the surface was covered in grooves and crevices did not support greater densities or a greater number of taxa than smooth wood where less than 10% of the surface was covered (Magoulick, 1998).

One of the main effects of woody debris is to reduce current velocity, increasing the retention of fine substrates and detritus (e.g. Palmer *et al*., 1996; Pretty and Dobson, 2004). These associated patches of sand and leaf litter have been shown to provide refugia for macro-invertebrates from disturbance, increasing the resistance of macro-invertebrate communities to flooding (Palmer *et al*., 1996). More complex woody debris traps more sediment (O'Connor, 1991), whilst groups of small sticks accumulate more organic matter than single logs (Scealy *et al*., 2007). Despite this, the size and branching of wood in debris dams appeared to have no effect on whether macro-invertebrates accumulated in associated fine sediments during floods (Palmer *et al*., 1996). Greater abundances and richness of macro-invertebrates may also reflect differences in predator distribution among woody debris and neighbouring patches, with lower abundances of fish in bundles of 16 sticks than 8 and compared to reference patches (Schnieder and Winemiller, 2008). Woody debris may also act as a direct food resource, and has been shown to contribute significantly to the carbon flux of streams (Elosegi *et al.*, 2007). This however is likely to be less important than other factors since obligate xylophagous species are uncommon (Hoffman and Hering, 2000). Colonisation of wood and plastic of similar dimensions and surface area by similar abundances of macro-invertebrates supports this theory (Hofer and Richardson, 2007). Whilst there is evidence that the complexity of the surface (O'Connor, 1991) and patches (Scealy *et al.*, 2007) of woody debris may influence macro-invertebrates independently of surface area, in the majority of studies surface area has not been considered.

Leaf litter also provides an important habitat for macro-invertebrates. In lowland streams, leaf litter support a distinct macro-invertebrate assemblage from other habitat types, and macro-invertebrates have been shown to preferentially colonise experimental leaf patches over adjacent natural and artificial substrates (Palmer *et al.*, 2000; Quinn *et al.*, 2000). Furthermore, the density of macro-invertebrates has widely been shown to increase with the biomass of leaf litter occurring in streams (Egglishaw, 1964; Harper *et al.*, 1992; Ruetz *et al.*, 2006). Studies investigating the effects of leaf diversity have however shown that assemblages colonising mixtures are not richer or more diverse than those colonising single leaf species (Abelho, 2009). Effects on macro-invertebrates appear to be complex, with non-additive effects on macro-invertebrate assemblage composition, abundance and diversity (Leroy and Marks, 2006; Abelho, 2009; Sanpera-Calbet *et al.*, 2009). Structural effects are however confounded by the fact that leaves from different tree species vary in their decomposition rate and palatability, and that these factors themselves may be affected in leaf mixtures (Dobson, 1994; Dudgeon and Wu, 1999; Leroy and Marks, 2006; Sanpera-Calbet *et al.*, 2009).

Early evidence indicated that the majority of species whose abundance increased with the volume of detritus feed directly on it (Egglishaw, 1964). Since then many experimental studies have compared the colonisation of artificial and natural leaves, largely showing that leaf litter is primarily colonised for its value as a food resource rather than simply as an additional surface for colonisation (Dobson *et al.*, 1992;

Richardson, 1992; Dudgeon and Wu, 1999; Gjerløv and Richardson, 2004; Hofer and Richardson, 2007). For example, only shredder-detritivores preferentially colonised experimental cages containing alder leaves compared to artificial leaves or just natural substrates, which supported similar abundances (Gjerløv and Richardson, 2004). Evidence that shredder densities increase proportionally to the mass of experimental leaf packs but are not affected by leaf surface area support this conclusion (Ruetz *et al*., 2006), although artificial leaves have been shown to support greater abundances than adjacent bare substrates (Quinn *et al*., 2000). Furthermore, whilst leaf litter retention in streams may alter near-bed flows, changes are too inconsistent to explain variations in the abundances of shredders with increasing amounts of leaf litter (Dobson *et al*., 1992). Colonisation of leaf litter by non-shredders appears to be related to the retention of fine particulate organic matter, with greater abundances on natural leaves compared to artificial ones reflecting their greater retention (Richardson, 1992). Relatively few studies have considered the importance of leaf litter as a refuge from predation compared to other habitats and the effects remain unclear (Holomuzki and Hoyle, 1990; Reice, 1991; Ruetz *et al*., 2006). Densities of macro-invertebrates in leaf packs were not reduced by fish predation in contrast to densities on cobbles (Reice, 1991). Predation rates of *Gammarus minus* by green sunfish (*Lepomis cyanellus*) were also lower in leaf litter than gravel and silt/sand substrates in laboratory experiments, however in the field the presence of fish did not affect habitat use (Holomuzki and Hoyle, 1990). The provision of refugia from predation also appears to be unaffected by leaf pack mass or surface area (Ruetz *et al*., 2006).

Tree roots have also been shown to support distinct macro-invertebrate assemblages (Harper *et al*., 1992; Cogerino *et al*., 1995). Greater species richness and abundance occurred on tree roots than adjacent mineral substrates in a French stream (Beisel *et al*., 1998), attributed to the provision of refugia and the accumulation of plant detritus respectively (Beisel *et al*., 1998). There are, however, no known studies investigating the complexity of this habitat.

## 2.6. Habitat heterogeneity

As discussed previously, habitat structure can also be generated by variations in the composition and configuration of different structural features. Studies investigating habitat heterogeneity have typically been undertaken at the patch scale, either focusing on the composition of differently sized substrate mixtures or the composition and configuration of surrounding habitat patches.

### 2.6.1. Substrate size heterogeneity

Despite the influence of substrate size on macro-invertebrates, evidence for the effect of substrate size heterogeneity on macro-invertebrates remains scarce. Colonisation experiments in an alpine stream showed that the number of species colonising two-, three- and four-way combinations of >64 mm, 32-64 mm, 16-32 mm and 8-16 mm sized substrates was greater than for single substrates (Allan, 1975). Species richness did not, however, increase continuously with the number of substrates at all sites and there appeared to be no clear additive effect of the number of substrate types on species richness (Allan, 1975). Natural substrate diversity also showed no correlation with species diversity across eight sites, although the substrate composition of sites was extremely similar (Allan, 1975). There was also no response of macro-invertebrate diversity, richness or abundance to reach-scale manipulations of substrate size heterogeneity measured as $D_{84}$:$D_{50}$ (the ratio of the particle size larger than 84% of particles to the median particle size) (Brooks *et al*., 2002).

Other experimental studies have also provided inconsistent results (Wise and Molles, 1979; Williams, 1980; Erman and Erman, 1984). For example, Wise and Molles (1979) reported that baskets containing an equal mixture of small (10-25 mm) and large (>75 mm) gravel supported an intermediate abundance and richness compared to baskets containing single substrates. However, macro-invertebrates may have been responding to average particle size, which neither Allan (1975) or Wise and Molles (1979) accounted for (Erman and Erman, 1984). Despite controlling for this, Erman and Erman (1984) reported that neither differences in the number of size classes or their proportions had an effect on the abundance or number of taxa, whilst median particle size had a significant positive effect in agreement with a previous experiment. Williams (1980) also found that differences in substrate size class proportions had no significant effect when mean particle size was held constant, although the number of taxa was greater in random mixtures of the greatest heterogeneity compared to those just containing 32 mm sized particles.

As a result, few studies have considered the mechanisms responsible for these relationships. Erman and Erman (1984) reported that the most heterogeneous substrate mixtures accumulated a greater amount of detritus compared to single substrates. Abundance increased with the amount of detritus whilst richness declined, however these effects were removed before testing the effect of substrate heterogeneity (Erman and Erman, 1984). More heterogeneous mixtures contained greater amounts of detritus and fine sediment in Williams' (1980) study, although as discussed above fine sediment accumulation may have negative effects on macro-invertebrates (e.g. Richards and Bacon, 1994; Bo *et al*., 2007). Interstitial volume may also be reduced in substrate mixtures (Williams, 1980; Gayraud and Philippe, 2003).

### 2.6.2. Spatial habitat heterogeneity

The potential importance of spatial habitat heterogeneity on macro-invertebrate distribution has long been recognised (Pringle *et al*., 1988; Townsend, 1989). 'Landscapes' of habitat patches can be characterised in a number of ways, with measures of their composition and configuration increasing over the last 20 years with developments in the field of landscape ecology (Li and Reynolds, 1995; Palmer *et al*., 2000). Composition describes the number and diversity of different habitat types (Beisel *et al*., 1998, 2000; Boyero, 2003b; Brown, 2003) whilst configuration has been measured as the number and diversity of habitat patches (Beisel *et al*., 1998, 2000; Boyero, 2003b; Palmer *et al*., 2000), patch size (Palmer *et al*., 2000), patch shape and total patch boundary (Beisel *et al*., 2000; Boyero, 2003b; Palmer *et al*., 2000). Other studies have also used variability in patch size and patch compactness (the ratio of the length to the width of the patch) as a measure of configuration (Palmer *et al*., 2000; Boyero, 2003b), although not strictly included in the definition of heterogeneity given here. Enhancing habitat heterogeneity, for example through the addition of woody debris, boulders or gravel, is now a central aim of many restoration schemes, with the underlying assumption that habitat heterogeneity enhances biodiversity (Miller *et al*., 2010; Palmer *et al*., 2010; Feld *et al*., 2011).

It is predicted that greater species richness will occur in more heterogeneous habitat mosaics due to the availability of a greater range of niches (Shmida and Wilson, 1985; Townsend and Hildrew, 1994) and food resources (Beisel *et al*., 2000), and a reduction in the likelihood of competitive exclusion (Townsend and Hildrew, 1994). Heterogeneous habitat mosaics may also ameliorate the effects of disturbance and predation through the provision of refugia (Townsend and Hildrew, 1994; Brown, 2003). For example, slower flow conditions in some patches enable individuals to persist during flood events, with reduced distances between small patches with large perimeters increasing the ability of macro-invertebrates to re-colonise neighbouring patches (Lancaster, 2000). There is also evidence that habitat heterogeneity may reduce temporal variability in macro-invertebrate assemblages (Brown, 2003).

Direct evidence for the relationship between habitat heterogeneity and species richness remains scarce. Macro-invertebrate richness increased approximately 1.5 times with the diversity, evenness and contagion of patches with 1 x 2 m plots in a New Hampshire stream (Brown, 2003). Macro-invertebrate diversity and richness also significantly increased with the number of surrounding habitats and patches and mean area/perimeter ratio within a 2 m circle in a French stream, whilst dominance decreased with mean area/perimeter ratio, the relative area of the sampled patch and the number of patches (Beisel *et al*., 2000). Macro-invertebrate richness, evenness and abundance increased with substrate diversity within 15 x 15 cm plots, whilst evenness and abundance decreased with the variability of patch compactness (Boyero, 2003b). Reach-scale abundances of chironomids and copepods were related to a number of measures of leaf litter patch arrangement such as the perimeter of the largest patch, with models including spatial information explaining more variance in abundances than those without (Palmer *et al*., 2000).

Despite these results, there is a lack of consistency among and even within studies (Beisel *et al*., 1998, 2000; Palmer *et al*., 2000; Boyero, 2003b). For example, Beisel *et al*. (2000) reported that the evenness, diversity and dominance of habitats and patches were unimportant, whilst no heterogeneity measures influenced macro-invertebrate density or evenness. Furthermore, in an earlier study the richness and diversity of surrounding habitat patches within a 4 m circle did not determine macro-invertebrate assemblage structure (Beisel *et al*., 1998). The importance of habitat heterogeneity may also vary with the extent of the investigation. Beisel *et al*. (2000) investigated correlations between macro-invertebrate assemblage structure and the heterogeneity of mosaics within 0.5-4 m circles, reporting that the number of significant correlations was greatest for 2 m circles. One possible explanation is that the passive dispersal of macro-invertebrates, often over large distances, may override any movement of macro-invertebrates among neighbouring patches (Palmer *et al*., 2000). Seasonal trends in the number and arrangement of habitat patches themselves may also obscure the response of macro-invertebrates (Palmer *et al*., 2000).

Some recent reviews have reported that whilst most restoration projects successfully enhance habitat heterogeneity, few meet their anticipated ecological benefit of enhancing macro-invertebrate diversity (e.g. Jähnig *et al*., 2010; Miller *et al*., 2010; Palmer *et al*., 2010; Feld *et al*., 2011). Although a number of factors may limit the success or restoration schemes, including larger scale processes (Bond and Lake, 2003; Spänhoff and Arle, 2007), this has given rise to a critical discussion of the underlying theory (Palmer *et al*., 2010). Only one-third of the studies investigating natural habitat heterogeneity reviewed by Palmer *et al*. (2010) found a positive relationship with species diversity, leading the authors to conclude that the underlying assumption that habitat heterogeneity enhances species diversity is unsubstantiated.

## 2.7. Current challenges and research needs

This review has highlighted several challenges to assessing the relationship between macro-invertebrates and habitat structure, although many are not unique to rivers. Five of the most important are: i) the use of many inconsistently defined terms, ii) difficulties in quantifying habitat structure, iii) distinguishing different components of habitat structure, iv) separating the effects of habitat complexity and surface area, and v) the limitation of most studies to a single, typically small, spatial scale.

### 2.7.1. Definition of habitat structure

One of the main issues in the investigation of habitat structure is the use of a large number of inconsistently defined terms, a problem which is not unique to river ecology (McCoy and Bell, 1991; Tews *et al*., 2004). Together with difficulties quantifying habitat structure (see below), this has hindered comparisons among studies both within rivers and among ecosystems, limiting general understanding of the importance of habitat structure. McCoy and Bell (1991) recognised this problem and attempted to provide a general framework for the study of habitat structure by distinguishing three components: i) complexity, the absolute abundance of individual structural features per unit area or volume, ii) heterogeneity, the relative abundance of different structural features and iii) scale, the variation resulting from the area or volume in which habitat complexity or heterogeneity is measured. In this review, the definition of heterogeneity incorporates structure provided by the addition of different types of structural features in line with previous studies (e.g. Downes *et al*., 1998), although as Matias *et al*. (2007) point out this was not the original definition proposed by McCoy and Bell (1991). According to the definitions given here, studies using terms such as ‘surface heterogeneity’ (Erman and Erman, 1984) and ‘structural heterogeneity’ (Ferreiro *et al*., 2011) actually investigated complexity, whilst conversely studies using terms such as ‘substratum complexity’ actually investigated heterogeneity (Allan, 1975). Other studies use the terms ‘complexity’ and ‘heterogeneity’ interchangeably (e.g. Palmer *et al*., 2010). Furthermore, some studies investigating complexity actually manipulated heterogeneity. Comparisons between bricks with a rough surface or large crevices and rough bricks with large crevices tested the effect of increasing heterogeneity, although this was considered an effect of increasing complexity (Downes *et al*., 1998). Further complications arise because heterogeneity has also been defined as “the variability in a process or pattern over space or time” (Palmer and Poff, 1997). It also has another specific meaning in the field of landscape ecology, where heterogeneity encompasses not only variability but also complexity, which is used to refer to the composition and configuration of habitat patches (Li and Reynolds, 1995). As mentioned previously, a number of studies have therefore measured ‘substrate heterogeneity’ as the variability in substrate topography (e.g. Tockner and Ward 1999; Lepori *et al*., 2005), or used variability in patch size and compactness as a measure of habitat heterogeneity (e.g. Palmer *et al*., 2000; Boyero, 2003b). Future studies should therefore explicitly define the terms used, preferably within the context of a general framework such as the one proposed by McCoy and Bell (1991), and clarify whether habitat complexity and/or heterogeneity is being investigated/manipulated.

### 2.7.2. Measurement of habitat structure

One possible explanation for the inconsistency in relationships between habitat structure and macro-invertebrates is that habitat complexity in particular is difficult to quantify

(McCoy and Bell, 1991). As a result, many studies of complexity have been limited to qualitative or categorical descriptions, such as ‘simple’ and ‘complex’ (e.g. Taniguchi *et al*., 2003) or ‘rough’ or ‘smooth’ (e.g. Boyero, 2003a), which only provide information about basic patterns. Another issue is that measures of habitat structure may be collinear or measure only subtly different aspects of habitat structure, confounding relationships with macro-invertebrates. Despite this few studies have assessed whether different measures of complexity (e.g. Downes *et al*., 1995; Warfe *et al*., 2008) or heterogeneity (e.g. Boyero, 2003b) are collinear. Current studies suggest that this may be the case, but have not always taken this into account when subsequently investigating relationships with macro-invertebrates (e.g. Boyero, 2003b; Warfe *et al*., 2008). The use of a continuous measure to quantifying complexity would provide a more comprehensive understanding of possible relationships with macro-invertebrates. Ideally such a measure would also permit comparisons among studies and ecosystems. Determining which measures best describe complexity and heterogeneity may also enable an assessment of their ecological relevance.

### 2.7.3. Distinguishing the effects of habitat complexity and habitat heterogeneity

Few studies have explicitly assessed the independent effects of increasing the abundance of structural features (complexity) from the addition of different structural features (heterogeneity) (see definitions in Section 2.2.). One exception is research on the surface roughness and macro-algal cover of individual substrate particles, two structural features which have both been shown to increase species richness (see above). Surface roughness of paving bricks and the presence of macro-algae appeared to have additive effects on species richness but not the number of individuals, which was similar among rough and smooth substrates (Downes *et al*., 2000a). Rough bricks covered in macro-algae supported a significantly greater number of species than rough bricks with no macro-algae, smooth bricks with macro-algae or bare, smooth bricks (Downes *et al*., 2000a). As mentioned above, studies should carefully consider whether habitat complexity or heterogeneity is being investigated (e.g. Downes *et al*., 1998), and additional research is needed to separate their effects.

### 2.7.4. Separating the effects of habitat complexity and surface area

As mentioned previously, structurally complex habitats tend to have a greater surface area and may therefore support more species because they support a greater number of individuals (Coleman *et al*., 1982). In the majority of studies, however, surface area was either not considered as a separate factor or it correlated with complexity (Warfe *et al*., 2008). Furthermore, those studies considering surface area have largely controlled for surface area effects, for example by using artificial substrates of a constant surface area (e.g. O'Connor, 1991; Douglas and Lake, 1994; Taniguchi *et al*., 2003; Taniguchi and Tokeshi, 2004). There is therefore a need for investigations separating the effects of continuous variations in complexity and surface area on macro-invertebrates, of which there are surprisingly few (e.g. Downes *et al*., 1995; Boyero, 2003a).

### 2.7.5. Study design

Most studies quantifying habitat complexity have been conducted at a single, small spatial scale, and have typically involved artificial substrates (e.g. O'Connor, 1991; Downes *et al*., 1998; Warfe *et al*., 2008). Few studies have considered intermediate scale structure provided by the complexity of river bed surfaces or the effect of habitat structure at more than one spatial scale (e.g. Robson and Chester, 1999). Studies combining field surveys and manipulative in-stream experiments are also scarce (e.g. Robson and Barmuta, 1998). Whilst experiments have the advantage of enabling variation in other environment factors to be taken into account, the use of artificial substrates in particular may have limited realism and caution is required in extrapolating the results. In-stream experiments and field surveys which assess whether habitat structure is ecologically relevant at multiple scales are therefore required to advance current knowledge.

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# Chapter 3 Re-appraising the effects of habitat structure on river macro-invertebrates

## 3.1. Abstract

1. Although rivers are highly structured physically, generalisations about the exact consequences for macro-invertebrates remain elusive. Challenges include quantifying structure *per se* and differentiating the effects of complexity (i.e. the abundance of structural features), heterogeneity (i.e. the composition and spatial arrangement of different structural features) and surface area.

2. Three hypotheses about habitat structure were tested at the patch scale (0.0625 $m^2$) in tributaries of the Rivers Wye and Usk, mid-Wales (UK): i) greater habitat heterogeneity and surface complexity alter macro-invertebrate composition and increase diversity, richness and abundance, ii) complexity effects are distinct from increased surface area and iii) habitat structure is a major determinant of variations in macro-invertebrates among habitat types.

3. Surface complexity, described using fractal dimension, and surface area were estimated from river bed profiles at locations where macro-invertebrate were sampled. Habitat heterogeneity was determined within 1 m radii and described by Principal Components reflecting the patchiness of habitat mosaics (PC1) and evenness of patch sizes (PC2).

4. Bedrock was the least complex habitat, whilst pebbles were the most complex. Cobbles were surrounded by the least even habitat mosaics, occurring within significantly less even mosaics than gravel or bedrock.

5. Complexity (but not heterogeneity) increased macro-invertebrate diversity, richness and abundance independently of surface area, but only when habitat type was ignored. Differences in macro-invertebrate assemblages among habitat types were greater than along the gradients of habitat structure, and physical structure accounted for <10% of

this among habitat variation. Bedrock, silt and sand typically had reduced diversity, richness and abundance mostly of EPT taxa whilst silt had abundant Chironomidae.

6. These results give empirical support only to the first two hypotheses: habitat heterogeneity had no effect on macro-invertebrates whilst complexity effects were weak. The implication is that habitat type must affect macro-invertebrates through other factors such as porosity, hydraulic conditions, stability or resource distributions that transcend the effects of structure alone.

## 3.2. Introduction

Habitat structure is postulated to affect community composition in terrestrial, marine and freshwater ecosystems, with structural complexity and heterogeneity often increasing species diversity and abundance (MacArthur, 1965; MacArthur and Wilson, 1967; Shmida and Wilson, 1985; Bell *et al.*, 1991; Statzner and Moss, 2004; Tews *et al.*, 2004). In rivers in particular, where physical structure is a major feature, recognition of the potential importance of habitat structure to benthic organisms is long-standing (Percival and Whitehead, 1929; Whitehead, 1935; Jones, 1949). Here, where macro-invertebrates have been shown to be influenced by complexity and heterogeneity (e.g. Vinson and Hawkins, 1998), structural features have now become a central focus in river management and restoration (Harper *et al.*, 1992; Harper and Everard, 1998; Vaughan *et al.*, 2009; Palmer *et al.*, 2010; Feld *et al.*, 2011).

Despite this interest, generalisations about the exact relationship between habitat structure and macro-invertebrate communities in rivers remain elusive. For example, both macro-invertebrate richness and abundance are sometimes greater on plants with a complex architecture (Krecker, 1939; Rooke, 1986; Warfe *et al.*, 2008) but not always (Taniguchi *et al.*, 2003; Ferreiro *et al.*, 2011). Fewer than half of the studies reviewed by Vinson and Hawkins (1998) showed that macro-invertebrate richness increased with substrate size heterogeneity, whilst Palmer *et al.* (2010) concluded that the majority of restoration projects that increase habitat mosaic heterogeneity do not enhance macro-invertebrate diversity.

There are several challenges to assessing the relationship between organisms and habitat structure. Firstly, key components of habitat structure such as heterogeneity and complexity are often poorly defined or differentiated. Heterogeneity encompasses both the composition (number and relative abundance) and configuration (spatial arrangement) of different structural features (e.g. habitat patches or types) (Li and Reynolds, 1995). In contrast, complexity has been defined as the total abundance of structural features (e.g. crevices on the surface of a substrate particle), with more complex habitats having greater numbers of structural features within a fixed area (McCoy and Bell, 1991). This property is often described as the roughness, rugosity or topography of a surface.

Secondly, habitat structure is difficult to quantify (McCoy and Bell, 1991). For complexity, categorisations based on surface texture (e.g. Boyero, 2003a) or plant morphology (e.g. Taniguchi *et al.*, 2003) are likely to provide only a partial understanding of any relationship with macro-invertebrate communities. Quantitative measures have included the number and size of crevices on and between substrate particles (Sanson *et al*., 1995), as well as particle height and length (Evans and Norris, 1997), whilst other studies have used variation in substrate height to describe topography (e.g. Statzner *et al*., 1988; Tockner and Ward, 1999; Crosa and Buffagni, 2002; Muotka *et al*., 2002; Brooks *et al*., 2005; Lepori *et al*., 2005). Alternatively, fractal dimension provides a quantitative measure of the complexity of a line or surface (Mandelbrot, 1977, 1983). As the fractal dimension of a structural feature increases, it fills the space it occupies more completely. The majority of freshwater studies have focused on macrophytes (McAbendroth *et al*., 2005; Thomaz *et al*., 2008; Warfe *et al*., 2008; Dibble and Thomaz, 2009; Ferreiro *et al*., 2011; Mormul *et al*., 2011). Studies assessing the fractal dimension of river bed surfaces are scarce (Robson, 1995 cited in Robson and Chester, 1999; Schmid, 2000; Robson *et al*. 2002).

For habitat mosaic heterogeneity, numerous measures have been developed over the last 20 years in the field of landscape ecology (Palmer *et al*., 2000). Composition is described by the number and diversity of different habitat types (Beisel *et al*., 1998, 2000; Boyero, 2003b; Brown, 2003) whilst configuration can be described by the number and diversity of habitat patches (Beisel *et al*., 1998, 2000; Boyero, 2003b; Palmer *et al*., 2000), patch size (Palmer *et al*., 2000) and patch shape and boundary length (Beisel *et al*., 2000; Downes *et al*., 2005; Boyero, 2003b; Palmer *et al*., 2000). Other studies have also used variability in patch size as a measure of configuration (Palmer *et al*., 2000; Boyero, 2003b). Such a large number of measures have, however, further hindered understanding of the importance of habitat structure. Some may be collinear or measure only subtly different aspects of habitat structure in ways that confound relationships with macro-invertebrates (Boyero, 2003b; Warfe *et al*., 2008; Zuur *et al*., 2010). Conversely, individual measures may fail to capture all aspects of structural variation (Wilson *et al*., 2007). Assessing which measures or combination of measures best describe complexity and heterogeneity may therefore facilitate improved understanding of their ecological relevance.

A third problem in understanding the relationship between habitat structure and benthic organisms is that heterogeneity and complexity vary among habitat types such as differently sized substrates, macrophytes, tree roots and leaf litter (Robson, 1995 cited in Robson and Chester, 1999; Beisel *et al*., 1998, 2000; Palmer *et al*., 2000; Robson *et al*., 2002). Consequently, relationships between habitat structure and macro-invertebrate communities may partly reflect other physical or ecological differences among habitat types such as variations in current velocity or resource availability. Despite this, few studies have assessed how complexity and/or heterogeneity vary among habitat types or have appraised the contribution of habitat structure to variations in macro-invertebrates among differently sized substrates (Robson, 1995 cited in Robson and Chester, 1999; Beisel *et al*., 1998, 2000; Robson *et al*., 2002).

Finally, relationships between organisms and habitat structure may be confounded by variations in surface area. Complex habitats typically have a larger surface area and may therefore support more species due to a species-area effect (Arrhenius, 1921; Gleason, 1922; MacArthur and Wilson, 1967; Connor and McCoy, 1979; Coleman *et al*., 1982). Some studies have controlled for surface area effects by using artificial substrates of a constant surface area (O'Connor, 1991; Jeffries, 1993; Douglas and Lake, 1994; Taniguchi *et al*., 2003; Taniguchi and Tokeshi, 2004), whilst direct investigations of the species-area relationship have focused on individual substrate particles (Hart, 1978; Clements, 1987; Douglas and Lake, 1994; Heino and Korsu, 2008). Few studies have however attempted to assess the independent effects of continuous variations in complexity and area on macro-invertebrates in rivers (Downes *et al*., 1995; Boyero, 2003a; Warfe *et al*., 2008), whilst there are no known investigations on natural river bed surfaces.

This chapter tested the hypotheses that: i) greater habitat heterogeneity and surface complexity alter macro-invertebrate composition and increase diversity, richness and abundance, ii) complexity effects are distinct from increased surface area and iii) habitat structure is a major determinant of variations in macro-invertebrates among habitat types.

## 3.3. Methods

### 3.3.1. Study area

The Welsh Rivers Wye and Usk drain catchments of 4136 km$^2$ and 1358 km$^2$, respectively, flowing 215 km and 120 km into the Severn Estuary (Figure 1). The Wye rises in the Plynlimon Mountains (National Grid reference SN 795 864) and the Usk on the Black Mountain (National Grid reference SN 819 239).

Although the Wye catchment varies in geology, relief and land use between upper, middle and lower sub-catchments (Edwards and Brooker, 1982; Jarvie *et al.*, 2003), work for this study focused on the middle catchment draining Devonian Old Red Sandstone or marls. Land use here is dominated by rough/semi-improved pasture and tributaries are relatively un-polluted and calcareous (50-250 mg $CaCO_3$ $l^{-1}$), contrasting with the low pH of those in the acidified uplands and the moderately elevated biochemical oxygen demand (BOD) and nutrient concentrations of those in the lowlands (Ormerod and Edwards, 1987; Jarvie *et al.*, 2003; Clews and Ormerod, 2009). Average annual rainfall varies from 900-2100 mm depending on altitude (Environment Agency, 2008).

The land use and geology of the Usk catchment matches the middle Wye catchment and general water quality, BOD and nutrient concentrations are also similar (Environment Agency, 2007, 2009). River discharge varies closely with rainfall, which averages 1336 mm annually across the catchment (Environment Agency, 2007).

Fieldwork was conducted in 2008 (from September to October) at 3 x 10 m sections at three sites on the Edw, a tributary in the middle Wye catchment, and in 2009 (from July to August) at 3 x 10 m sections at single sites on the upper Usk and two of its tributaries, the Honddu and Tarrell (Table 1; Figure 1). Tributaries were selected to minimise any confounding effects of water quality, land use or channel modification and drained a mixture of rough/semi-improved pasture and broadleaved woodland.

### 3.3.2. Sampling design

Surface complexity and area of the river bed were determined within 0.25 x 0.25 m at five random locations within each 10 m section, from which macro-invertebrates were sampled and habitat heterogeneity determined within 1 m radii (Figure 1). In addition, a small number of samples were collected to ensure that all substrate sizes (excluding boulders and clay) within each 10 m section based on the Wentworth scale were sampled, resulting in a total of 108 samples of six habitat types (cobbles=47, pebbles=29, gravel=16, sand=4, silt=5 and bedrock=7).

### 3.3.3. Data collection

#### *Habitat heterogeneity*

The habitat mosaic within each 10 m section was mapped onto a 0.25 m grid. Visual assessments of substrate size were made based on the Wentworth scale (Wentworth, 1922).

#### *Surface complexity and area*

At each sampling location ten pin profiles of the river bed were obtained using a profiler consisting of 104 pins at 2.4 mm intervals (Figure 2). Using a sampling frame, the profiler was positioned at five 62.5 mm intervals parallel to the flow, and five perpendicular to it, each pin pushed down until it touched the river bed and the height of each pin recorded (Figure 3).

#### *Macro-invertebrates*

At each sampling location macro-invertebrates were kick-sampled for 1 minute immediately after pin profiles were taken using a standard net (0.25 x 0.25 m with a 1 mm mesh). The net was also held downstream during pin-profiling to ensure any disturbed macro-invertebrates were captured. Samples were preserved in 70% ethanol for subsequent analysis, when macro-invertebrates were identified to species level where possible and counted.

### 3.3.4. Data analysis

#### *Habitat heterogeneity*

Habitat maps were digitised in ArcMap version 9.2 using ArcScan (ESRI, 2006). Patch Analyst version 4.2 (Rempel *et al*., 2008) was used to calculate a range of previously published measures of the composition and the configuration of the habitat mosaic within a 1 m radius around the centre of each sampling location (Table 2). Samples at the edge of a 10 m section, where the habitat mosaic within the entire 1 m radius was not mapped, were removed from the heterogeneity analysis to avoid edge effects resulting in a total of 90 samples for which habitat heterogeneity was measured.

#### *Surface complexity and area*

The ten pin profiles from each sampling location were used to calculate mean fractal dimension in addition to the standard deviation in pin height, one of the most widely used measures of the topography of river bed surfaces. To calculate the mean fractal dimension each pin profile was first digitised in ArcMap version 9.2 using Hawth's Analysis Tools version 3.27 (Beyer, 2004). FracLac for ImageJ version 2.5 (Karperien, 1999) was used to calculate fractal dimension using the box-counting method, in which a line or outline is covered with a series of grids and the number of occupied grid squares counted (Mandelbrot, 1983; Fielding, 1992). The slope coefficient from the regression of the number of grid squares occupied versus the square size on log-log axes then provides an estimate of fractal dimension (Mandelbrot, 1983). The analysis was restricted to grid squares between twice the distance between pins and one-fifth of the profile length to avoid fractal dimension estimates of less than 1, which can occur when the same number of grid squares is occupied at very large consecutive grid sizes (Buczkowski *et al*., 1998; Halley *et al*., 2004). For a small proportion of profiles where this did not remove the effects of these artefacts, fractal dimension estimates were replaced by a value of 1.

An index of surface area for each sampling location was estimated as the square of the mean profile length (Kostylev *et al*., 2005).

Prior to further analysis, Principal Component Analyses (PCAs) extracted from correlation matrices were used to examine collinearity among measures of: i) habitat heterogeneity and ii) surface complexity and area from all sampling locations and identify the measure(s) describing the main aspects of variation (Zuur *et al*., 2010). Reducing the number of variables also reduced the risk of Type I errors - incorrectly rejecting the null hypothesis - when analysing relationships with macro-invertebrate assemblages.

Differences in habitat structure among habitat types were analysed using a One-Way ANOVA followed by a Tukey test or a Kruskal-Wallis test followed by Mann-Whitney tests, for which P=0.003 (=0.05/15) was used as the critical level of significance to reduce the chance of Type I errors.

### *Macro-invertebrates*

Total abundance, taxonomic richness and taxonomic diversity (using Shannon's diversity index) were calculated. Macro-invertebrate abundances were then analysed using Detrended Correspondence Analysis (DCA) in Canoco for Windows version 4.54 to assess variation in composition (ter Braak and Šmilauer, 1997). This type of unconstrained ordination was selected as the DCA gradient lengths indicated that taxon response curves were non-linear and Correspondence Analysis showed a potential arch effect (ter Braak and Šmilauer, 2002). Abundance was normalised using a logarithmic transformation prior to subsequent analyses, with the exception of DCA.

### *Relationships between habitat type, habitat structure, surface area and macro-invertebrates*

Relationships between habitat type, measures selected from each PCA and macro-invertebrates were analysed using Generalised Linear Mixed Models in ASReml version 3 (VSN International Ltd, 2009) or, where a random effect was not required, Generalised Linear Models in R version 2.9.2 (R Development Core Team, 2009). A random effect was used to control for possible dependence on site, but was excluded when it was not significant. Models were checked for validity through assessment of standardised residuals and refined using stepwise deletions at the P=0.05 level of significance. Models were refined from two initial models: i) excluding and ii) including habitat type as a fixed effect. In models where a random effect was required and habitat type was significant, differences in macro-invertebrates among pairs of habitat types were analysed using Wald tests.

Hierarchical partitioning using the hier.part package (Walsh and MacNally, 2008) in R was subsequently used to explore the variance in macro-invertebrates explained independently by habitat type, habitat heterogeneity, fractal dimension, surface area and by their joint effects. Two samples for which surface complexity and area were not measured were removed along with those at the edge of 10 m sections resulting in a total of 89 samples for which variance was investigated.

## 3.4. Results

### *Habitat structure and surface area*

For habitat heterogeneity, 58% and 22% of the variation was explained by the first two principal components (Figure 4). The number of patches (loading: 0.384), number of habitat types (loading=0.361) and total edge (loading=0.361) increased along Heterogeneity PC1 whilst mean patch size (loading=-0.379) and mean patch edge (loading=-0.352) declined. Patch diversity (loading=0.377) and habitat diversity (loading=0.365) increased along Heterogeneity PC2 whilst both the standard deviation and coefficient of variance in patch size declined (loading=-0.577 and -0.484 respectively). In other words, moving from low to high scores, PC1 represented a gradient of increasingly patchy habitat mosaics whilst PC2 represented a gradient of increasing evenness of patch sizes within the mosaic. Sand and gravel were surrounded by more patchy mosaics than other habitat types, with cobbles occurring in the least patchy mosaics, although these differences were not significant (H=7.61; P=0.180; DF=5) (Figure 5). Heterogeneity PC2 scores did however vary significantly among habitat types (F=4.06; P=0.002; DF=5, 84), with cobbles surrounded by mosaics of less even patch size than bedrock or gravel (P<0.05) (Figure 5).

For surface complexity and area, 60% and 35% of the variation was explained by the first two principal components. Surface area (loading=0.713) and standard deviation in pin height (loading=0.694) increased on Complexity PC1, whilst fractal dimension (loading=0.962) increased on Complexity PC2. In other words, PC1 represented a gradient of increasing relief resulting in larger surface areas whilst PC2 represented a gradient of increasing surface complexity which was distinct from surface area. Since these gradients were so well described by surface area and fractal dimension, these measures were used in subsequent analyses with macro-invertebrates. Fractal dimension varied significantly among habitat types (F=25.83; P<0.001; DF=5, 100), being lowest for bedrock (P<0.05) (Figure 5). Fractal dimension was also significantly lower for cobbles than gravel or pebbles and significantly lower for sand than pebbles (P<0.05). Surface area also varied significantly among habitat types (H=51.71; P<0.001; DF=5) with cobbles>pebbles>gravel>bedrock and cobbles>pebbles>sand=silt (P<0.003) (Figure 5).

### *Macro-invertebrate composition*

In DCA, two axes explained 21% of the variation in composition, with axis 1 explaining 15%. Axis 1 scores generally separated the upper Usk from tributaries of the Usk and Wye (Figure 6). Chironomidae, *Psychomyia pusilla* and *Hydroptila* spp. tended to increase along this axis whilst Simuliidae, *Sialis lutaria* and *Isoperla grammatica* declined (Table 3). Axis 2 scores largely reflected differences between tributaries of the Rivers Wye and Usk (Figure 6), with less widely collected species such as *Helodes* sp., *Helobdella stagnalis, Chaetopteryx villosa* and Tipulidae increasing whilst *Hydropsyche angustipennis*, *Gyrinus* sp., *Diplectrona felix* and *Glossosoma boltoni* declined (Table 3). Macro-invertebrates occurring on bedrock, silt and sand were predominantly a sub-set of those occurring in other habitat types (Figure 7).

### *Relationships between habitat type, habitat structure, surface area and macro-invertebrates*

Macro-invertebrate diversity was positively correlated with fractal dimension whilst richness and abundance were positively correlated with both fractal dimension and surface area (Table 4). DCA axis 1 scores were negatively correlated with surface area, indicating that as surface area decreased macro-invertebrate assemblages contained fewer Ephemeroptera, Plecoptera and Trichoptera (Table 4). Macro-invertebrates were not significantly correlated with Heterogeneity PCs.

When habitat type was included in the models macro-invertebrate diversity, richness, and abundance and DCA axis 1 scores only varied significantly among habitat types (Table 5). None of the measures of habitat structure or surface area were significantly correlated with macro-invertebrates. Diversity was significantly lower on bedrock than in other habitat types except for silt, where it was significantly lower than on sand, gravel, pebbles and cobbles ($P<0.05$) (Table 6). Richness was significantly lower for bedrock than gravel, pebbles and cobbles ($P<0.05$) (Table 6). Abundance was significantly lower on bedrock and sand than on silt, gravel, pebbles and cobbles ($P<0.05$) (Table 6). DCA axis 1 scores were significantly greater for silt than other habitat types and significantly greater for sand than gravel, pebbles and cobbles ($P<0.05$) (Table 6). In other words, bedrock, silt and sand typically had reduced diversity, richness and abundance mostly of Ephemeroptera, Plecoptera and Trichoptera whilst silt had abundant Chironomidae. DCA axis 2 scores did not vary significantly among habitat types and were not significantly correlated with Heterogeneity PCs, fractal dimension or surface area.

Hierarchical partitioning explained the redundancy of fractal dimension and surface area when habitat type was included in the models (Figure 8). Fractal dimension and surface area independently explained a maximum of 5% and 4% of the variance in macro-invertebrates. With the exception of DCA axis scores, around half of the $R^2$ values for fractal dimension and surface area comprised joint effects, predominantly with habitat type. In contrast, habitat type explained 6-30% of the variance in macro-invertebrates independently of fractal dimension and surface area. With the exception of macro-invertebrate richness, around one-quarter of the $R^2$ values at most were attributed to habitat type in combination with Heterogeneity PCs, fractal dimension and/or surface area.

## 3.5. Discussion

Theoretical and empirical links between habitat structure and the distribution of macro-invertebrates are well documented. Heterogeneous habitat mosaics and structurally complex habitats are expected to provide a greater range of niches and reduce the likelihood of competitive exclusion, enabling a greater number of species to co-exist (Shmida and Wilson, 1985; Townsend and Hildrew, 1994). They may ameliorate the effects of disturbance and predation through the provision of refugia (Townsend and Hildrew, 1994; Brown, 2003). For example, slower flow conditions in some patches enable individuals to persist during flood events, with large patch perimeters and reduced distances between patches increasing the ability of macro-invertebrates to re-colonise neighbouring patches (Lancaster, 2000). Heterogeneous habitat mosaics and structurally complex habitats may also support a greater range and abundance of food resources. For example, complex habitats have been shown to accumulate greater abundances of epilithon (Gawne and Lake, 1995; Sanson *et al*., 1995).

This study provided mixed results for these predictions. Contrary to the first hypothesis, habitat heterogeneity did not correlate with macro-invertebrate diversity, richness or abundance or alter composition. Relationships between the heterogeneity of habitat mosaics and macro-invertebrates have been reported in some studies (Beisel *et al*., 2000; Boyero, 2003b; Brown, 2003) but not others (Beisel *et al*., 1998), and there is a lack of consistency among and even within studies (Beisel *et al*., 1998, 2000; Boyero, 2003b). The importance of habitat heterogeneity may also vary with the extent of the investigation, although the number and significance of significant relationships with macro-invertebrates were greatest within 1 m radii in a previous study indicating this to be a sufficient extent to assess the surrounding habitat mosaic mapped with a resolution of 0.1-0.25 m (Beisel *et al*., 2000). It is possible however that neighbouring patches in the current study were insufficiently distinct and that gradients of substrate size rather than distinct boundaries occurred between patches. Alternatively, macro-invertebrates may not have responded to the composition and configuration of neighbouring patches even though the habitat type of the sampled patch was important. The generally passive dispersal of macro-invertebrates over frequently large distances may override any movement of macro-invertebrates among neighbouring patches (Palmer *et al*., 2000). Seasonal trends in the number and arrangement of habitat patches themselves may also obscure the response of macro-invertebrates (Palmer *et al*., 2000).

In contrast to habitat heterogeneity, the diversity, richness and abundance of macro-invertebrates correlated positively with surface complexity in agreement with the first hypothesis. More complex natural and artificial surfaces have been shown to support a greater macro-invertebrate diversity, richness and abundance in some studies (Douglas and Lake, 1994; Downes *et al*., 1998, 2000; Taniguchi and Tokeshi, 2004), whilst others have reported inconsistent results among different structural features and different aspects of assemblage structure (Downes and Jordan, 1993; Downes *et al*., 1995; Robson and Barmuta, 1998; Robson and Chester, 1999). Fractal dimension has previously been shown to provide an ecologically relevant measure of complexity, with habitats of a greater fractal dimension supporting greater abundances and/or species richness in freshwater (Taniguchi *et al*., 2003; Thomaz *et al*., 2008; Warfe *et al*., 2008; Dibble and Thomaz, 2009; Mormul *et al*., 2011) and marine ecosystems (Gee and Warwick, 1994; Beck, 2000; Kostylev *et al*., 2005), although other studies have found contrasting results (Hills *et al*., 1999; Attrill *et al*., 2000; Johnson *et al*., 2003; McAbendroth *et al*., 2005; Ferreiro *et al*., 2011). Fractal dimension appeared to be largely independent of variations in surface area in agreement with some studies of macrophytes in rivers (Ferreiro *et al*., 2011; cf. Warfe *et al*., 2008), whilst more complex habitat types did not necessarily have a greater surface area. The results of the PCA suggest that previous studies employing standard deviation may have been describing variations in surface area rather than structure *per se*. This may have caused difficulties in interpretation and account for some of the conflicting results in previous literature (e.g. Lepori *et al*., 2005).

In agreement with the second hypothesis, complexity effects were not simply the result of increased surface area, supporting studies showing that increasingly complex artificial substrates and macrophytes of a constant surface area support greater macro-invertebrate richness and abundance (O'Connor, 1991; Jeffries, 1993; Douglas and Lake, 1994; Taniguchi *et al*., 2003; Taniguchi and Tokeshi, 2004). Richness and abundance did however increase with surface area, indicating that surface area effects may also be an important determinant of species richness on river bed surfaces as well as individual substrate particles (Hart, 1978; Clements, 1987; Douglas and Lake, 1994). Nevertheless, it should be noted that both surface complexity and area were derived from two-dimensional profiles which are unable to capture overhangs or the three-dimensional shape of crevices and spaces (Sanson *et al*., 1995). It was also assumed that intersecting profiles were representative of the surface as a whole. Surfaces with the same fractal dimension may also vary in the number of crevices or spaces of different sizes that are available to organisms (Sanson *et al*., 1995).

Overall, surface complexity appeared to be a minor determinant of variations in macro-invertebrates among habitat types, whilst heterogeneity was not important. This result was in contrast to the third hypothesis. Habitat type explained a much larger proportion of the variance in macro-invertebrates than habitat structure, with hierarchical partitioning suggesting that, excluding macro-invertebrate richness, complexity and heterogeneity explained up to around one-quarter of the differences among habitat types. Lower diversity, richness and abundance are characteristic of communities on bedrock and small substrates (Percival and Whitehead, 1929; Hynes, 1970; Harper *et al*., 1995). Richness and abundance have also been shown to increase from small to large substrates, such as from gravel to cobbles, in some studies (e.g. Allan, 1975; Erman and Erman, 1984) but not others (e.g. Minshall and Minshall, 1977; Rabeni and Minshall, 1977; Williams and Mundie, 1978; Wise and Molles, 1979). Whilst bedrock had the lowest fractal dimension, indicating that it essentially provided a two-dimensional habitat for macro-invertebrates, silt and sand had a similar fractal dimension to larger substrates. This may reflect the tendency for silt and sand to have uneven surfaces, but may also be attributed to the ease with which pins could accidentally be pushed below the surface. Cobbles supported a similar macro-invertebrate assemblage to pebbles and gravel despite having a lower fractal dimension, reflecting the fact that fewer substrate particles were encountered along each profile relative to profile length. Previous studies have reported a similar result for 10 cm transects, with bedrock and boulder-cobble riffles having fractal dimensions of 1.02 and 1.029 respectively (Robson, 1995 cited in Robson and Chester, 1999). Bedrock appears to consistently provide a simple habitat for macro-invertebrates, whilst cobbles may provide greater complexity than bedrock or gravel along transects of approximately 1-10 m measured at a resolution of one-tenth of the transect length, corresponding to the fact at this extent cobble surfaces are the least uniform (Robson, 1995 cited in Robson and Barmuta, 1998; Robson *et al*., 2002).

The results of this study therefore give empirical support only to the first two hypotheses. The implication is that other factors are more important determinants of variations in macro-invertebrates among habitat types. Substrates of different sizes may vary in other structural characteristics such as porosity, which might not be reflected by differences in their surface complexity. Interstitial spaces in large and medium substrates provide an important habitat for macro-invertebrates, whilst the low porosity of small substrates is one of the most important factors limiting colonisation (Hynes, 1970; Harper *et al*., 1995). Other factors such as hydraulic conditions, stability and resource distributions are also likely to be important (e.g. Beisel *et al*., 1998) and may transcend the effects of habitat structure alone. However, separating the effects of such factors presents a major challenge because many of them may be dependent upon each other in natural systems (Allan, 1995). Progress in this area requires experimental investigation and should be prioritised, given that habitat structure is postulated widely to affect ecosystem structure and function and these ideas are central to river management and restoration.

In summary, habitat heterogeneity had no effect on macro-invertebrates whilst complexity effects, despite being independent of surface area, were weak and largely attributed to habitat type. Differences in macro-invertebrate assemblages among habitat types were greater than along the gradients of habitat structure, and physical structure accounted for <10% of this among habitat variation. Bedrock, silt and sand typically had reduced diversity, richness and abundance mostly of EPT taxa whilst silt had abundant Chironomidae.

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## 3.7. Tables and Figures

Table 1. Location of 10 m sections on tributaries of the Rivers Wye and Usk.

| River | Tributary | 10 m section | Grid reference |
|---|---|---|---|
| Wye | Edw-site 1 | 1 | SO 11825 57329 |
| | | 2 | SO 11673 57140 |
| | | 3 | SO 11635 57052 |
| | Edw-site 2 | 4 | SO 11440 54270 |
| | | 5 | SO 11508 54153 |
| | | 6 | SO 11601 53966 |
| | Edw-site 3 | 7 | SO 09926 48026 |
| | | 8 | SO 09716 47876 |
| | | 9 | SO 09621 47845 |
| Usk | Usk-site 1 | 1 | SN 81761 26868 |
| | | 2 | SN 81917 26979 |
| | | 3 | SN 81960 27054 |
| | Honddu-site 1 | 1 | SO 02961 35463 |
| | | 2 | SO 03206 35271 |
| | | 3 | SO 03239 35251 |
| | Tarrell-site 1 | 1 | SO 01268 26914 |
| | | 2 | SO 01042 26958 |
| | | 3 | SO 00936 26964 |

Table 2. Measures describing habitat heterogeneity within 1 m radii of sampling locations.

| Composition |
|---|
| Number of habitat types |
| Habitat diversity (calculated using Shannon Diversity Index) |
| **Configuration** |
| Mean patch size |
| Patch size standard deviation |
| Patch size coefficient of variance |
| Mean perimeter/area ratio |
| Mean patch edge |
| Total edge |
| Number of patches (regardless of habitat type) |
| Patch diversity (calculated using Shannon Diversity Index) |

Table 3. Detrended Correspondence Analysis (DCA) loadings of macro-invertebrates.

| **Taxon** | **DCA axis 1** | **Taxon** | **DCA axis 2** |
|---|---|---|---|
| Chironomidae | 4.11 | *Helodes* sp. | 5.09 |
| *Wiedemannia* spp. | 4.02 | *Helobdella stagnalis* | 5.09 |
| *Habrophlebia fusca* | 3.97 | *Chaetopteryx villosa* | 4.83 |
| *Psychomyia pusilla* | 3.93 | Tipulidae | 4.80 |
| *Orectochilus villosus* | 3.92 | *Glossosoma* spp. | 4.17 |
| *Hydroptila* spp. | 3.87 | *Chloroperla tripunctata* | 3.90 |
| *Radix peregra* | 3.87 | *Chelifera stigmatica* | 3.70 |
| *Polycentropus flavomaculatus* | 3.85 | *Psycoda* spp. | 3.66 |
| *Hydrocyphon* sp. | 3.81 | Hydrobiidae | 3.65 |
| *Perla bipunctata* | 3.77 | Dytiscidae | 3.63 |
| *Polycelis felina* | 3.76 | *Baetis fuscatus* | 3.57 |
| *Caenis rivulorum* | 3.74 | *Lype* sp. | 3.51 |
| *Planaria torva* | 3.73 | *Dinocras cephalotes* | 3.49 |
| *Hydroporus* sp. | 3.71 | *Hydropsyche instabilis* | 3.46 |
| Tabanidae | 3.69 | *Leuctra geniculata* | 3.40 |
| *Esolus parallelepipedus* | 3.66 | *Polycelis felina* | 3.19 |
| *Polycentropus kingi* | 3.60 | *Serratella ignita* | 3.15 |
| *Centroptilum pennulatum* | 3.57 | Sciomyzidae | 2.95 |
| *Micronecta poweri* | 3.55 | *Halesus radiatus* | 2.90 |
| *Ecdyonurus venosus* | 3.48 | *Potamophylax latipennis* | 2.87 |
| Empididae | 3.47 | *Leuctra* spp. | 2.85 |
| *Phagochata vitta* | 3.41 | *Helophorus brevipalpis* | 2.85 |
| Hydracarina | 3.38 | *Leuctra fusca* | 2.80 |
| Oligochaeta | 3.35 | *Hydraena gracilis* | 2.78 |
| *Tinodes waeneri* | 3.34 | *Limnophila* sp. | 2.67 |
| *Haliplus lineatocollis* | 3.34 | *Orectochilus villosus* | 2.63 |
| *Centroptilum luteoum* | 3.34 | *Baetis* spp. | 2.62 |
| *Chloroperla torrentium* | 3.34 | *Hydrocyphon* sp. | 2.62 |
| *Oreodytes sanmarkii* | 3.22 | *Hydroporus* sp. | 2.60 |
| *Plectocnemia conspersa* | 3.14 | *Erpobdella testacea* | 2.50 |
| *Limnophila* sp. | 3.10 | *Baetis scambus* | 2.45 |
| *Hydropsyche siltalai* | 3.08 | *Baetis rhodani* | 2.41 |
| *Oulimnius tuberculatus* | 3.03 | *Oreodytes sanmarkii* | 2.36 |
| *Odontocerum albicorne* | 2.99 | Tabanidae | 2.32 |
| *Helodes* sp. | 2.92 | *Baetis muticus* | 2.28 |
| *Helobdella stagnalis* | 2.92 | *Plectocnemia conspersa* | 2.17 |
| *Dinocras cephalotes* | 2.90 | Hydracarina | 2.16 |
| *Baetis muticus* | 2.88 | *Perla bipunctata* | 2.05 |
| *Chaetopteryx villosa* | 2.82 | Chironomidae | 1.98 |
| *Platambus maculatus* | 2.82 | *Chloroperla torrentium* | 1.97 |
| *Glossiphonia complanata* | 2.82 | *Odontocerum albicorne* | 1.87 |
| Heptageniidae | 2.74 | *Sialis lutaria* | 1.84 |
| *Paraleptophlebia submarginata* | 2.70 | Simuliidae | 1.77 |
| *Ephemera danica* | 2.66 | *Esolus parallelepipedus* | 1.77 |
| Tipulidae | 2.49 | *Hydroptila* spp. | 1.75 |
| *Dicranota* spp. | 2.41 | *Wiedemannia* spp. | 1.74 |
| Limnephilidae | 2.38 | *Planaria torva* | 1.72 |
| *Elmis aenea* | 2.36 | *Limnius volckmari* | 1.71 |
| *Sericostoma personatum* | 2.28 | *Ecdyonurus venosus* | 1.69 |

Table 3 continued.

| Taxon | DCA axis 1 | Taxon | DCA axis 2 |
|---|---|---|---|
| *Hydropsyche* spp. | 2.24 | *Polycentropus kingi* | 1.64 |
| *Leuctra geniculata* | 2.22 | *Radix peregra* | 1.59 |
| Ceratopogonidae | 2.21 | *Caenis rivulorum* | 1.57 |
| *Ancylus fluviatilis* | 2.20 | *Polycentropus flavomaculatus* | 1.55 |
| *Nemoura avicularis* | 2.19 | *Psychomyia pusilla* | 1.51 |
| *Micropterna lateralis* | 2.03 | *Habrophlebia fusca* | 1.48 |
| *Helophorus brevipalpis* | 1.97 | *Goera pilosa* | 1.48 |
| *Serratella ignita* | 1.92 | Empididae | 1.41 |
| *Gammarus pulex* | 1.89 | *Hydropsyche siltalai* | 1.41 |
| *Chloroperla tripunctata* | 1.87 | *Micropterna lateralis* | 1.40 |
| *Glossosoma boltoni* | 1.79 | *Centroptilum pennulatum* | 1.38 |
| *Hydropsyche angustipennis* | 1.78 | *Potamopyrgus antipodarum* | 1.34 |
| *Ecdyonurus* spp. | 1.69 | *Perlodes microcephalus* | 1.30 |
| *Baetis fuscatus* | 1.66 | *Rhyacophila dorsalis* | 1.25 |
| *Potamophylax latipennis* | 1.63 | *Micronecta poweri* | 1.25 |
| *Hydraena gracilis* | 1.53 | Oligochaeta | 1.17 |
| *Limnius volckmari* | 1.49 | *Tinodes waeneri* | 1.16 |
| *Ecdyonurus torrentis* | 1.49 | *Haliplus lineatocollis* | 1.16 |
| *Erpobdella testacea* | 1.48 | *Centroptilum luteoum* | 1.16 |
| *Psycoda* spp. | 1.47 | *Ancylus fluviatilis* | 1.12 |
| *Pisidium* spp. | 1.45 | *Pisidium* spp. | 1.08 |
| *Leuctra fusca* | 1.39 | *Platambus maculatus* | 1.04 |
| *Nemoura erratica* | 1.37 | *Glossiphonia complanata* | 1.04 |
| Sciomyzidae | 1.33 | Limnephilidae | 1.00 |
| *Chelifera stigmatica* | 1.31 | *Ecdyonurus torrentis* | 0.96 |
| *Halesus radiatus* | 1.17 | *Ephemera danica* | 0.82 |
| *Baetis* spp. | 1.14 | *Ecdyonurus* spp. | 0.80 |
| Dytiscidae | 1.11 | *Hydropsyche* spp. | 0.79 |
| *Baetis scambus* | 1.00 | *Oulimnius tuberculatus* | 0.78 |
| *Protonemura meyeri* | 0.93 | *Elmis aenea* | 0.73 |
| *Perlodes microcephalus* | 0.90 | *Paraleptophlebia submarginata* | 0.73 |
| *Gyrinus* sp. | 0.87 | *Dicranota* spp. | 0.36 |
| Hydrobiidae | 0.83 | *Phagochata vitta* | 0.32 |
| *Erpobdella octoculata* | 0.61 | *Hydropysche pellucidula* | 0.28 |
| *Hydropsyche pellucidula* | 0.52 | *Isoperla grammatica* | 0.00 |
| *Baetis rhodani* | 0.42 | *Gammarus pulex* | -0.04 |
| *Agapetus* spp. | 0.39 | *Sericostoma personatum* | -0.14 |
| *Rhithrogena semicolorata* | 0.32 | *Erpobdella octoculata* | -0.20 |
| *Glossosoma* spp. | 0.30 | *Protonemura meyeri* | -0.20 |
| *Potamopyrgus antipodarum* | 0.29 | *Rhithrogena semicolorata* | -0.41 |
| *Diplectrona felix* | 0.27 | Heptageniidae | -0.52 |
| *Hydropysche instabilis* | 0.27 | *Nemoura erratica* | -0.85 |
| *Lype* sp. | 0.25 | Ceratopogonidae | -1.37 |
| *Goera pilosa* | 0.20 | *Nemoura avicularis* | -1.39 |
| *Rhyacophila dorsalis* | 0.08 | *Agapetus* spp. | -1.89 |
| *Leuctra* spp. | 0.06 | *Glossosoma boltoni* | -2.03 |
| *Isoperla grammatica* | 0.04 | *Diplectrona felix* | -2.06 |
| *Sialis lutaria* | -0.16 | *Gyrinus* sp. | -3.43 |
| Simuliidae | -0.45 | *Hydropsyche angustipennis* | -3.46 |

Table 4. Relationships between macro-invertebrates, including Detrended Correspondence Analysis (DCA) axis scores, and habitat heterogeneity, fractal dimension and an index of surface area ($m^2$) when habitat type is not included as a fixed effect. Only significant fixed effects are shown.

| Model | Fixed effects | Coefficient (± 95% confidence interval) | F | P | DF | Random effect |
|---|---|---|---|---|---|---|
| Diversity | Fractal dimension | 3.34±1.77 | 13.6 | <0.001 | 1, 104 | - |
| Richness | Fractal dimension | 26.47±19.36 | 7.18 | 0.009 | 1, 103 | Site |
| | Surface area | 60.05±39.76 | 8.76 | 0.004 | 1, 103 | Site |
| Log(abundance) | Fractal dimension | 5.86±3.55 | 6.43 | 0.013 | 1, 103 | Site |
| | Surface area | 2.24±1.73 | 10.48 | 0.002 | 1, 103 | Site |
| DCA axis 1 score | Surface area | -7.54±4.72 | 9.80 | <0.001 | 1, 104 | Site |
| DCA axis 2 score | NS | - | - | NS | - | Site |

Table 5. Relationships between macro-invertebrates, including Detrended Correspondence Analysis (DCA) axis scores, and habitat type, habitat heterogeneity, fractal dimension and an index of surface area ($m^2$). Only significant fixed effects are shown.

| Model | Fixed effects | F | P | DF | Random effect |
|---|---|---|---|---|---|
| Diversity | Habitat type | 8.03 | <0.001 | 5, 102 | - |
| Richness | Habitat type | 3.88 | 0.003 | 5, 102 | Site |
| Log(abundance) | Habitat type | 8.08 | <0.001 | 5, 102 | Site |
| DCA axis 1 score | Habitat type | 14.46 | <0.001 | 5, 102 | Site |
| DCA axis 2 score | NS | - | NS | - | Site |

Table 6. Predicted macro-invertebrate diversity, richness, abundance and Detrended Correspondence Analysis (DCA) axis 1 score among habitat types ± 95% confidence intervals, with lower/upper confidence intervals given for back-transformed abundance.

| Model | Habitat type | | | | | |
|---|---|---|---|---|---|---|
| | Bedrock | Silt | Sand | Gravel | Pebbles | Cobbles |
| Diversity | 1.11±0.29 | 1.16±0.35 | 1.72±0.39 | 1.99±0.19 | 1.90±0.14 | 1.80±0.11 |
| Richness | 8±6 | 12±7 | 14±7 | 15±4 | 13±4 | 12±4 |
| Abundance | 10±6/12 | 94±55/131 | 17±10/26 | 63±28/49 | 80±32/53 | 65±24/39 |
| DCA axis 1 score | 2.19±0.72 | 3.58±0.75 | 2.52±0.77 | 1.90±0.66 | 1.87±0.65 | 1.80±0.64 |

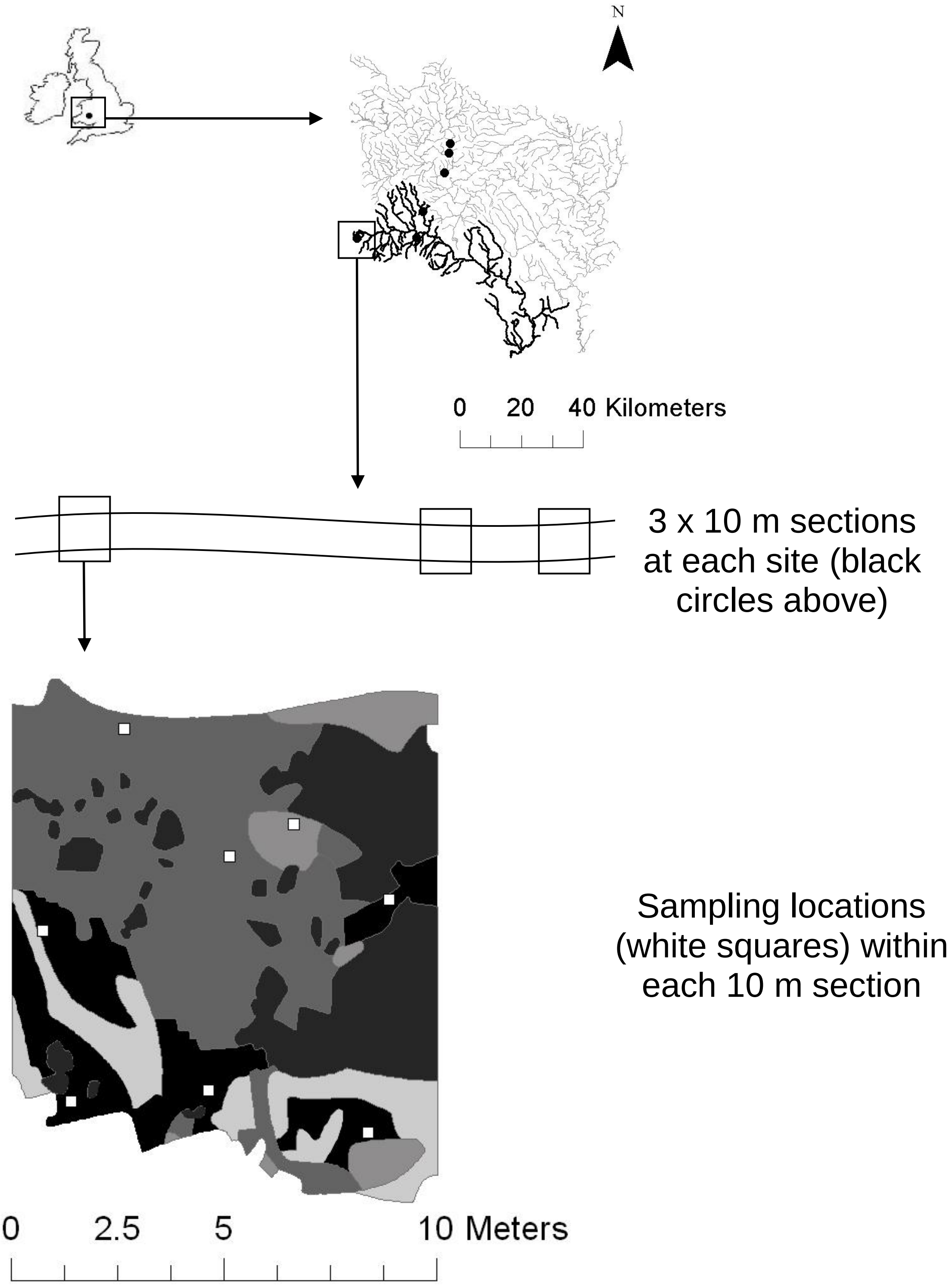

Figure 1. Sampling design showing 0.25 x 0.25 m sampling locations (white squares) within 10 m sections nested within sites (black circles) on tributaries of the Rivers Wye and Usk (in bold).

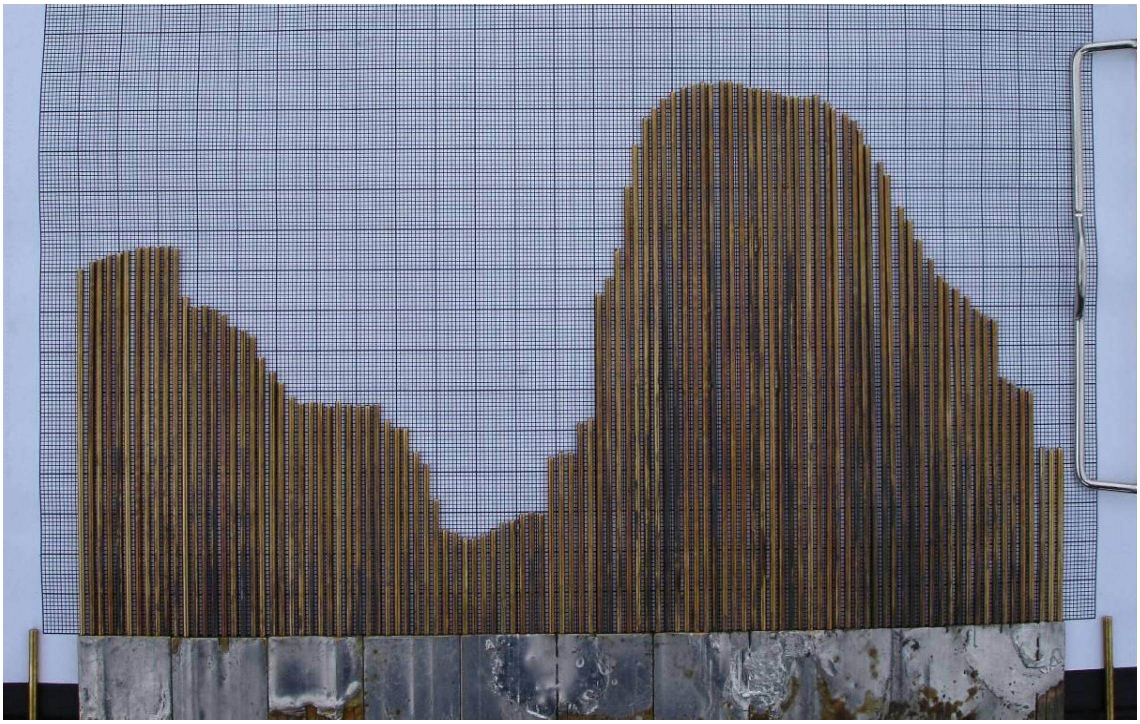

Figure 2. Profiler and example of a river bed profile used to assess surface complexity and area for 0.25 x 0.25 m sampling locations.

Figure 3. Sampling frame (0.25 x 0.25 m) showing the location of holes for positioning the profiler.

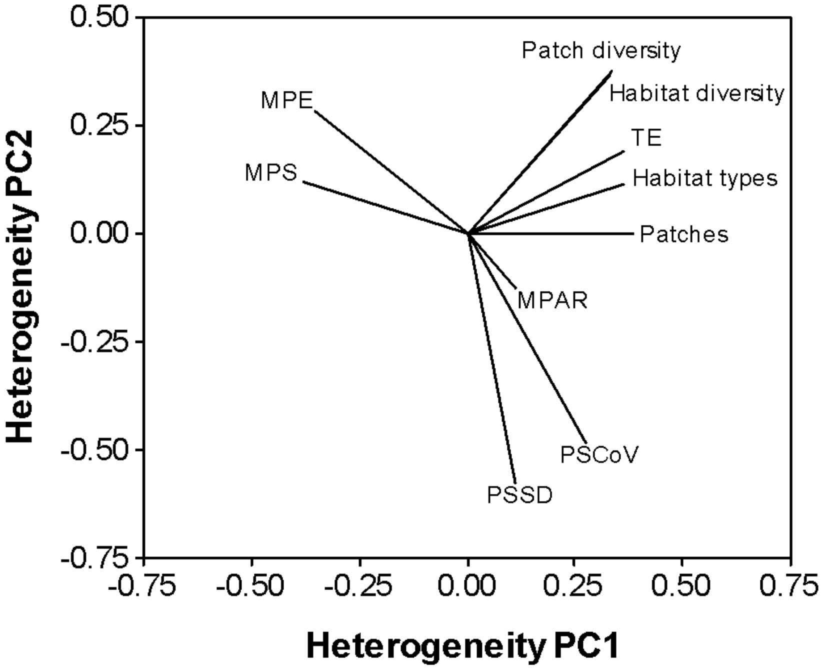

Figure 4. Principal Component Analysis (PCA) loading plot of habitat heterogeneity measures. MPS=mean patch size, MPE=mean patch edge, TE=total edge, Habitat types=number of habitat types, Patches=number of patches, MPAR=mean perimeter/area ratio, PSCoV=patch size coefficient of variance and PSSD=patch size standard deviation.

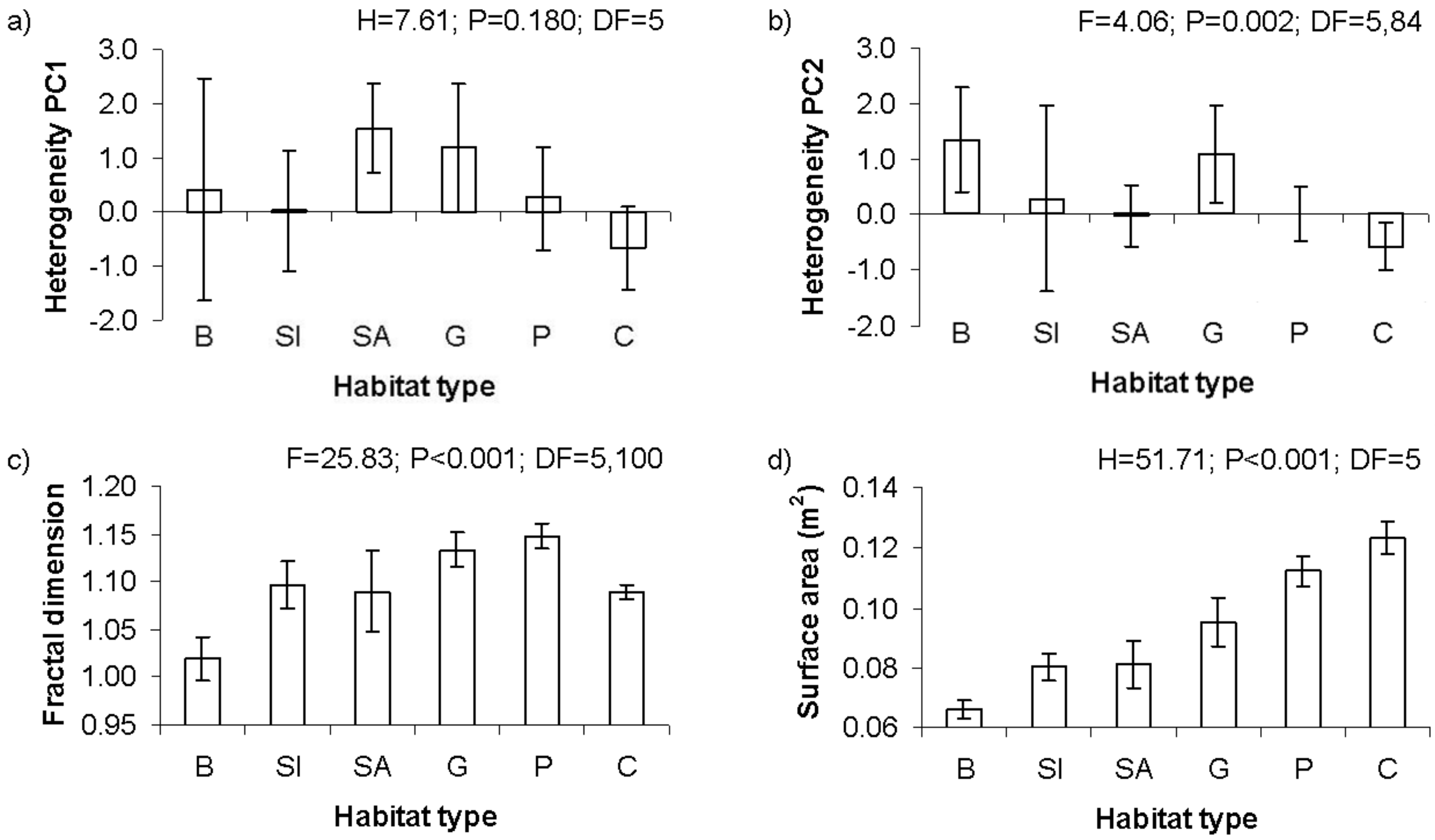

Figure 5. a) Heterogeneity Principal Component (PC) 1, b) Heterogeneity PC2, c) fractal dimension and d) an index of surface area ($m^2$) among habitat types. Bars represent mean values, error bars represent 95% confidence intervals. B=bedrock, SI=silt, SA=sand, G=gravel, P=pebbles and C=cobbles.

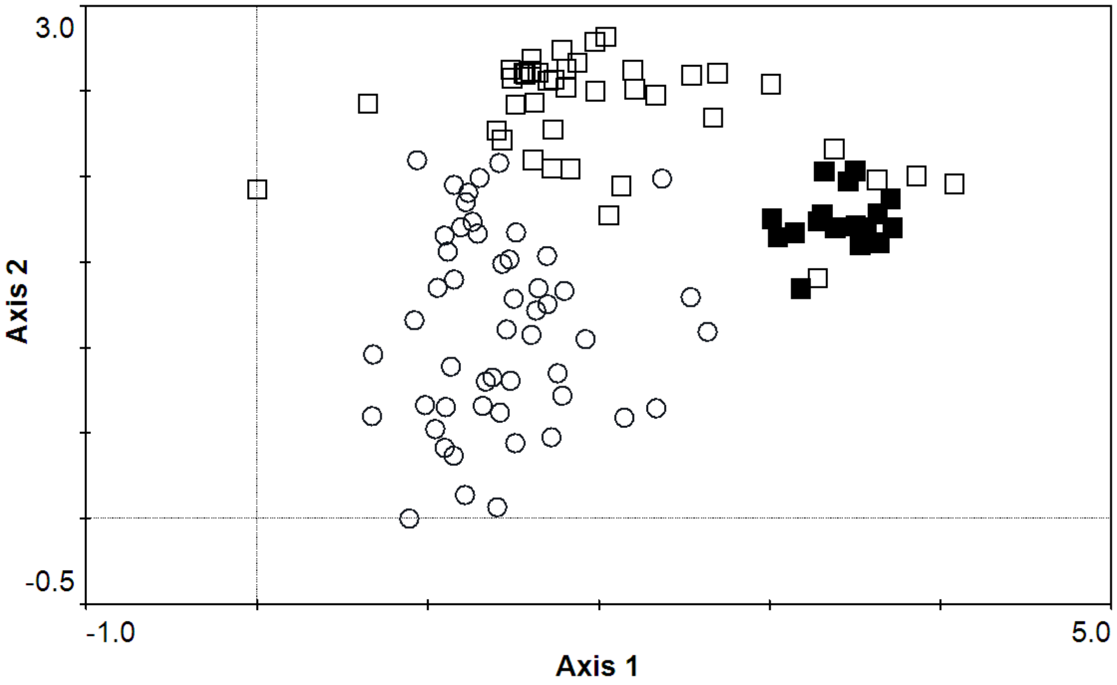

Figure 6. Detrended Correspondence Analysis (DCA) score plot with samples grouped by tributary. Samples from a tributary of the River Wye = ○, the upper River Usk = ■ and tributaries of the Usk = □.

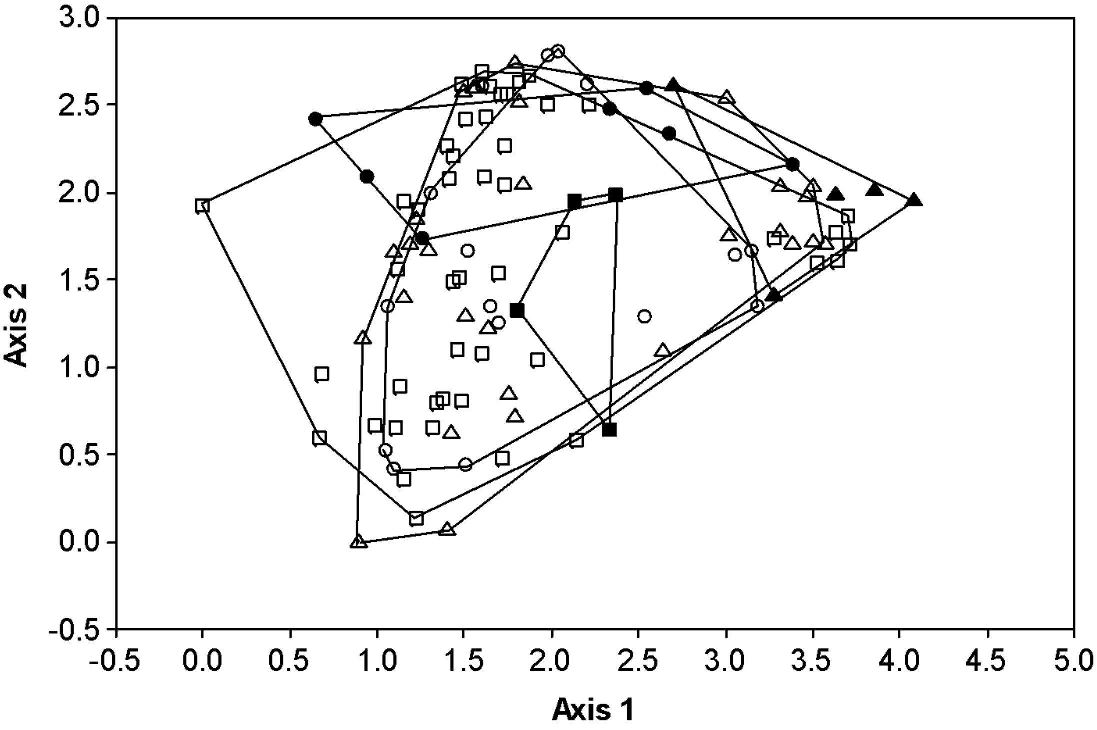

Figure 7. Detrended Correspondence Analysis (DCA) score plot with samples grouped by habitat type. Bedrock = ●, silt = ▲, sand = ■, gravel = ○, pebbles = △ and cobbles = □.

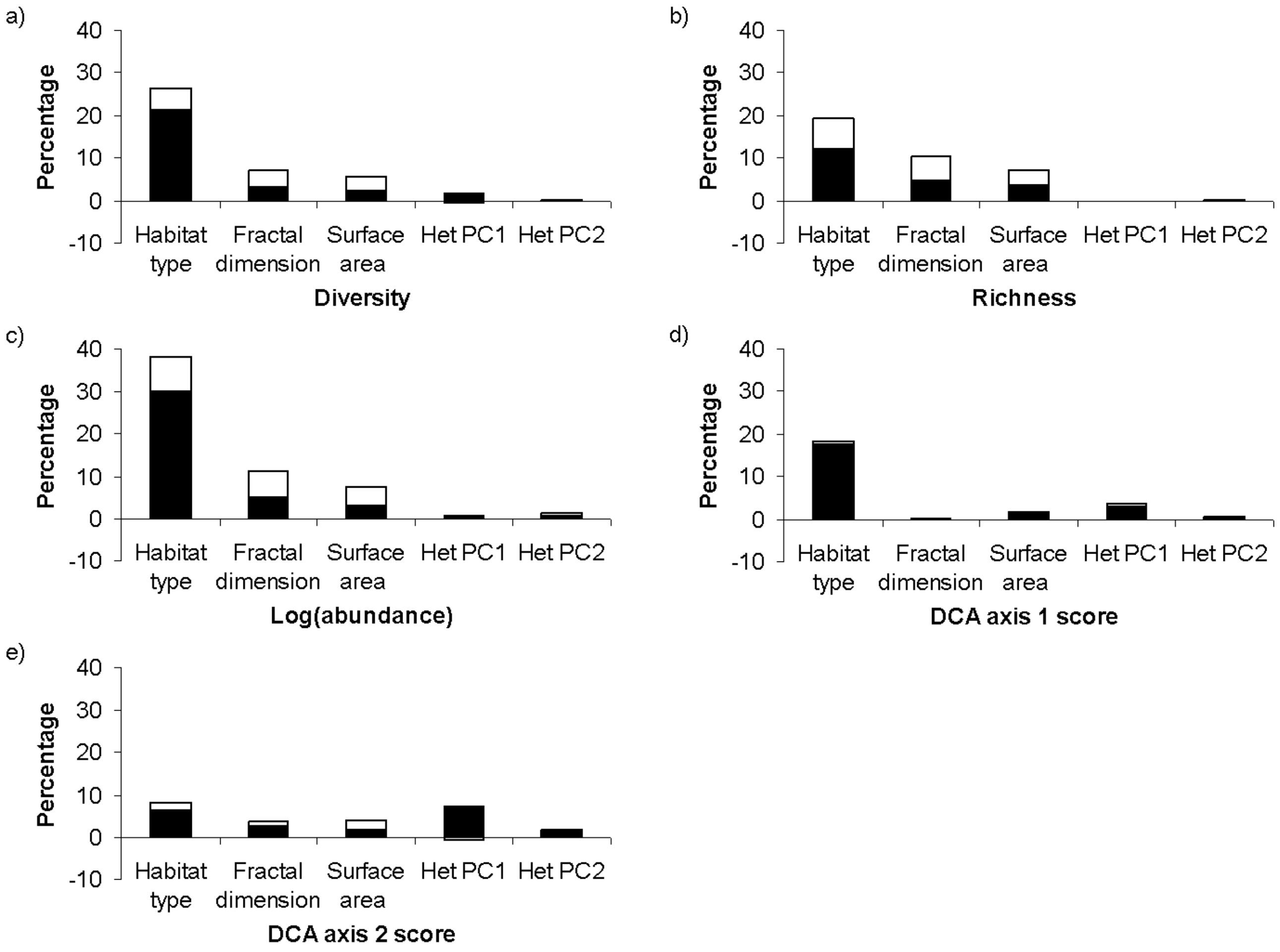

Figure 8. Percentage of the joint (□) and independent (■) variance in macro-invertebrate a) diversity, b) richness, c) logarithmically transformed abundance, d) Detrended Correspondence Analysis (DCA) axis 1 score and e) DCA axis 2 score explained independently and jointly by habitat type, fractal dimension, an index of surface area ($m^2$) and Heterogeneity Principal Components (PCs) based on hierarchical partitioning.

# Chapter 4 Higher-level filters transcend the local effects of physical habitat complexity on river macro-invertebrates

## 4.1. Abstract

1. Physical habitat structure is considered to be fundamental to the distribution, composition and body size of organisms. Documented relationships between structure and organisms are however inconsistent with one possible explanation being that variations in other physical factors act as higher-level filters on species composition. Such effects are likely in highly physically-structured systems such as rivers which are characterised by variations in multiple physical factors.

2. Based on these prepositions, two hypotheses were tested experimentally by manipulating patches of benthic habitat in a British upland river: i) greater surface complexity and interstitial volume increase macro-invertebrate diversity, richness and abundance whilst altering body size and composition independently of surface area and ii) variation among habitats in other conditions - in this case flow type - transcend the effects of structure alone. Experimental baskets containing cobbles, pebbles, gravel or 50:50 mixtures were deployed in riffles and glides to create variation in surface complexity, surface area and interstitial volume independent of flow type.

3. Flow type explained significant variations in macro-invertebrate richness, abundance, composition and mean body size, with riffles supporting richer assemblages containing greater numbers of individuals of smaller mean body size. Whilst substrates with greater surface areas supported an increased proportion of small- (<5 mm) relative to intermediate- (5-9 mm) sized individuals, surface complexity and interstitial volume had no measurable effects.

4. As one of the few experiments attempting to separate the effects of habitat structure and flow on river macro-invertebrates, this study does not support the hypothesis that local habitat structure determines their assemblage composition and structure. Given the relationship between discharge and channel form in rivers, this implies that widely observed differences in assemblages among habitats result from flow-related effects,

rather than habitat structure *per se*. Species distributions may vary along other physical dimensions and cannot be assumed to result from habitat structure unless the effects of other physical factors are excluded.

## 4.2. Introduction

Physical habitat structure is considered to be a fundamental factor affecting organism distribution and diversity in terrestrial, marine and freshwater ecosystems (McCoy and Bell, 1991; Tews *et al*., 2004). It is widely predicted that more complex habitats will support greater diversity and abundances of organisms by providing a greater range of niches (Shmida and Wilson, 1985; Townsend and Hildrew, 1994), refuges from disturbance and predation (e.g. Power, 1992; Rice *et al*., 2007) and a greater abundance of resources (e.g. Gawne and Lake, 1995; Sanson *et al*., 1995). More complex habitats may also provide more usable space for small individuals and are therefore expected to have a greater relative abundance of small organisms than simple habitats (Morse *et al*., 1985; Gee and Warwick, 1994). Body size in turn influences an organism's energetic requirements and resource exploitation with implications for ecological processes and numerous life-history traits such as lifespan (Schmid *et al*., 2000; Brown *et al*., 2007).

Despite the interest habitat structure has received in ecology, there are still however disparities among theory and empirical evidence. Many studies have documented how relationships between habitat structure and animal communities vary among structural features, species, locations, seasons and spatial scales, whilst others have found no relationship (e.g. Beck, 1998; Downes and Jordan, 1993; Downes *et al*., 1995; Vinson and Hawkins, 1998; Johnson *et al*., 2003; Taniguchi and Tokeshi, 2004; Tews *et al*., 2004; Nakaoka, 2005; Meager *et al*., 2011). One possible explanation is that variations in other physical factors act as higher-level 'filters' on species composition, with species that do not possess the necessary trait characteristics unable to persist in a habitat even if the physical structure is suitable (Poff, 1997). The concept of environmental trait filters has received much attention since the 'habitat templet concept' of Southwood (1977) and such filters may mediate key processes structuring communities even in complex ecosystems such as tropical forests (e.g. Lebrija-Trejos *et al*., 2010). Environmental trait filters provide a framework for assessing which scales give rise to the greatest influence on communities (e.g. Algar *et al*., 2011), as well as predicting the distribution of organisms (e.g. Chessman and Royal, 2004) (Poff, 1997). Assessing which traits are filtered out within particular habitats as well as determining clear species-habitat associations also has conservation importance (Roy and de Blois, 2006). One of the major challenges in testing these ideas is that habitat structure may be correlated with higher-level filters, potentially confounding observational studies and necessitating an experimental approach.

Environmental trait filters have generated much interest in river ecosystems (e.g. Poff, 1997; Wiens, 2002; Lamoroux *et al*., 2004; Statzner *et al*., 2004) which are naturally highly structured across a hierarchy of spatial scales (Vaughan *et al*., 2009). Variation in multiple physical factors such as hydraulic conditions and resource distributions at a range of scales have been shown to influence river communities (e.g. Vinson and Hawkins, 1998), whist there is also long-standing interest in the role of habitat structure in the distribution and abundance of benthic organisms (Percival and Whitehead, 1929, 1930; Whitehead, 1935; Jones, 1949). Among these, macro-invertebrates are a widespread, abundant and highly diverse group, which have a range of body sizes (Hynes, 1970). They exemplify the conflicting results from studies of habitat structure, with equivocal effects of different structural features and on different aspects of assemblage composition, structure and body size (e.g. Downes *et al*., 1995, 1998; Gayraud and Philippe, 2001). Rivers therefore provide a good system in which to examine hypotheses about the relative importance of habitat structure and possible explanations for the lack of a consistent relationship with organisms.

Crucially, distinguishing the effects of habitat structure from other physical factors that affect organisms often requires experimentation because complex interactions occur in the field. In rivers, hydraulic conditions at the reach scale determine substrate size, which affects characteristics such as surface complexity, surface area and porosity (Allan, 1995). Conversely, substratum characteristics govern near-bed flow conditions (Davis and Barmuta, 1989). Experimental studies attempting to separate the ecological effects of habitat structure and flow are however few (Minshall and Minshall, 1977; Rabeni and Minshall, 1977; Lancaster and Mole, 1999; Boyero, 2003; Scealy *et al*., 2007). A further complication arises from the fact that habitat structure is confounded by other physical factors. For example, more complex habitats typically have a larger surface area than simple ones and may therefore support a greater number of species as a result of a species-area effect (Arrhenius, 1921; Gleason, 1922; MacArthur and Wilson, 1967; Connor and McCoy, 1979; Coleman *et al*., 1982), but studies separating the relative effects of complexity and surface area on freshwater macro-invertebrate communities are scarce (Downes *et al.*, 1995; Boyero, 2003; McAbendroth *et al.*, 2005; Becerra-Muñoz and Schramm, 2007; Thomaz *et al.*, 2008; Warfe *et al.*, 2008).

In this chapter, I aim to distinguish the role of local habitat structure, flow conditions and surface area on river macro-invertebrates, using fractal dimension to quantify surface complexity as evidence suggests it may not be correlated with surface area (Chapter 3). Two hypotheses were tested: i) greater surface complexity and interstitial volume increase macro-invertebrate diversity, richness and abundance whilst altering body size and composition independently of surface area and ii) variation among habitats in other conditions - in this case flow type - transcend the effects of structure alone.

## 4.3. Methods

### 4.3.1. Study area

The River Wye rises in the Plynlimon Mountains, draining a catchment of 4136 $km^2$ and flowing 215 km into the Severn Estuary (Figure 1). The catchment is divided into upper, middle and lower sub-catchments which vary in geology, relief and land use (Edwards and Brooker, 1982; Jarvie *et al.*, 2003). Ordovian or Silurian sandstones, shales, grits and mudstones in the upper catchment are replaced by Devonian Old Red Sandstone or marls in the middle and lower catchment where this study took place. Land use is dominated by rough semi-intensive pasture with some arable farmland in the south-east lowlands. Tributaries in the middle of the catchment are relatively un-polluted and calcareous (50-250 mg $CaCO_3$ $l^{-1}$), contrasting with the low pH of those in acidified uplands and the moderately elevated biochemical oxygen demand and nutrient concentrations of those in the lowlands (Ormerod and Edwards, 1987; Jarvie *et al.*, 2003; Clews and Ormerod, 2009). Average annual rainfall varies from 900-1200 mm depending on altitude (Environment Agency, 2008).

The experiment was conducted in 2009 (from mid-August to late September 2009) in a 50 m section of the River Edw, a fourth-order tributary in the middle Wye catchment (SO 09926 48026) (Figure 1). This tributary was selected to minimise any confounding effects of water quality, land use or channel modification and drained a mixture of rough/semi-improved pasture and broadleaved woodland.

### 4.3.2. Sampling design

Variation in fractal dimension, surface area and interstitial volume was generated by filling six plastic baskets (21 x 21 x 7.5 cm covered in 1.5 cm plastic mesh to prevent loss of substrate particles) with each the following: i) cobbles (typical diameter 80 mm), ii) pebbles (typical diameter 30 mm), iii) gravel (typical diameter 20 mm), iv) a 50:50 mixture of cobbles and pebbles based on volume, v) a 50:50 mixture of pebbles and gravel and vi) a 50:50 mixture of cobbles and gravel (Figure 2). Baskets were sunk level with the river bed at random locations distributed equally among riffles and glides, two distinct flow types based on velocity and Froude number (Padmore, 1998). Baskets were deployed for between five and six weeks (due to the time required to retrieve them), considered sufficient for colonisation by reasonably representative macro-invertebrate assemblages (Mason, 2002), during a period of base flows.

### 4.3.3. Data collection

Following exposure, baskets were enclosed with a standard kick-sample net (250 x 250 mm with a 1 mm mesh) and retrieved carefully to minimise disturbance to the arrangement of substrate particles.

#### *Surface complexity and area*

Following removal, ten pin profiles of the surface of each basket were obtained using a profiler consisting of 87 pins at 2.4 mm intervals (Figure 3). Once the mesh was removed, the profiler was positioned at five 52.5 mm intervals in each direction using a sampling frame, each pin pushed down until it touched the surface and the height of each pin recorded (Figure 4).

### *Interstitial volume*

Each basket was submerged carefully in a bucket filled with 5 l of water and the volume of water displaced measured to the nearest 100 $cm^3$ (≡ 3% of the basket volume). Interstitial volume was then estimated as the difference between the geometric volume of the basket (3002.5 $cm^3$) and the volume of water displaced.

### *Macro-invertebrates*

The contents of each basket were preserved in 70% ethanol for subsequent analysis, when macro-invertebrates were identified to species level where possible and counted.

## 4.3.4. Data analysis

### *Surface complexity and area*

The ten pin profiles from each basket were used to calculate the mean fractal dimension. Each pin profile was first digitised in ArcMap version 9.2 using Hawth's Analysis Tools version 3.27 (Beyer, 2004). FracLac for ImageJ version 2.5 (Karperien, 1999) was used to calculate fractal dimension using the box-counting method, in which a line or outline is covered with a series of grids of increasing size and the number of occupied grid squares counted (Mandelbrot, 1983; Fielding, 1992). The slope coefficient from the regression of the number of grid squares occupied versus the square size on log-log axes then provides an estimate of fractal dimension (Mandelbrot, 1983). The analysis was restricted to grid squares between twice the distance between pins and one-fifth of the profile length to avoid fractal dimension estimates of less than 1, which can occur when the same number of grid squares is occupied at very large consecutive grid sizes (Buczkowski *et al*., 1998; Halley *et al*., 2004). For a small number of profiles where this did not remove the effects of these artefacts, fractal dimension estimates were replaced by a value of 1.

An index of surface area for each basket was estimated from the square of the mean profile length (Kostylev *et al*., 2005).

Differences in surface complexity, surface area and interstitial volume among substrate types were analysed using a One-Way ANOVA followed by a Tukey test to assess whether they were comparable to natural substrates.

### *Macro-invertebrates*

Total abundance, taxonomic richness and taxonomic diversity (using Shannon's diversity index) were calculated for each basket. Logarithmically (x+1) transformed macro-invertebrate abundances were then analysed using Principal Component Analysis (PCA) extracted from a covariance matrix to assess variation in composition. This type of unconstrained ordination was selected as the Detrended Correspondence Analysis gradient lengths indicated that taxon response curves were linear (ter Braak and Šmilauer, 2002).

The body lengths of Ephemeroptera, Plecoptera and Trichoptera (EPT), which made up approximately 50% of the macro-invertebrates in each basket on average, were determined to the nearest 0.5 mm. Measurements were made along the dorsal edge, excluding antennae, cerci and anal gills, for a maximum of 50 randomly selected individuals of each species and each taxon identified above species level. Mean body size and the proportion of individuals <5 mm, 5-9 mm, 10-14 mm and 15-20 mm were used for analyses.

### ***Relationships between structural characteristics, surface area, flow type and macro-invertebrates***

Relationships between habitat structural characteristics, surface area, flow type and macro-invertebrates were analysed using Generalised Linear Mixed Models in ASReml version 3 (VSN International Ltd, 2009) or, where random effects were not required, Generalised Linear Models in R version 2.9.2 (R Development Core Team, 2009). Random effects were used to control for possible dependence on sampling date and basket location, but were excluded when they were not significant. Macro-invertebrate abundance and PCA axis 1 scores were modelled using Generalised Least Squares in the nlme package in R (Pinheiro *et al*., 2009) to allow for unequal variances among flow types. Error and link functions are given in Table 1. Models for proportions of individuals in body size classes included a scaling parameter to account for overdispersion. Models were checked for validity through assessment of standardised residuals and refined using stepwise deletions at the P=0.05 level of significance.

## 4.4. Results

### ***Structural characteristics and surface area among substrate types***

Fractal dimension (F=6.09; P<0.001; DF=5, 30), surface area (F=13.07; P<0.001; DF=5, 30) and interstitial volume (F=2.96; P=0.028; DF=5, 30) varied significantly among substrate types (Figure 5). Fractal dimension was significantly greater for pebbles than cobbles and cobbles/gravel whilst surface area was significantly greater for cobbles and cobbles/pebbles than gravel, pebbles/gravel and cobbles/gravel, and for pebbles than gravel (P<0.05). Interstitial volume was significantly greater for gravel than cobbles/pebbles (P<0.05).

### ***Macro-invertebrate composition***

In PCA, two axes explained 63% of the variation in composition, with axis 1 explaining 57% (Figure 6). Axis 1 scores completely separated riffles from glides (Figure 7) and represented increasing numbers of fast-flow specialists such as *Hydropsyche siltalai*, *Baetis rhodani* and *Rhithrogena semicolorata* whilst *Erpobdella octoculata*, *Paraleptophlebia submarginata* and *Plectrocnemia conspersa* declined. On axis 2, *Sericostoma personatum*, *Glossosoma* spp. and *Ancylus fluviatilis* increased whilst *Hydropsyche pellucidula*, *Baetis rhodani and Simulidae* declined, but this far weaker axis was excluded from subsequent analyses and it appeared to have no relevance to structural characteristics or flow type.

### ***Relationships between structural characteristics, surface area, flow type and macro-invertebrates***

Macro-invertebrate richness (by c 50%), abundance (by c 300%) and PCA axis 1 scores were significantly greater for riffles than glides, whilst mean body size was smaller by approximately 15% (Tables 1 and 2).

The proportion of individuals <5 mm also increased with the surface area of a basket whilst the proportion of individuals 5-9 mm decreased (Table 1). As the estimated surface area of a basket increased from 0.08 $m^2$ to 0.18 $m^2$ the proportion of individuals <5 mm increased from 51% to 62%, whist the proportion of individuals 5-9 mm decreased from 43% to 28% (Table 3).

Macro-invertebrate diversity, richness, abundance, PCA axis 1 score and mean body size did not vary with fractal dimension or interstitial volume (Table 1). In other words, in contrast to the effects of flow type, neither macro-invertebrate assemblage composition nor structure varied with surface complexity or interstitial volume.

## 4.5. Discussion

This study is one of the first experimental attempts to separate habitat complexity from the ecological effects of flow velocity in rivers - two of the major factors thought to influence river organisms. Whilst individually both have been widely studied, experimental studies separating their influences are rare (Minshall and Minshall, 1977; Rabeni and Minshall, 1977; Lancaster and Mole, 1999; Boyero, 2003; Scealy *et al.*, 2007). Even fewer have assessed the ecological effects of a gradient of substratum complexity (e.g. Taniguchi and Tokeshi, 2004).

Whilst habitat structure provided by the substratum is considered one of the most important local factors affecting macro-invertebrates in rivers (Hynes, 1970), the results of this study suggest that the effects of other physical factors - in this case flow type - are greater. Substrate size and roughness typically emerge as being of secondary importance to flow effects in the field (Quinn and Hickey, 1994; Mérigoux and Dolédec, 2004; Brooks *et al.*, 2005; cf. Beisel *et al.*, 1998), although situations occur in which flow velocity and surface complexity are related to different aspects of macro-invertebrate assemblage composition and structure (Downes *et al.*, 1995). Previous experimental studies have however indicated that both flow and habitat complexity have effects on organisms, and that these effects may be interactive (Rabeni and Minshall, 1977; Lancaster and Mole, 1999; Boyero, 2003). Placement of large pebbles, a substrate type associated with erosional areas, into riffles (erosional areas) and pools (depositional areas) indicated that both substrate and current velocity were important

(Minshall and Minshall, 1977). In a laboratory flume, *Baetis rhodani* was only able to persist in its preferred fast current velocities on rough substrates (Lancaster and Mole, 1999). In contrast, a broad categorisation of flow types was sufficient to explain most of the variation in macro-invertebrate richness, abundance, composition and mean body size in this study, despite the fact that near-bed flow conditions are likely to have varied within riffles and glides (Davies and Barmuta, 1989; Padmore, 1998). Evidence that riffles may support a greater richness and abundance of organisms than slower-flowing habitats is long-standing, and riffles in this study were characterised by baetid and heptageniid mayflies, hydropsychid caddisflies, stoneflies and simuliids in agreement with previous studies (Hynes, 1970; Minshall and Minshall, 1977; Logan and Brooker, 1983; Brown and Brussock, 1991). In the present study, greater abundances of these taxa in riffles resulted in high unevenness of assemblages, and subsequently riffles did not support a greater diversity than glides.

Surface complexity provided by substratum roughness, crevices and bed topography can increase macro-invertebrate diversity, richness and abundance (Douglas and Lake, 1994; Downes *et al*., 1998; Robson and Chester, 1999) as well as alter assemblage composition (Downes *et al*., 1998, 2000a). Interstices in large and medium sized substrates also provide an important habitat for macro-invertebrates (Harper *et al*., 1995; Robertson and Wood, 2010), with experimental studies showing that gravel and cobbles containing interstitial spaces supported a greater richness and abundance than cement-embedded substrates with few interstices (Flecker and Allan, 1984). Conversely, reduction in interstitial volume, for example due to the accumulation of fine substrates, decreases the richness and abundance of macro-invertebrates (Richards and Bacon, 1994; Gayraud and Philippe, 2001, 2003; Bo *et al*., 2007). Other studies have however found that only certain structural features are important (Downes *et al*., 1995), or that there are only effects on aspects of assemblage structure (Downes *et al*., 2000a, 2000b) or particular species (Downes and Jordan, 1993). Similarly contrasting results have been found among macro-invertebrate assemblages on freshwater macrophytes (e.g. Jeffries, 1993; Taniguchi *et al*., 2003; Thomaz *et al*., 2008; Warfe *et al*., 2008; Dibble and Thomaz, 2009; Mormul *et al*., 2011; cf. McAbendroth *et al*., 2005; Ferreiro *et al*., 2011) and among the fauna of rocky shores (Beck, 1998; Johnson *et al*., 2003; Kostylev *et al*., 2005; Meager *et al*., 2011; cf. Attrill *et al*., 2000).

The results of this study are consistent with the hypothesis that other physical factors act as higher-level filters on species composition. Whilst small body size may be an adaptation to both substrate size and complex near-bed hydraulic conditions (Lamouroux *et al.*, 2004), other studies have shown that flow conditions are the main filter acting on body size (Mérigoux and Dolédec, 2004). Smaller body size reduces drag and enables macro-invertebrates to avoid hydraulic stress by remaining within the boundary layer (Hynes, 1970; Lamouroux *et al.*, 2004; Townsend and Thompson, 2007). Macro-invertebrates also exhibit ontogenetic shifts in hydraulic habitat use, with early instars of species such as *Baetis rhodani, Rhithrogena semicolorata* and *Hydropsyche siltalai* occupying areas of greater current velocity than late instars to ensure occurrence in approximately constant hydraulic conditions (Statzner and Borchardt, 1994; Hanquet *et al.*, 2004).

Previous studies have reported weak relationships between substrate size and the body size distribution of macro-invertebrates (Bourassa and Morin, 1995; Solimini *et al.*, 2001). However, habitats of a greater fractal dimension typically support a greater proportion of small organisms than simple ones in a number of ecosystems (Morse *et al.*, 1985; Shorrocks *et al.* 1991; Williamson and Lawton, 1991; Gunnarsson, 1992; Gee and Warwick, 1994; Kostylev *et al.*, 2005; McAbendroth *et al.*, 2005; Ferreiro *et al.*, 2011; Meager *et al.*, 2011). Previous experimental studies in rivers have shown that individuals of smaller average body size occurred on plates of greater fractal dimension (Taniguchi and Tokeshi, 2004), whilst rough substrates with small crevices supported a greater proportion of small individuals than smooth substrates (Downes *et al.*, 1998). Interstitial space is also considered to have an important effect on body size since this determines the ability of species to penetrate the substratum (Gayraud and Philippe, 2001; Robertson and Wood, 2010). Smaller body size enables organisms to occupy small interstices between substrate particles, whilst larger macro-invertebrates are restricted to large pore spaces (Gayraud and Philippe, 2001; Schmid and Schmid-Araya, 2010). Long, cylindrical body shapes also enable organisms to move within very fine sediments (Williams and Hynes, 1974; Lamouroux *et al.*, 2004; Omesová *et al.*, 2008). Studies have also shown that body size scales with the fractal dimension of the boundary between pore spaces and substrate particles, with the proportion of intermediate sized individuals increasing with pore complexity (Schmid *et al.*, 2002).

Contrary to the majority of previous studies assessing the relative importance of habitat complexity and surface area, surface area did not have an effect on macro-invertebrate richness or abundance (Downes *et al*., 1995; Attrill *et al*., 2000; Boyero, 2003; Kostylev *et al*., 2005; Becerra-Muñoz and Schramm, 2007; Thomaz *et al*., 2008; Warfe *et al*., 2008; cf. McAbendroth *et al*., 2005). The increased proportion of small- (<5 mm) relative to intermediate- (5-9 mm) sized individuals on substrates with greater surface areas is difficult to explain, however it is possible that this was not an effect of area in the strictest sense, and that larger substrates, which tended to have a larger surface area, were more exposed to size-selective predation, near-bed hydraulic conditions or different resource conditions (Townsend and Thompson, 2007).

As with all ecological experiments this study had some limitations, and care is therefore needed in transferring the results to real field circumstances. Firstly, uncontrolled variations in environmental conditions are likely to have generated variations among baskets and restrict the extent to which results can be extrapolated. Secondly, whilst previous studies have reported mixed effects of substrate size and composition on interstitial volume (Gayraud and Philippe, 2001, 2003), differences in interstitial volume among substrates in this study may have been reduced by the accumulation of fine substrates or detritus during exposure or below the level of detection by water displacement (Gayraud and Philippe, 2003). In addition, both surface complexity and area were derived from two-dimensional profiles which are unable to capture overhangs or the three-dimensional shape of crevices and spaces and may not be representative of the surface as a whole. Surfaces which have a different number of crevices or spaces of different sizes may also have the same fractal dimension (Sanson *et al*., 1995). Thirdly, time constraints resulted in the survey being conducted during late summer, by which time some insects may have emerged as adults, with likely effects on assemblage composition and body size distribution (Hynes, 1970). Limited colonisation time did not however appear to result in the absence of taxa occurring naturally on the substratum during a similar period in the previous year. With two exceptions all of the taxa found in gravel, pebbles and cobbles at the study site during the field survey in September 2008 colonised experimental baskets, and on average baskets supported greater macro-invertebrate diversity, richness and total abundance than natural gravel, pebbles and cobbles at both the study site and across sites (Chapter 3). Furthermore, variations among substrate types reflected those among natural substrates for both fractal dimension (cobbles<gravel<pebbles) and surface area (gravel<pebbles<cobbles), although average fractal dimension tended to be lower than for natural substrates and average surface area tended to be greater relative to the sampled area (Chapter 3).

These data therefore only give empirical support to the second hypotheses: habitat structure had no effect on macro-invertebrates. Instead, macro-invertebrate assemblage composition and structure appeared to vary along other physical dimensions which filtered species based on their body size. Given the relationship between discharge and channel form in rivers, this implies that observed differences in assemblages among habitats result from flow related-effects, rather than habitat structure *per se*. Species distributions may vary along other physical dimensions and cannot be assumed to result from habitat structure unless the effects of other physical factors are excluded.

In summary, whilst substrates with greater surface areas supported an increased proportion of small- (<5 mm) relative to intermediate- (5-9 mm) sized individuals, surface complexity and interstitial volume had no measurable effect on macro-invertebrates. Instead, flow type explained significant variations in macro-invertebrate richness, abundance, composition and mean body size, with riffles supporting richer assemblages containing greater numbers of individuals of smaller mean body size.

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## 4.7. Tables and Figures

Table 1. Relationships between macro-invertebrates, including Principal Component Analysis (PCA) axis 1 score, and fractal dimension, an index of surface area ($m^2$), interstitial volume ($cm^3$) and flow type. Only significant fixed effects are shown.

| Model | Fixed effects | Coefficient (± 95% confidence interval) | F or Chi-sq | P | DF | Random effects | Error function | Link function |
|---|---|---|---|---|---|---|---|---|
| Diversity | NS | - | - | NS | - | - | Gaussian | Identity |
| Richness | Flow type | 0.38±0.11 | 44.19 | <0.001 | 1, 34 | - | Poisson | Log |
| Abundance | Flow type | 831.17±202.62 | 61.05 | <0.001 | 1, 34 | - | Gaussian | Identity |
| PCA axis 1 score | Flow type | 4.17±0.67 | 140.66 | <0.001 | 1, 34 | - | Gaussian | Identity |
| Mean body size | Flow type | -0.14±0.10 | 7.63 | 0.009 | 1, 34 | - | Gamma | Log |
| Proportion <5 mm | Surface area | 4.71±4.11 | 5.05 | 0.032 | 1, 34 | Location | Binomial | Logit |
| Proportion 5-9 mm | Surface area | -6.52±3.89 | 9.55 | 0.003 | 1, 34 | Location | Binomial | Logit |
| Proportion 10-14 mm | NS | - | - | NS | - | Location | Binomial | Logit |
| Proportion 15-20 mm | NS | - | - | NS | - | Date | Binomial | Logit |

Table 2. Predicted macro-invertebrate diversity, richness, abundance, Principal Component Analysis (DCA) axis 1 score and mean body size among flow types ± 95% confidence intervals where available.

| **Model** | **Flow type** | |
|---|---|---|
| | **Riffle** | **Glide** |
| Richness | 41±2 | 28±1 |
| Abundance | 1127 | 295 |
| PCA axis 1 score | 4.66 | 0.48 |
| Mean body size | 4.86±0.17 | 5.58±0.19 |

Table 3. Predicted proportions of individuals <5 mm and 5-9 mm at the minimum (0.08 $m^2$) and maximum (0.18 $m^2$) surface areas recorded (to 2 d.p) ± 95% confidence intervals.

| **Model** | **Surface area** | |
|---|---|---|
| | **0.08 $m^2$** | **0.18 $m^2$** |
| Proportion <5 mm | 0.51±0.10 | 0.62±0.11 |
| Proportion 5-9 mm | 0.43±0.09 | 0.28±0.08 |

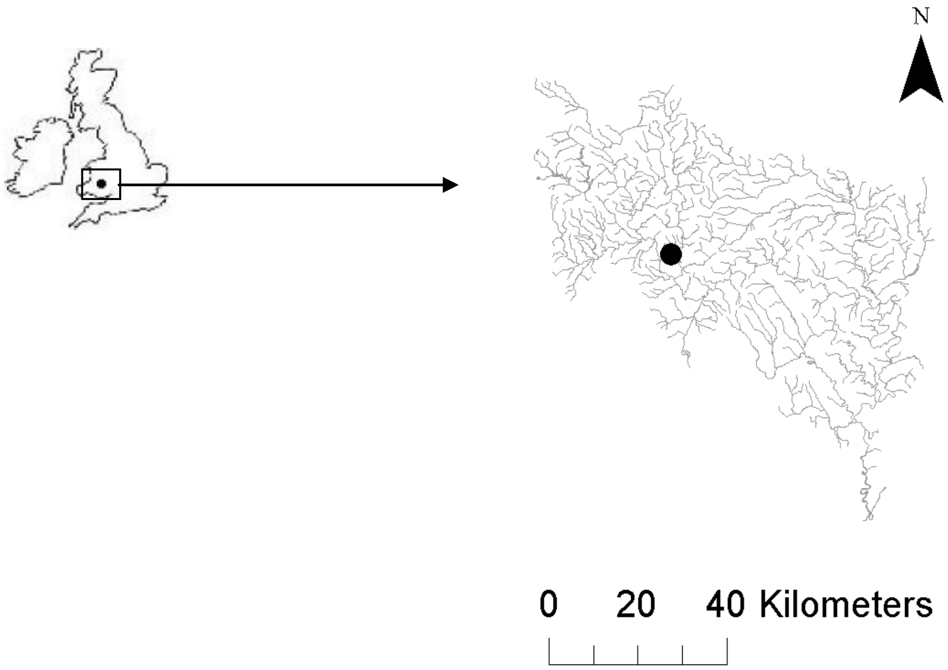

Figure 1. Location of the River Edw, a tributary of the River Wye.

Figure 2. Examples of experimental baskets filled with cobbles, pebbles, gravel and 50:50 mixtures.

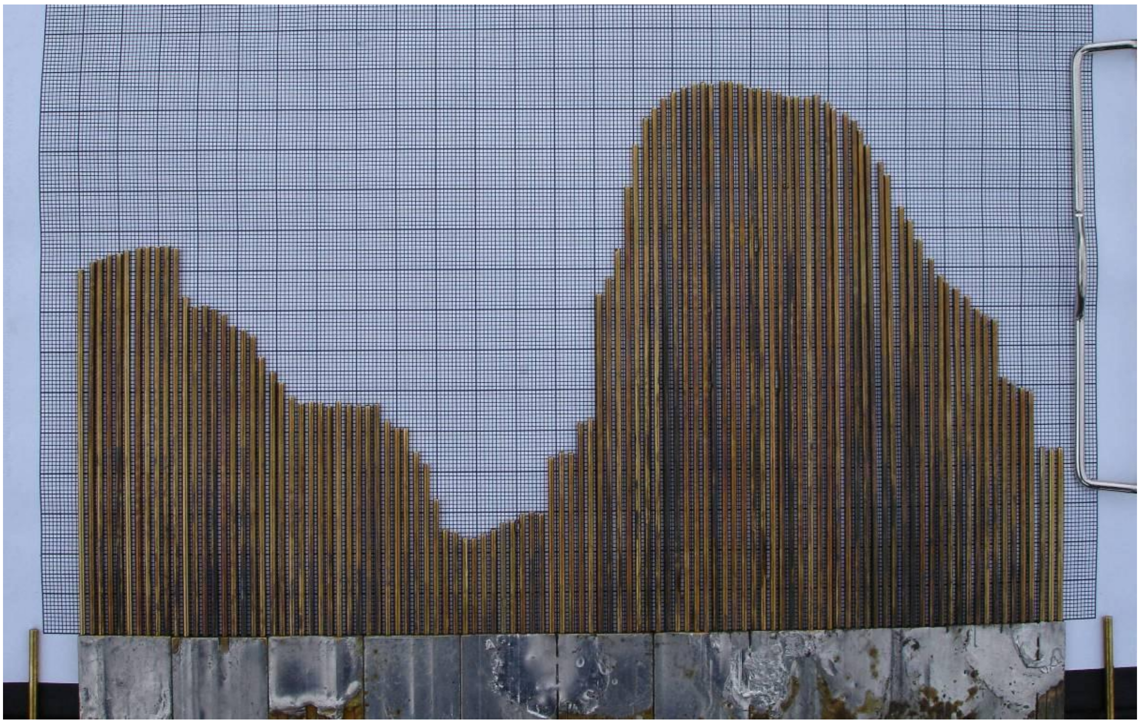

Figure 3. Profiler and example profile used to assess surface complexity and area.

Figure 4. Sampling frame (0.25 x 0.25 m) showing the location of holes for positioning the profiler.

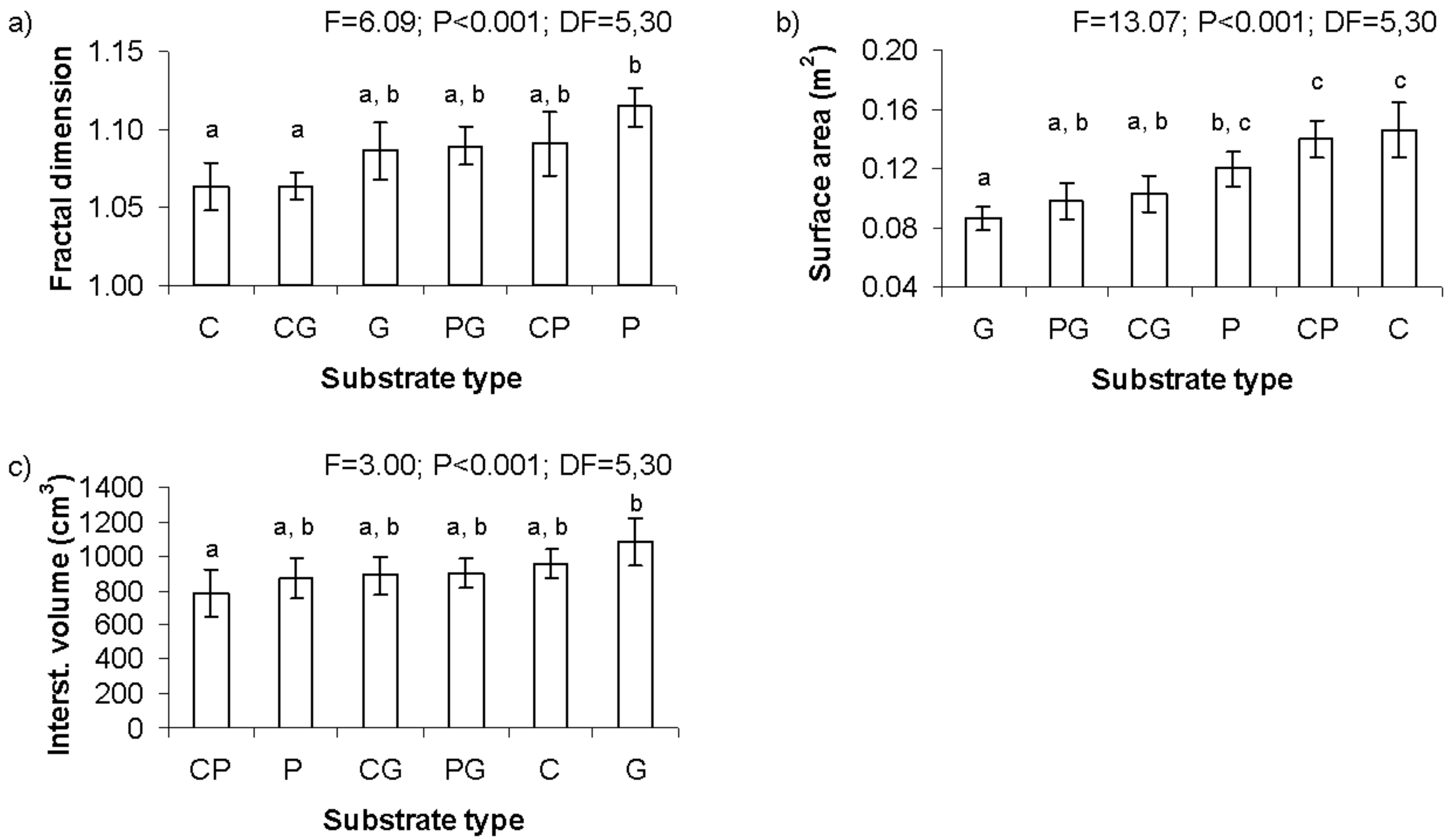

Figure 5. a) Fractal dimension, b) an index of surface area ($m^2$) and c) interstitial volume ($cm^3$) among cobbles (C), pebbles (P), gravel (G) and 50:50 mixtures. Bars represent mean values, error bars represent 95% confidence intervals. Substrate types with the same letter were not significantly different at $P=0.05$.

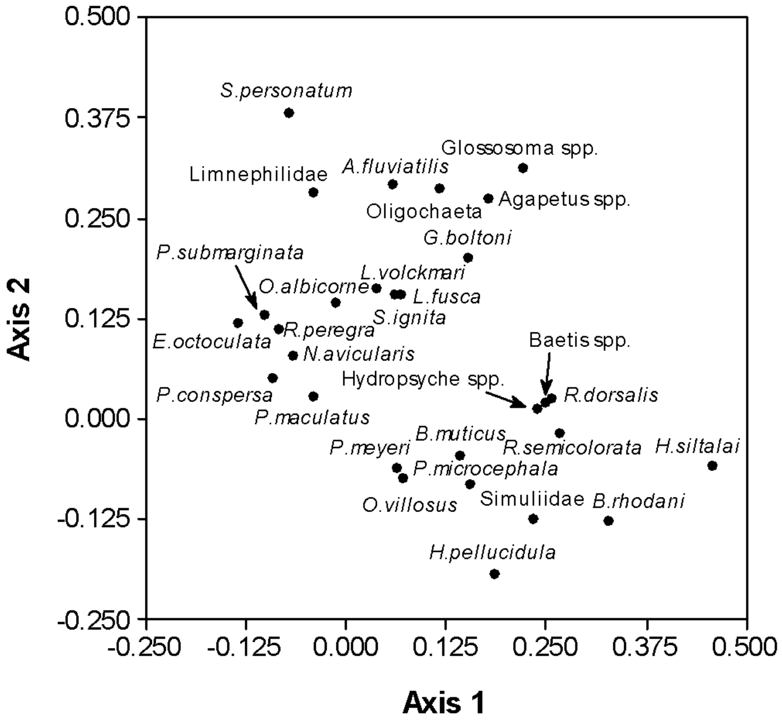

Figure 6. Principal Component Analysis (PCA) loading plot of macro-invertebrates. For clarity not all taxa are shown.

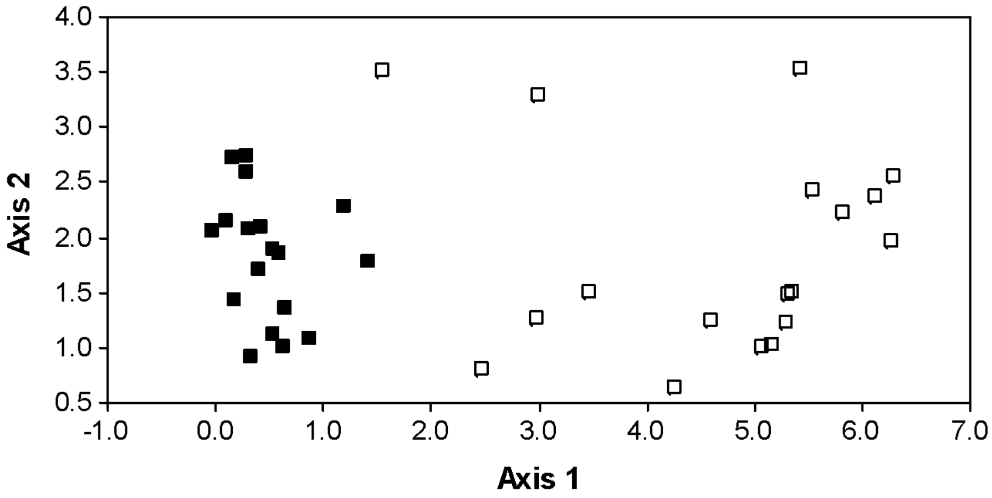

Figure 7. Principal Component Analysis (PCA) score plot with samples grouped by flow type. Glides = ■ and riffles = □.

# 5. Effects of habitat identity and heterogeneity on macro-invertebrate assemblages, nestedness and species traits in rivers

## 5.1. Abstract

1. Physical modifications can represent a major ecological impairment in rivers by affecting organisms and ecological processes. Physical habitat management has therefore become an important focus for river management and conservation, founded on the assumption that greater habitat heterogeneity supports greater biodiversity. Variations in habitat identity and heterogeneity might also affect the organisation of assemblages, for example because species are ‘nested’ among habitats or reaches. Direct evidence to support these assumptions is, however, surprisingly scarce.

2. Three hypotheses were tested at the patch and reach scale in tributaries of the Rivers Wye and Usk, mid-Wales (UK): i) mineral habitats (defined below) support macro-invertebrate assemblages of lower taxonomic diversity, richness, abundance and trait diversity, which are nested sub-sets of those in organic habitats (defined below), ii) homogeneous reaches support assemblages of lower taxonomic diversity, richness, abundance and trait diversity, which are nested sub-sets of those in heterogeneous reaches and iii) nestedness is attributed to differences in trait diversity and composition among assemblages.

3. Habitat heterogeneity (the number of patches, the number of habitats and habitat diversity) was determined for 18 x 10 m river sections. Within each section, macro-invertebrate assemblages were sampled randomly from organic (macrophytes, moss, leaf litter and tree roots) and mineral habitats (boulders, cobbles, pebbles, gravel, sand, silt and bedrock).

4. At the patch scale, some organic habitats - in particular macrophytes and moss - supported greater macro-invertebrate diversity, abundance and trait diversity, as well as disproportionally greater richness, than some mineral habitats - in particular bedrock. Macro-invertebrate assemblages were highly nested, with those occurring on bedrock,

boulders and cobbles appearing to be sub-sets of those occurring on macrophytes, moss and tree roots. Nested assemblages had lower trait diversity and altered habitat and feeding trait representation.

5. River sections containing fewer habitats supported macro-invertebrate assemblages of lower abundance, but not richness, taxonomic diversity or trait diversity, which appeared to be weakly nested sub-sets of those in heterogeneous sections. This is probably because habitats in homogeneous sections were not sub-sets of those in heterogeneous ones.

6. These results support the first and third hypotheses with two major implications for river conservation. Firstly, conserving or restoring patches of macrophytes and moss is likely to enhance local richness and prevent a reduction in trait diversity in stony-bed rivers. Secondly, restoration strategies that maximise the area of species-rich habitats are likely to enhance species richness more than those simply increasing habitat heterogeneity. Further research is required to assess the generality of these results.

## 5.2. Introduction

Rivers naturally consist of mosaics of physical habitat across a range of scales, with their heterogeneity varying laterally, longitudinally and in relation to stream size (Tockner and Ward, 1999; Ward *et al.*, 2002). Like many other ecosystems, rivers have been extensively modified as a result of widespread human influences, with activities such as channel engineering, water abstraction and flow regulation causing direct and indirect changes to river habitats (Malmqvist and Rundle, 2002). Whilst recognition of the importance of physical habitat is long-standing in river ecology (Percival and Whitehead, 1929; Whitehead, 1935; Jones, 1949), it has only become a central focus for river management and conservation in recent decades (e.g. Harper and Everard, 1998). This change of emphasis is in part due to improvements in water quality, which has typically dominated river management, highlighting the effects of changes in physical habitat on river biodiversity (Vaughan *et al.*, 2009). In Europe, the Water Framework Directive (2000/60/EC) has also been a major legislative driver, stating explicitly that hydromorphology should support 'good ecological status' (European Commission, 2000). This and parallel conservation legislation elsewhere has increased initiatives to restore rivers, a trend which is expected to continue (Lake *et al.*, 2007; England *et al.*, 2008).

Whilst some river restoration strategies aim to return rivers as closely as possible to natural conditions, the most common restoration measures involve adding woody debris, boulders or gravel to river reaches (Feld *et al.*, 2011). This strategy is based on a 'building-block' approach to enhancing habitat heterogeneity, in which different habitats are progressively added to a river channel as though they were units resembling building blocks (Petersen *et al.*, 1992). This approach is perceived as being more manageable than attempting to influence individual species or factors such as natural disturbance regime (Harper *et al.*, 1995; Palmer *et al.*, 2010). Central to this habitat-based approach is the widespread assumption that greater habitat heterogeneity supports greater biodiversity (Harper and Everard, 1998; Palmer *et al.*, 2010). Greater species richness is expected to arise from a greater range of niches (Shmida and Wilson, 1985; Townsend and Hildrew, 1994), a reduction in the likelihood of competitive exclusion (Townsend and Hildrew, 1994), provision of refugia from the effects of disturbance and predation (Townsend and Hildrew, 1994; Brown, 2003) and a greater range of food resources (Beisel *et al.*, 2000). Direct evidence to support this basic assumption remains scarce (e.g. Beisel *et al.*, 2000; Brown, 2003; Palmer *et al.*, 2010), whilst critical reviews suggest that the restoration of physical heterogeneity in rivers is not guaranteed to enhance biodiversity (Palmer *et al.*, 2010; Feld *et al.*, 2011). Closer evaluation of the underlying theory about the relationship between habitat heterogeneity and biodiversity in rivers therefore seems important to river conservation and restoration.

Alongside the requirement to re-appraise basic theory linking diversity to habitat structure in rivers is a need to incorporate recent perspectives that move beyond assemblage composition to emphasise ecological function. Reductions in species diversity are postulated to affect ecosystem functioning as species with particular traits are replaced by others (Loreau *et al.*, 2001; Palmer *et al.*, 2010). Such changes in trait composition can aid in diagnosing changes in environmental conditions whilst providing a mechanistic understanding of the factors responsible for organisms' responses. This approach is becoming increasingly widespread, particularly in Europe where trait information for macro-invertebrates is readily available (Statzner *et al.*, 2001). Underlying this approach is the hypothesis that environmental variations among habitats act as a templet onto which species traits have evolved (Southwood, 1977). In rivers, these ideas have been recast as the River Habitat Templet (Townsend and Hildrew, 1994) and combined with evidence to reveal how trait composition varies among habitats (Usseglio-Polatera, 1994; Usseglio-Polatera *et al.*, 1999). Assessments of macro-invertebrate traits in relation to habitat heterogeneity are, however, still rare (e.g. Jähnig *et al.*, 2009), despite their potential to provide insights into how habitat heterogeneity affects assemblage structure and ecological function (Beisel *et al.*, 1998).

A further important consideration in understanding habitat structure in rivers is the need to appraise how species assemblages are organised in space, and in particular the extent to which species are nested among the habitat patches that comprise reach scale mosaics. Often regarded as the inverse of beta diversity, high degrees of nestedness occur when assemblages in species-poor locations are sub-sets of those in richer locations, with evidence suggesting that this pattern might be widespread across a range of taxa and environments (Patterson and Atmar, 1986; Wright *et al.*, 1998; Leibold and Mikkelson, 2002). The effect has been attributed to a number of mechanisms including differences in extinction probabilities, variations in colonisation, gradients in habitat quality along which species vary in their precise requirements, and gradients in habitat heterogeneity (Hylander *et al*., 2005; Heino *et al*., 2010). One major implication for conservation biology is that species protection in highly nested assemblages could be optimised by preserving species-rich locations (Malmqvist and Hoffsten, 2000). More directly in relation to habitat degradation or restoration, nestedness may help to determine how habitat modification affects species diversity in any given location (Cook, 1995).

Despite long-standing recognition of nestedness in conservation biology more generally (Patterson, 1987; Cutler, 1994), there have been relatively few applications to macro-invertebrate assemblages in rivers and even fewer attempts to assess the potential mechanisms (Omesová *et al*., 2008; Heino *et al*., 2009, 2010). Most studies across ecosystems have focused on extinction and colonisation as possible underlying mechanisms whilst the importance of habitat quality or heterogeneity has received relatively little interest (Cutler, 1994; Hylander *et al*., 2005; Heino *et al*., 2010). In rivers, organic habitats such as macrophytes, moss, tree roots and woody debris could represent ‘high quality’ habitats, contributing disproportionately to macro-invertebrate species richness based on their coverage of the river channel (Harper *et al*., 1995; Shupryt and Stelzer, 2009). Relatively species-poor assemblages in ‘low quality’ mineral habitats such as bedrock or silt may therefore represent nested sub-sets of these assemblages, supporting species which vary in their precise environmental requirements. There is however also a general scarcity of studies examining whether species nestedness in macro-invertebrate assemblages is attributed to the representation of certain traits (e.g. Omesová *et al*., 2008; Heino, 2009; Heino *et al*., 2009; Larsen and Ormerod, 2010). Moreover, habitat heterogeneity could have major implications for reach scale species diversity by promoting nestedness in assemblages if homogeneous sites contain a sub-set of the habitats occurring in heterogeneous ones (Hylander *et al*., 2005).

This chapter tested the hypotheses that: i) mineral habitats support macro-invertebrate assemblages of lower taxonomic diversity, richness, abundance and trait diversity, which are nested sub-sets of those in organic habitats, ii) homogeneous reaches support assemblages of lower taxonomic diversity, richness, abundance and trait diversity, which are nested sub-sets of those in heterogeneous reaches and iii) nestedness is attributed to differences in trait diversity and composition among assemblages.

## 5.3. Methods

### 5.3.1. Study area

The Welsh Rivers Wye and Usk drain catchments of 4136 km$^2$ and 1358 km$^2$ respectively, flowing 215 km and 120 km into the Severn Estuary (Figure 1). The Wye rises in the Plynlimon Mountains (National Grid reference SN 795 864) and the Usk on the Black Mountain (National Grid reference SN 819 239).

Although the Wye catchment varies in geology, relief and land use between upper, middle and lower sub-catchments (Edwards and Brooker, 1982; Jarvie *et al.*, 2003), work for this study focused on the middle catchment draining Devonian Old Red Sandstone or marls. Land use here is dominated by rough/semi-improved pasture and tributaries are relatively un-polluted and calcareous (50-250 mg $CaCO_3$ $l^{-1}$), contrasting with the low pH of those in the acidified uplands and the moderately elevated biochemical oxygen demand (BOD) and nutrient concentrations of those in the lowlands (Ormerod and Edwards, 1987; Jarvie *et al.*, 2003; Clews and Ormerod, 2009). Average annual rainfall varies from 900-2100 mm depending on altitude (Environment Agency, 2008).

The land use and geology of the Usk catchment matches the middle Wye catchment and general water quality, BOD and nutrient concentrations are also similar (Environment Agency, 2007, 2009). River discharge varies closely with rainfall, which averages 1336 mm annually across the catchment (Environment Agency, 2007).

Fieldwork was conducted in 2008 (from September to October) at 3 x 10 m sections at three sites on the Edw, a tributary in the middle Wye catchment, and in 2009 (from July to August) at 3 x 10 m sections at single sites on the upper Usk and two of its tributaries, the Honddu and Tarrell (Table 1; Figure 1). Tributaries were selected to minimise any confounding effects of water quality, land use or channel modification and drained a mixture of rough/semi-improved pasture and broadleaved woodland.

### 5.3.2. Data collection

#### *Habitat heterogeneity*

The habitat mosaic within each river section was mapped onto a 0.25 m grid. Visual assessments of substrate size were made based on the Wentworth scale (Wentworth, 1922) whilst patches of macrophytes were classified by species and patches of moss were classified as ‘moss’.

#### *Macro-invertebrates*

Within each river section, macro-invertebrates within 0.25 x 0.25 m were kick-sampled for 1 minute using a standard net (0.25 x 0.25 m with a 1 mm mesh) at five random locations at which mineral habitats occurred and additional random locations to ensure all habitats occurring in patches covering 0.25 x 0.25 m were sampled. This resulted in a total of 153 samples of 18 habitats (bedrock=7, clay=1, silt=5, sand=4, gravel=16, pebbles=29, cobbles=47, boulders=13, leaf litter=3, tree roots=8, woody debris=1, *Callitriche* sp.=1, *Oenanthe crocrata*=1, *Mentha aquatica*=1, *Myriophyllum* sp.=1 and moss=15). Samples were preserved in 70% ethanol for subsequent analysis, when macro-invertebrates were identified to species level where possible and counted. Due to an insufficient number of samples, those from *Callitriche* sp., *Oenanthe crocrata*, *Mentha aquatica* and *Myriophyllum* sp. are subsequently referred to as ‘macrophytes’ and were treated as a single habitat type in subsequent analyses. Samples from clay and woody debris were also scarce, and were excluded from subsequent analyses unless otherwise stated.

### 5.3.3. Data analysis

#### *Macro-invertebrates*

Total abundance, taxonomic richness and taxonomic diversity (using Shannon’s diversity index) were calculated from the raw macro-invertebrate data. Trait diversity for each sample was calculated based on available information for 13 biological species traits (Table 2) (Schmidt-Kloiber and Hering, 2011 and references therein). Group-wide averages were used for taxa identified above species level. Fuzzy coding was used to determine the affinity of each taxon for the categories of each trait and each affinity score re-scaled as a proportion of 1 (Chevenet *et al.*, 1994). For each sample, affinity scores were then multiplied by log(x + 1) abundance of each taxon and the sum of taxon affinity scores for each category of each trait used to produce an abundance-weighted trait profile (Dolédec *et al.*, 2000; Archaimbault *et al.*, 2005). Trait diversity for each sample was calculated as the average diversity (using Shannon's diversity index) across traits (Larsen and Ormerod, 2010).

### ***Macro-invertebrates among habitats***

Differences in abundance, taxonomic diversity and trait diversity among organic and mineral habitats were analysed using General Linear Mixed Models (GLMMs) in ASReml version 3 (VSN International Ltd, 2009). Abundance was normalised using a logarithmic transformation prior to analysis. A random effect was used to control for dependence on site. Models were checked for validity through assessment of standardised residuals and refined using stepwise deletions at the P=0.05 level of significance. In models where habitat was significant, differences in macro-invertebrates among pairs of organic and mineral habitats were analysed using Wald tests.

To assess whether organic habitats supported disproportionately greater macro-invertebrate species richness, a sample-based rarefaction curve (Gotelli and Colwell, 2001) was generated for each habitat by randomising sample order 100 times using the vegan package (Oksanen *et al.*, 2010) in R version 2.9.2 (R Development Core Team, 2009). Rarefaction rather than accumulation curves based on a single order of samples were used to avoid variations in the shape of the curve resulting from sample order and produce smooth curves facilitating comparisons among habitats (Colwell and Coddington, 1994; Gotelli and Colwell, 2001). Differences in rarefaction curves among organic and mineral habitats were analysed using General Linear Models in R following a reciprocal transformation to linearise the relationship. Rarefied taxonomic richness was compared between the sampled area of organic habitats (0.0625 $m^2$) and areas of mineral habitats up to five times as large (0.325 $m^2$) using the contrast package (Kuhn, 2009) in R.

Patterns of macro-invertebrate nestedness were determined using the binary matrix nestedness temperature calculator (BINMATNEST) (Rodríguez-Gironés and Santamaría, 2006). BINMATNEST calculates the temperature (T) of a presence-absence species matrix by re-ordering rows and columns to maximise nestedness. For perfectly nested matrices, in which species-poor assemblages are perfect sub-sets of richer assemblages and rare species are only found in rich assemblages, T=0 ˚C, whilst for totally random matrices where species are often unique to individual assemblages, T=100 ˚C (Cook, 1995; Rodríguez-Gironés and Santamaría, 2006). The temperature method is insensitive to matrix size and the original ranking of samples (Cook, 1995; Wright *et al.*, 1998). The statistical significance of T was determined from comparisons with 400 random matrices generated using null model 3, in which the probability that a cell in a random matrix is occupied equals the average occupancy of its row and column in the actual matrix (Rodríguez-Gironés and Santamaría, 2006). This null model is the most conservative but reduces Type I errors - incorrectly rejecting the null hypothesis (Rodríguez-Gironés and Santamaría, 2006). To determine whether the macro-invertebrate assemblages of mineral habitats were nested sub-sets of those from organic ones, differences in average sample rank in the maximally packed matrix among habitats were analysed using a Kruskal-Wallis test followed by Mann-Whitney tests among pairs of organic and mineral habitats. P=0.002 (=0.05/28) was used as the critical level of significance for Mann-Whitney tests to reduce the chance of Type I errors. Relationships between nestedness and habitat heterogeneity (see below) were analysed using Spearman's rank correlations. P=0.017 (=0.05/3) was used as the critical level of significance to reduce the chance of Type I errors. To assess whether nestedness in macro-invertebrate assemblages was attributed to differences in trait diversity and composition, sample rank was related to trait diversity and the proportions of selected microhabitat/substrate preferences and feeding types using Spearman-rank correlations. P=0.05/number of categories for a particular trait was used as the critical level of significance to reduce the chance of Type I errors.

### ***Habitat heterogeneity***

Habitat maps were digitised in ArcMap version 9.2 using ArcScan (ESRI, 2006). Patch Analyst version 4.2 (Rempel *et al.*, 2008) was used to calculate the number of patches, the number of habitats and habitat diversity (using Shannon’s diversity index) within each river section.

### *Relationships between habitat heterogeneity and macro-invertebrates*

Relationships between the number of patches, the number of habitats and habitat diversity within a river section and macro-invertebrates, including from clay and woody debris, were analysed using GLMMs using the package asreml version 2 (Butler, 2006) in R. A random effect was used to control for dependence on river section as well as pseudoreplication. Models were checked for validity through assessment of standardised residuals and refined using stepwise deletions at the P=0.05 level of significance.

## 5.4. Results

### *Macro-invertebrates among habitats*

Macro-invertebrates varied significantly among habitats based on several metrics (Table 3). Taxonomic diversity was significantly greater on tree roots, macrophytes and moss than on boulders or bedrock; leaf litter than on boulders; tree roots than silt but also gravel than in moss (P<0.05) (Table 4). Abundance was significantly greater on macrophytes and moss than in mineral habitats, and in leaf litter and tree roots than on bedrock and sand (P<0.05). Trait diversity was significantly greater on leaf litter, tree roots, macrophytes and moss than on bedrock, and on macrophytes and moss than in pebbles, cobbles and boulders (P<0.05). No other differences in macro-invertebrates among organic and mineral habitats were significant.

Rarefaction curves, showing the accumulation of species with increasing area, varied significantly among habitats (F=499.85; P<0.001; DF=10, 129) (Figure 2). Sample-based rarefied taxonomic richness was significantly greater on macrophytes than in areas of bedrock, silt, sand and boulders five times as large, cobbles three times as large and gravel and pebbles twice as large (P<0.05). Richness in moss was significantly greater than on areas of bedrock five times as large, silt and boulders three times as large, sand twice as large and in equivalent areas of gravel, pebbles and cobbles

($P<0.05$). Richness in leaf litter was significantly greater than on areas of bedrock four times as large and in equivalent areas of silt, sand and boulders ($P<0.05$) but less than equivalent areas of gravel and pebbles ($P<0.05$) and not significantly different from an equivalent area of cobbles ($P=0.11$). Richness in tree roots was significantly greater than on areas of bedrock four times as large, boulders twice as large and in equivalent areas of silt, sand and cobbles ($P<0.05$) but less than equivalent areas of pebbles ($P<0.05$) and not significantly different from an equivalent area of gravel ($P=0.38$).

### *Nestedness*

Macro-invertebrate assemblages were highly nested ($T=7.3$ ˚C; $P<0.001$) (Figure 3), with sample rank varying significantly among habitats ($H=54.06$; $P<0.001$; $DF=10$) (Table 5). Boulders, cobbles and pebbles supported assemblages that were apparently nested sub-sets of those on macrophytes whilst bedrock, boulders and cobbles had nested assemblages compared to moss ($P<0.002$). Bedrock supported assemblages that were nested compared to tree roots ($P<0.002$). Assemblages from river sections with fewer habitats were apparently weakly nested sub-sets of those from river sections with a greater number of habitats ($r_s=-0.32$; $P<0.001$; $n=151$), however there was no correlation between sample rank and the number of patches ($r_s=-0.04$; $P=0.654$; $n=151$) or habitat diversity ($r_s=0.00$; $P=0.973$; $n=151$).

Nested assemblages had significantly lower trait diversity than species-rich assemblages ($r_s=-0.63$; $P<0.001$; $n=151$) and were characterised by a greater representation of taxa preferring coarse gravel to cobbles, whilst richer assemblages were characterised by a greater representation of taxa preferring algae and woody debris (Table 6). Richer assemblages were also characterised by a greater representation of miners, xylophagous taxa, filter feeders, predators and parasites (Table 6).

### *Relationships between habitat heterogeneity and macro-invertebrates*

River sections containing a greater number of habitats supported assemblages that were greater in abundance but not taxonomic diversity, richness or trait diversity, than homogeneous ones (Table 7). Macro-invertebrate assemblages were unrelated to the number of patches or habitat diversity.

## 5.5. Discussion

Several important results emerged from this study, providing support for the first and last hypothesis but only limited support for the second. Firstly, some organic habitats - in particular macrophytes and moss - supported greater macro-invertebrate diversity, richness, abundance and trait diversity than some mineral habitats - in particular bedrock. Secondly, macro-invertebrate assemblages appeared to be highly nested among habitats. The combination of nestedness and trait analysis also revealed that nestedness may be attributed to differences in trait diversity and composition, with nested assemblages having reduced trait diversity and altered habitat and feeding trait representation. Finally, despite differences in macro-invertebrates among habitats, habitat heterogeneity had no effect on macro-invertebrate diversity, richness or trait diversity, and in general did not promote nestedness in species assemblages. The implications of these results are discussed below.

Previous studies have shown that both macrophytes (Percival and Whitehead, 1929; Wright *et al*., 1983, 1992; Armitage and Cannan, 2000; Harrison, 2000) and moss (Percival and Whitehead, 1929; Brusven *et al*., 1990; Suren, 1991; Downes *et al*., 1995; Beisel *et al*., 1998; Cattaneo *et al*., 2004) support greater abundances and richness of macro-invertebrates than a number of mineral habitats. Leaf litter and tree roots are also important habitats for macro-invertebrates, with tree roots supporting greater macro-invertebrate richness and abundance than a range of mineral habitats (Beisel *et al*., 1998), whilst leaf litter enhances the abundance of some species (Egglishaw, 1964; Palmer *et al*., 2000). In this study, leaf litter and tree roots supported similar, if not lower, diversity, richness and abundance than gravel, pebbles and cobbles, however bedrock, silt, sand, bedrock and boulders were typically characterised by lower richness and abundance in agreement with previous studies (Percival and Whitehead, 1929; Hynes, 1970). In addition, the results suggest that macrophytes and moss in particular supported disproportionately greater macro-invertebrate richness than areas of mineral habitats several times as large, in agreement with previous studies (Harper *et al*., 1995; Shupryt and Stelzer, 2009). Greater trait diversity on macrophytes and moss compared to most mineral habitats, and in leaf litter and tree roots compared to on bedrock, is likely to reflect the greater range of niche opportunities for organisms. For example, whilst macrophytes provide a food resource for shredders and miners (Harper *et al*., 1995; Harrison, 2000), leaves and stems also accumulate periphyton and particulate organic matter providing food for other functional feeding groups which also occur in other habitats (Diehl, 1992; Harper *et al*., 1995; Harrison, 2000; Wright and Smock, 2001). Moss also accumulates a large amount of detritus as well as acting as a direct food source (Glime and Clemons, 1972; Suren, 1991; Suren and Winterbourn, 1992; Beisel *et al*., 1998; Clenaghan *et al*., 1998).

Macro-invertebrate assemblages showed a highly nested structure despite use of the most conservative null model for significance testing, with assemblages occurring on bedrock, boulders and cobbles appearing to be sub-sets of those occurring on macrophytes, moss and tree roots. Such a low matrix temperature is unusual for aquatic invertebrates, which generally exhibit a higher matrix temperature than vertebrate or terrestrial organisms due to their diverse composition and varied responses to environmental conditions (Boecklen, 1997; Wright *et al*., 1998; Malmqvist and Hoffsten, 2000; Omesová *et al*., 2008; Heino *et al*., 2009, 2010). Nestedness appeared to be attributed to differences in trait diversity and composition among assemblages, with reduced trait diversity and altered representation of some traits in nested assemblages possibly reflecting a reduced range of food resources in species-poor, typically mineral habitats. Supported by evidence that taxonomic richness and trait diversity was greater in organic habitats compared to some mineral habitats, these results indicate that the loss or modification of species-rich habitats in rivers is likely to be accompanied by altered or impaired ecological function. Furthermore, these results suggest that differences in habitat quality may influence nestedness in macro-invertebrates, in agreement with previous studies in rivers (Omesová *et al*., 2008; Heino *et al*., 2010), lakes (Heino and Muotka, 2005) and ponds (McAbendroth *et al*., 2005). Variations in environmental conditions among habitats were not measured directly, and other mechanisms such as colonisation-extinction dynamics may however be important (Heino *et al*., 2009, 2010).

Greater heterogeneity theoretically increases the range of ecological niches and reduces the likelihood of competitive exclusion enabling a greater number of species to co-exist (Shmida and Wilson, 1985; Townsend and Hildrew, 1994), increases the availability of refugia ameliorating the effects of disturbance and predation (Townsend and Hildrew, 1994; Brown, 2003), and increases the range and abundance of food resources (Beisel *et* *al*., 2000). Based on these assumptions it was hypothesised that river sections of greater habitat heterogeneity might, on average, support assemblages of greater taxonomic diversity, richness and trait diversity. However, these results support an increasing number of studies which suggest that habitat heterogeneity is not guaranteed to increase biodiversity (Palmer *et al*., 2010; Feld *et al*., 2011). Furthermore, whilst different habitats have been shown to support assemblages with specific groups of species traits (Townsend and Hildrew, 1994; Usseglio-Polatera *et al*., 1999), the implication is that enhancing habitat heterogeneity may not increase trait diversity and subsequently ecological function. These findings are probably attributed to the fact that habitats occurring in homogeneous river sections were not perfect sub-sets of those in heterogeneous ones, with species-rich organic habitats occurring in homogeneous river sections and some heterogeneous river sections containing a greater number of species-poor mineral habitats compared to homogenous ones. As a result, the assemblages from homogeneous river sections were at most weakly nested sub-sets of the assemblages occurring in heterogeneous ones, indicating that there may be high beta diversity among river sections and that homogeneous reaches may therefore make an important contribution to gamma diversity at the catchment scale.

Caution is required in extrapolating the results to the reach scale and beyond because they are based on patch scale samples. The sampling strategy employed did not permit pooling of samples to determine macro-invertebrate metrics for each river section and therefore further studies are required to determine the effect of habitat heterogeneity on macro-invertebrates at the reach scale, as well as the contribution of different habitats based on their relative area. Furthermore, only a low number of samples were obtained from some habitats, which for mineral habitats such as clay reflected their low frequency of occurrence and coverage, but for organic habitats was also an artefact of the sampling strategy.

These results therefore provide support for the first and last hypothesis with two major implications for river conservation. Firstly, conserving or restoring patches of macrophytes and moss is likely to preserve local richness in stony-bed rives and prevent a reduction in trait diversity with likely consequences for ecosystem processes (Hooper *et al*., 2005; Cardinal *et al*., 2006). Secondly, restoration strategies that maximise the area of species-rich habitats may enhance species richness more than those simply increasing habitat heterogeneity. However, since not all species will be supported by the most species-rich habitats, or heterogeneous reaches, river management should still maintain habitat and reach variety. Further research is required to assess the generality of these results.

In summary, macrophytes and moss in particular supported greater macro-invertebrate diversity, abundance and trait diversity, as well as disproportionally greater richness, than some mineral habitats, in particular bedrock. Macro-invertebrate assemblages appeared to be highly nested among habitats, with nested assemblages having lower trait diversity and altered habitat and feeding trait representation. Finally, habitat heterogeneity had no effect on macro-invertebrate diversity, richness or trait diversity, and in general did not promote nestedness in species assemblages.

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## 5.7. Tables and Figures

Table 1. Location of 10 m river sections on tributaries of the Rivers Wye and Usk.

| River | Tributary | 10 m section | Grid reference |
|---|---|---|---|
| Wye | Edw-site 1 | 1 | SO 11825 57329 |
| | | 2 | SO 11673 57140 |
| | | 3 | SO 11635 57052 |
| | Edw-site 2 | 4 | SO 11440 54270 |
| | | 5 | SO 11508 54153 |
| | | 6 | SO 11601 53966 |
| | Edw-site 3 | 7 | SO 09926 48026 |
| | | 8 | SO 09716 47876 |
| | | 9 | SO 09621 47845 |
| Usk | Usk-site 1 | 1 | SN 81761 26868 |
| | | 2 | SN 81917 26979 |
| | | 3 | SN 81960 27054 |
| | Honddu-site 1 | 1 | SO 02961 35463 |
| | | 2 | SO 03206 35271 |
| | | 3 | SO 03239 35251 |
| | Tarrell-site 1 | 1 | SO 01268 26914 |
| | | 2 | SO 01042 26958 |
| | | 3 | SO 00936 26964 |

Table 2. Species traits and categories.

| Trait | Categories |
|---|---|
| Microhabitat/substrate preference | mud (grain size < 0.063 mm)<br>silt, loam, clay (grain size < 0.063 mm)<br>sand (grain size 0.063-2 mm)<br>fine to medium-sized gravel (grain size 0.2-2 cm)<br>coarse gravel to hand-sized cobbles (grain size 2-20 cm)<br>stones, boulders, bedrock (grain size > 20 cm)<br>algae<br>macrophytes<br>particulate organic matter<br>woody debris<br>edge of water bodies, moist substrates |
| Current preference | limnophil<br>limno to rheophil<br>rheo to limnophil<br>rheophil<br>rheobiont<br>indifferent |
| Feeding type | grazers/scrapers<br>miners<br>xylophagous taxa<br>shredders<br>gatherers/collectors<br>active filter feeders<br>passive filter feeders<br>predators<br>parasites<br>other |
| Respiration | tegument<br>gill<br>plastron<br>spiracle (aerial)<br>hydrostatic vesicle (aerial)<br>tapping of air stores of aquatic plants<br>extension/excursion to surface |
| Locomotion type | swimming/skating<br>swimming/diving<br>burrowing/boring<br>sprawling/walking<br>(semi) sessile<br>other |
| Dispersal capacity | low<br>high<br>unknown |
| Dissemination strategy | aquatic passive<br>aquatic active<br>aerial passive<br>aerial active |

Table 2 continued.

| **Trait** | **Categories** |
|---|---|
| Life duration | ≤1 year<br>>1 year |
| Reproduction | ovoviviparous<br>free isolated eggs<br>cemented isolated eggs<br>fixed clutches<br>free clutches<br>clutches in vegetation<br>terrestrial clutches<br>asexual<br>parasitic |
| Reproductive life cycles per year | semivoltine<br>univoltine<br>bivoltine<br>trivoltine<br>multivoltine<br>flexible |
| r- K- strategy | r-strategist<br>K-strategist |
| Resistance form | eggs, statoblasts<br>cocoons<br>housings against desiccation<br>diapause or dormancy<br>quiescense<br>none |

Table 3. Significance of differences in taxonomic diversity, log-transformed abundance and trait diversity among habitats.

| Model | F | P | DF |
|---|---|---|---|
| Taxonomic diversity | 6.44 | <0.001 | 10, 104 |
| Log(abundance) | 9.46 | <0.001 | 10, 104 |
| Trait diversity | 3.57 | <0.001 | 10, 104 |

Table 4. Predicted taxonomic diversity, back-transformed abundance and trait diversity among habitats ± 95% confidence intervals, with lower/upper confidence intervals given for back-transformed abundance. B=bedrock, Si=silt, Sa=sand, G=gravel, P=pebbles, C=cobbles, Bo=boulders, L=leaf litter, T=tree roots, Ma=macrophytes and Mo=moss.

| Model | Mineral habitats | | | | | | | Organic habitats | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | Si | Sa | G | P | C | Bo | L | T | Ma | Mo |
| Taxonomic diversity | 1.19±0.34 | 1.23±0.39 | 1.69±0.42 | 1.97±0.23 | 1.87±0.19 | 1.82±0.16 | 1.08±0.25 | 1.70±0.48 | 2.01±0.31 | 1.80±0.44 | 1.61±0.24 |
| Abundance | 10±5/12 | 89±54/136 | 18±11/31 | 64±30/57 | 78±33/59 | 66±27/45 | 37±18/35 | 93±63/193 | 54±29.63 | 415±268/762 | 264±126/240 |
| Trait diversity | 0.63±0.10 | 0.92±0.11 | 0.77±0.12 | 0.79±0.08 | 0.75±0.06 | 0.74±0.05 | 0.72±0.08 | 0.87±0.14 | 0.85±0.13 | 0.82±0.13 | 0.85±0.07 |

Table 5. Median sample rank in the maximally packed matrix among habitats. Values given in brackets are the range.

| B | Si | Sa | G | P | C | Bo | L | T | Ma | Mo |
|---|---|---|---|---|---|---|---|---|---|---|
| 141 (46) | 85 (106) | 101 (72) | 73 (127) | 51 (139) | 88 (131) | 131 (113) | 74 (99) | 53 (109) | 3 (12) | 23 (99) |

Table 6. Spearman's rank correlation coefficients ($r_s$) between the representation of trait categories and sample rank (n=151) in the maximally packed matrix. Significant correlations at the P value corrected for the number of trait categories are shown in bold.

| **Trait** | **Categories** | **$r_s$** | **P** |
|---|---|---|---|
| Microhabitat/substrate preference | silt, loam, clay | -0.098 | 0.234 |
| | sand | -0.195 | 0.017 |
| | fine to medium-sized gravel | 0.093 | 0.260 |
| | coarse gravel to hand-sized cobbles | **0.280** | **<0.001** |
| | stones, boulders, bedrock | 0.186 | 0.023 |
| | algae | **-0.270** | **<0.001** |
| | macrophytes | -0.196 | 0.016 |
| | particulate organic matter | -0.106 | 0.195 |
| | woody debris | **-0.286** | **<0.001** |
| Feeding type | grazers/scrapers | 0.101 | 0.216 |
| | miners | **-0.419** | **<0.001** |
| | xylophagous taxa | **-0.431** | **<0.001** |
| | shredders | 0.048 | 0.560 |
| | gatherers/collectors | 0.032 | 0.700 |
| | active filter feeders | **-0.327** | **<0.001** |
| | passive filter feeders | **-0.270** | **<0.001** |
| | predators | **-0.432** | **<0.001** |
| | parasites | **-0.408** | **<0.001** |

Table 7. Relationships between macro-invertebrates and the number of patches, number of habitats and habitat diversity within 10 m river sections. Only significant fixed effects are shown.

| **Model** | **Fixed effects** | **Coefficient (± 95% confidence interval)** | **F** | **P** | **DF** |
|---|---|---|---|---|---|
| Taxonomic diversity | NS | - | - | - | - |
| Richness | NS | - | - | - | - |
| Log(abundance) | Number of habitats | 0.104±0.09 | 5.69 | 0.030 | 1, 151 |
| Trait diversity | NS | - | - | - | - |

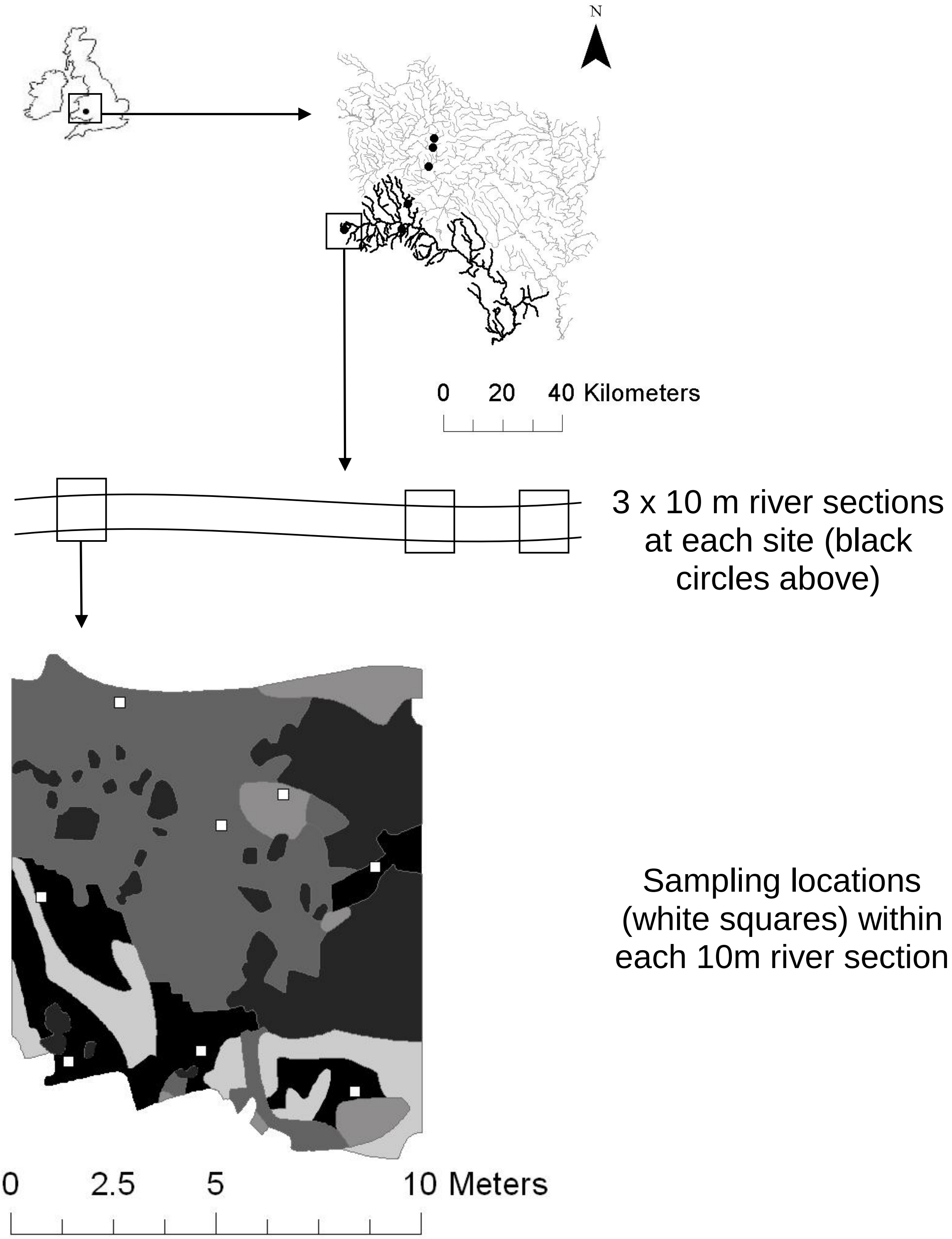

Figure 1. Sampling design showing 0.25 x 0.25 m sampling locations (white squares) within 10 m river sections nested within sites (black circles) within the catchments of the Rivers Wye and Usk (shown in bold).

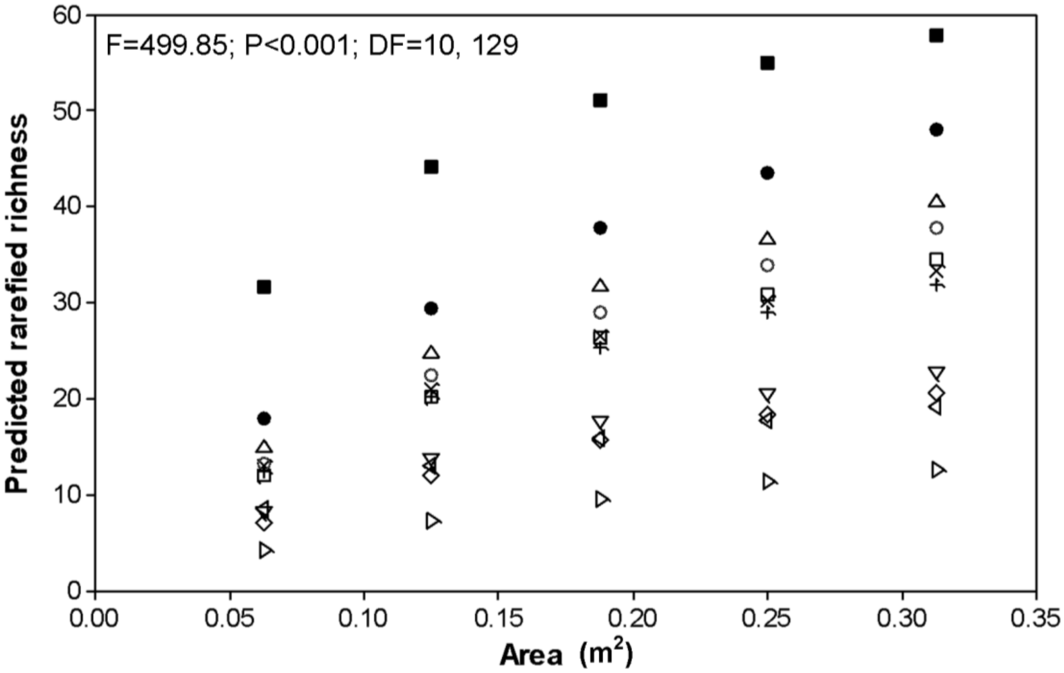

Figure 2. Predicted sample-based rarefaction curves among habitats. Bedrock = ▷, silt = ◁, sand = ▽, gravel = ○, pebbles = △, cobbles = □, boulder = ◊, leaf litter = +, tree roots = x, macrophytes = ■ and moss = ●. For clarity confidence intervals are not shown.

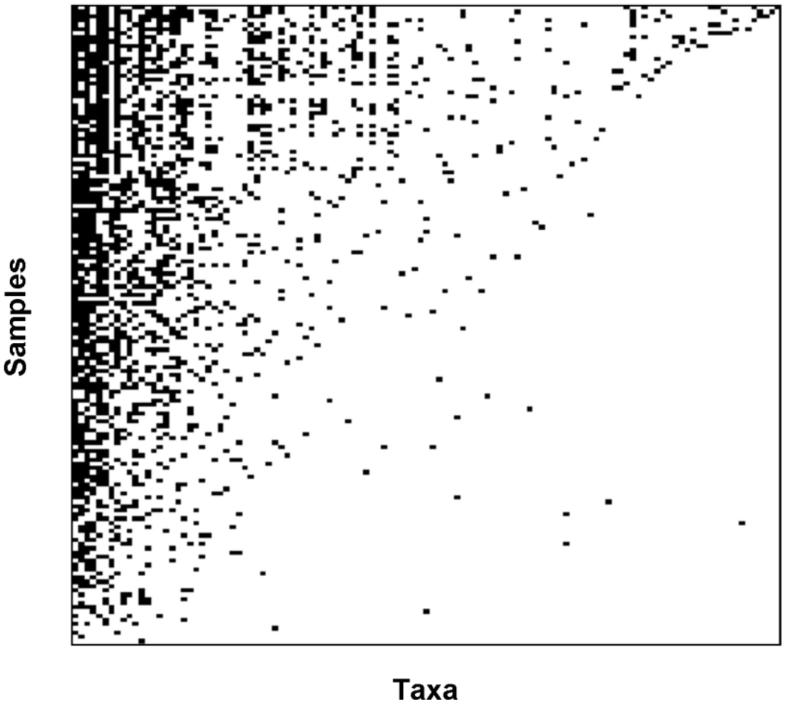

Figure 3. Matrix of samples x taxa packed to maximise nestedness (i.e. minimise unexpected presences and absences). Black squares represent presence.

# Chapter 6 General Discussion

## 6.1. Synthesis

Despite evidence that more structured habitats support greater biodiversity in some terrestrial and aquatic ecosystems (Bell *et al*., 1991; Tews *et al*., 2004), previous studies investigating habitat structure in rivers have reported mixed effects with increased complexity and heterogeneity sometimes giving rise to increased species richness but sometimes not (e.g. Downes *et al*., 1995; Beisel *et al*., 2000). Whilst such conflicting results call into question basic ecological theory about the effects of habitat structure on organisms and ecological processes, a simpler explanation is that habitat structure is difficult to quantify, which has also been a major obstacle to making general conclusions regarding its effects. These ideas formed the wider context for this thesis, which quantified basic aspects of the relationship between benthic habitat structure and macro-invertebrates using a combination of field surveys and a field experiment. It also provided one of the first studies attempting explicitly to identify, and then separate as far as possible, potential confounding effects on macro-invertebrates from other sources of variation among habitats of contrasting physical structure.

On a more detailed point, this study was also among the first to quantify the complexity of natural river bed surfaces using fractal dimension which, despite some drawbacks (discussed below), provides a measure that is not habitat-specific and therefore permits comparison among studies and ecosystems. Although fractal dimension can provide an ecologically relevant assessment of habitat complexity in freshwaters, one of the main findings of Chapter 3 was that variations in surface complexity only had weak effects on macro-invertebrates, and only when habitat type was ignored. Effects were independent of surface area, but overall surface complexity was a minor determinant of variations in macro-invertebrate diversity, richness and abundance among habitat types indicating that other variations at this scale, for example in hydraulic conditions or resource distributions, must play an important role in determining the distribution of organisms. Whilst differences in macro-invertebrate assemblages among mineral habitats are long-established (e.g. Percival and Whitehead, 1929), many previous studies have either not assessed the contribution of habitat structure to variations in assemblages among habitat types or not fully accounted for other factors that might confound structural effects in such variable environments as rivers.

The second of these points became particularly apparent in the survey results from Chapter 3, where it appeared likely that the effects of habitat structure on macro-invertebrates might be overridden by variations in local flow pattern. The importance of flow has long been established in rivers, for example being used to explain greater species diversity in substrates located in riffles (gravel/pebbles/cobbles) compared to those more typically found in glides and pools (sand/silt) (Hynes, 1970). Chapter 4 therefore aimed to assess such a possibility experimentally - surprisingly one of the few experimental studies attempting to separate habitat structure and flow effects on macro-invertebrates. Despite creating highly significant variations in surface complexity similar to those among natural substrates, the results were entirely consistent with field observations from Chapter 3. Instead, variations in flow appeared to explain differences in macro-invertebrate assemblages. The implication is that observed differences in assemblages among mineral habitats in rivers result from flow-related effects or processes rather than habitat structure *per se*. Selective filtering of organisms based on their body size appeared to be involved, consistent with the likely evolutionary effects of such a major aspect of river ecosystems. This does not mean that habitat structure has no importance to benthic organisms, but any such effects may be secondary to conditions, processes or resource distributions related to flow pattern among habitat types.

In addition to the above themes, this thesis provided an assessment of how macro-invertebrate assemblages in rivers are organised spatially. Chapter 5 provided evidence that macro-invertebrate assemblages may be highly nested among river habitat types, even when judged against a conservative null model for significance testing. In other words, the species found in the least rich habitat types in rivers, often those with mineral composition, appeared to be sub-sets of those found in the most species rich habitats, usually composed of organic materials such as macrophytes. This contrasts with previous studies, which have reported only weak nestedness, at best, despite being based on more liberal null models (Heino, 2009). The combination of nestedness and trait analysis also revealed that nested assemblages had reduced trait diversity and altered trait representation. Supported by evidence that taxonomic richness and trait diversity was greater in organic habitats compared to some mineral habitats, in particular bedrock, these results indicate that the loss or modification of species-rich habitats in rivers is likely to be accompanied by altered or impaired ecological function.

Despite variations in macro-invertebrate assemblages among habitat types, measures quantifying the composition and configuration of habitats consistently had no effect on macro-invertebrates at the patch (Chapter 3) or section (Chapter 5) scale. Direct evidence to support the widespread assumption that greater habitat heterogeneity supports greater biodiversity remains scarce and the results of this study highlight the need for further evaluation of the underlying theory (Palmer *et al*., 2010). Explanations for the absence of this relationship in rivers are generally unknown, but may reflect the fact that habitat heterogeneity is not measured in an ecologically relevant way or that larger scale factors are of greater importance (Heino, 2009).

## 6.2. Limitations and caveats

Despite similarities between the surveys and experiment in the apparent responses of macro-invertebrates to habitat structure at the patch and section scale, like all studies there were limitations with the approach taken that require brief discussion. Whilst the combination of field surveys and an experiment provided both *realism* in measurements from actual benthic habitats (survey) and *control* of some factors (experiment), caveats include: i) the time-scale over which studies could be undertaken, ii) variations that were beyond experimental control, iii) limitations in measurements and sampling strategy, iv) experimental artefacts and v) limitations in scaling-up from the work done (patch to section) to the scales emphasised in management (reach to catchment).

### 6.2.1. Timescales

Patterns in the distribution of organisms in rivers develop over periods ranging from very short (< days) to very long (seasons, years, decades or much longer) and the processes involved are equally short- to long-term. Seasonal variations in the distinctiveness of habitats and the distribution of habitat patches, as well as factors such as the availability of food resources, will also affect organisms. The results from Chapters 3 and 5 represent only a snapshot of the habitat structure available to organisms, and of their distributions within it. Particular difficulties in this study were also weather related, with exceptionally high summer flows in 2008 delaying initial fieldwork until September. Since many insects have univoltine life cycles and summer emergence periods some species may have emerged as adults by this time (Hynes, 1970).

### 6.2.2. Uncontrollable variations

There are likely to be uncontrolled variations in environmental conditions even in the best-designed ecological experiment, and this generates variations among experimental units and restricts the extent to which results can be extrapolated. One specific issue also arose in the field survey. Following a survey of a tributary of the River Wye in 2008, a second survey was conducted on the neighbouring River Usk catchment to increase the natural range of habitats and habitat heterogeneity encountered. Despite the selection of tributaries of similar geology, water chemistry and land use, this inevitably introduced additional variation in macro-invertebrate assemblages, most clearly seen in assemblage composition among sites and catchments in Chapter 3. However, unlike the majority of previous studies, this thesis considered whether habitat structure was significant after variation among sites was taken into account.

### 6.2.3. Measurement and sampling limitations

As discussed above, a general limitation of studies investigating habitat structure arises from the fact that it is difficult to quantify. The main drawback of the methods used in Chapters 3 and 4 is that surface complexity and area were derived from two-dimensional profiles, whilst organisms and their habitats are distinctly three-dimensional. As discussed in Chapters 3 and 4, profiles are unable to capture overhangs or the three dimensional shape of crevices or interstitial spaces and may not be representative of the surface as a whole (Sanson *et al.*, 1995). In the field survey, there were also difficulties obtaining profiles from fine substrates such as silt. As an index, fractal dimension also suffers from the disadvantage that surfaces with the same value may provide a different number of crevices or spaces of different sizes (Sanson *et al.*, 1995). Furthermore, the sampling strategy adopted resulted in a varying and in some cases low number of samples among habitats, which may have reduced the sensitivity of the analyses used in Chapters 3 and 4 and restricted possible analyses in Chapter 5.

### 6.2.4. Experimental artefacts

Caveats associated with the experiment, including those discussed above, were discussed in Chapter 4, and care is needed in transferring the results to real field circumstances because they are limited in both spatial and temporal extent. Time constraints resulted in the survey being conducted during late summer, which as discussed above is likely to have affected assemblage composition as well as body size distribution (Hynes, 1970). Limited colonisation time did however not appear to result in the absence of taxa found at the study site during a similar period in the previous year (Chapter 3). Furthermore, generated variations in fractal dimension and surface area were similar to those among natural substrates.

### 6.2.5. Issues of scaling-up

This is another widespread limitation in ecological research, which is typically carried out at small extents (< metres) and small timescales (days to months) whilst ecological management and restoration must address larger and longer-term factors. In this study, habitat structure was investigated at the patch and section scale to improve understanding at the scale of individual organisms, but also to inform river management and restoration at the reach scale. As discussed in Chapter 5, caution is required in extrapolating the results to the reach scale as they are based on patch scale samples. Furthermore, factors varying among sites and catchments, as well larger scale factors acting beyond the catchment, were not investigated.

## 6.3. Implications for river management and conservation

Notwithstanding the above limitations, the results of this thesis have several important implications for river management and conservation. Dominant themes are that: i) management or restoration of habitat heterogeneity might not guarantee increased biodiversity, ii) management to restore key habitat types might deliver biodiversity gains and iii) flow types should be more fully integrated into management for hydromorphology.

### 6.3.1. Management or restoration of habitat heterogeneity

The absence of a clear link between habitat heterogeneity and biodiversity could be one of the most important and far-reaching implications of this work. Although this inference depends on being able to scale-up results from patch scale to reach scale or beyond, as well as from macro-invertebrates to other groups of organisms, it is supported by an increasing array of field studies and reviews that also suggest that habitat heterogeneity is not guaranteed to increase biodiversity (Palmer *et al*., 2010; Feld *et al*., 2011). Limitations in the way and scale at which previous studies have however quantified heterogeneity mean that improved field evaluations are likely to be required. Furthermore, these conclusions do not refute the suggestion that the physical structure of river systems *per se*, as encompassed by hydromorphology, is important for biodiversity.

### 6.3.2. Management to deliver biodiversity gains

In contrast to habitat heterogeneity, evidence from Chapter 5 suggests that certain organic habitat types support greater macro-invertebrate diversity, abundance and trait diversity, as well as disproportionately greater macro-invertebrate richness, than particular mineral habitats. Conserving or restoring the most species-rich habitats would therefore be expected to deliver biodiversity enhancement on a reach-to-reach basis and prevent a loss of trait diversity with possible consequences for ecological function. The results from Chapter 5 also indicate that maximising the area of species-rich habitats should be considered. Two important caveats before implementing this idea are that: i) further data are required from other locations and river types to confirm the generality of these conclusions and ii) the absence of perfect nestedness among habitats will mean that not all species will be supported by the most species-rich habitats, so that management should still maintain habitat variety.

### 6.3.3. Management for hydromorphology

Evidence from both survey and experimental approaches of the apparent effects of flow type on macro-invertebrate assemblages illustrates the importance of velocity, near-bed hydraulics or related factors to benthic organisms. Whilst the Water Framework Directive (2000/60/EC) emphasises hydromorphology in supporting 'good ecological status', emphasis so far has been placed on structural aspects of rivers (European Commission, 2000). The results of this study support the view that management for flow pattern, for example through careful abstraction licensing and flow regulation, should be integrated into hydromorphology more fully when considering river management for organisms (Vaughan *et al.*, 2009).

## 6.4. Wider ecological implications

The results reported in this thesis not only contribute to understanding of the importance of habitat structure in rivers but also have broader relevance because the basic theory being tested has significance for ecology in general. Rivers are among the most highly structured ecosystems making them a highly valuable system in which to examine the ecological effects of physical structure. Care is however needed in extrapolating the results from rivers to other ecosystems because heterogeneity is so marked. Notwithstanding these points, there are some important general implications. Firstly, as discussed in Chapter 1, research in other ecosystems might benefit from a standardisation of the definition and measurement of habitat structure, and the methods used here have some strengths and weaknesses. Secondly, variations in species and body size distributions cannot be assumed to result from variations in habitat structure unless other factors known to influence organisms among habitats are excluded. Factors that confound the effect of habitat structure are likely to occur also in other ecosystems and include variations in surface area. These, and other themes, will be developed further during the publication of the results from this thesis.

## 6.5. Future research needs

Future research needs identified above and in individual chapters can be broadly categorised as: i) methodological improvements and ii) improvements in study design.

### 6.5.1. Methodological improvements

Characterisation of the three-dimensional structure available to organisms within a particular habitat would provide a much better understanding of the importance of habitat complexity. One approach would be to generate Digital Elevation Models of the river bed surface. Whilst this could be achieved using a greater density of pin profile data, more sophisticated methods are now being developed which can characterise three-dimensional habitat structure in fine detail. One of these is digital photogrammetry, which involves taking a pair of photographs at a set distance apart to provide a stereo image of the river bed surface. The feasibility of this approach has already been demonstrated for natural river beds and river bed casts although a complex correction is required for the effects of refraction at the water surface (Butler *et al.*, 2002). An alternative method is laser altimetry or Light Detection And Ranging (LiDAR), which directly measures three-dimensional topography (Lefsky *et al.*, 2002). Whilst terrestrial scanners typically have wavelengths of 900-1064 nm, green LiDAR (also known as bathymetric LiDAR) can measure elevations under shallow water using wavelengths near 532 nm, although there are issues such as irregular reflectance (Lefsky *et al.*, 2002; Marcus and Fonstad, 2010). Whilst both approaches require specialist software, possible measurements of surface structure are subsequently limitless and include roughness measurements and surface fractal dimension (e.g. Sun *et al.*, 2006).

Developments in such remote sensing technologies over the last two decades mean that mapping spatial and temporal variations in river ecosystems at the reach and catchment scale are now possible (e.g. Marcus and Fonstad, 2010). Potential applications include measuring river bed elevation at the reach scale (e.g. Lane, 2000) and automated mapping of habitat distribution. Wider application of such approaches would greatly improve understanding of the dynamic contribution of different habitats and habitat heterogeneity to reach scale diversity. Furthermore, this could greatly improve understanding of the limitations of river restoration schemes, where larger scale factors may override any local improvements and the permanence of increases in habitat heterogeneity may be variable (Palmer *et al.*, 2010).

### 6.5.2. Improvements in study design

Few studies have assessed whether relationships between habitat structure and macroinvertebrates determined by small scale, predominantly experimental, studies are replicable or have predictive capability at different locations or among river types (e.g. Downes *et al.*, 2000). Future studies should consider the importance of multiple scale factors, as well as location within the spatial structure of the river network, in determining the distribution and diversity of organisms (Grant *et al.*, 2007; Brown *et al.*, 2011). Manipulations of habitat heterogeneity at the reach scale would also provide an important advancement.

Both alpha and beta diversity, and their contribution to gamma diversity, should be considered in river conservation (Heino, 2009; Clark *et al.*, 2010). As identified in Chapter 5, further studies are required to determine the contribution of different habitats to diversity at the reach scale, as well as the contribution of reaches of different heterogeneity to catchment scale diversity. The partitioning of diversity across spatial scales, such that gamma diversity at one scale becomes alpha diversity at a larger scale, would enable such an assessment (Stendera and Johnson, 2005). Such an approach is highly amenable to studying the organisation of species diversity in rivers since they are hierarchically nested systems (Frissell *et al.*, 1986; Clark *et al.*, 2010).

Finally, despite the potential of species traits to provide a mechanistic understanding of the response of organisms to habitat structure and an assessment of its importance to ecological function, studies in rivers have been dominated by a taxonomic approach. Further research is required to determine if habitat complexity *per se* has effects on species traits other than body size, and whether modification or enhancement of habitat heterogeneity has effects on trait representation and diversity at the reach scale. This may also provide a transferable understanding of the species at risk from habitat modification among geographic regions (Larsen and Ormerod, 2010). Given that river restoration may act as a disturbance, and that species in restored reaches may be filtered based on the possession of traits enabling persistence, a trait-based approach could also benefit the development of river restoration targets (Tullos *et al.*, 2009).

## 6.6. Conclusions

Despite quantifying variations in physical structure, habitat complexity and heterogeneity had consistently weak effects on macro-invertebrates at the scales studied and were only a minor determinant of variations in macro-invertebrates among mineral habitat types. Instead, flow type explained variations in macro-invertebrate richness, abundance and composition, and appeared to filter organisms based on their body size. Macro-invertebrate assemblages occurring in some mineral habitats, typically with lower macro-invertebrate diversity, richness, abundance and trait diversity, appeared to be nested sub-sets of those occurring in organic habitats. Nested assemblages also had reduced trait diversity and altered trait representation. Finally, this thesis demonstrates the benefits of combining field surveys and experiments to identify, and where possible separate, the ecological effects of confounding physical factors, and future improvements in methodology and study design will further benefit river management and conservation as well as ecology in general.

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