The relationships between stressors, macroinvertebrate community structure and leaf processing in stream ecosystems.

By Joanne B. Harkness

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Summary.

In the face of major threats to global biodiversity, and in order that ecosystem managers might act to reduce the impacts of anthropogenic stressors on ecosystems, it is critical that we understand how ecosystem structure and function respond to stressors. I focussed on investigating the relationship between macroinvertebrate community structure and function, measured as the rate of leaf processing. An initial meta-analysis of previous studies revealed no association between structure and function in streams exposed to three distinct pollutant stressors (heavy metal contamination, acidification and organic pollution). Interpretation of patterns was hindered by low sample sizes, and so a field study was conducted to clarify patterns in response to heavy metal contamination. Stream sites were located in Cornwall and Lanarkshire. Associations between structure and function were driven by the direct effects of stressors in Cornwall, but not in Lanarkshire. The results indicate that the only way to assess function effectively in natural streams may be to make direct assessment of functional aspects of the system, in addition to structural assessment.

Experimental stream mesocosms were used to determine whether structure reveals function, in so much that the rates of leaf processing by mixed-species assemblages were predictable from the rates of species in isolation. Rates of leaf processing were greater than predicted, indicative of complementarity between shredder species. Finally, species-specific feeding trials were used to determine the effect of fungal species richness on rates of leaf processing by macroinvertebrate shredders. While there was some evidence for complementarity between fungal species, which resulted in increases in leaf processing between 1 and 3 fungal species, overall there was no effect of increasing fungal species richness. Results of both experimental studies indicate that the relationship between structure and function is idiosyncratic. The implication of this for the management of freshwater ecosystems is that it is difficult to predict the consequences of species' losses for ecosystem processes.

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1. General introduction.

1.1. Introduction.

1.1.1. The issue: human activities are affecting ecosystems.

Recent expansion of the human population is associated with an increase in human domination of the Earth's ecosystems (Cohen 1996; Vitousek *et al.* 1997; Palmer *et al.* 2004). Humans are causing adverse effects on ecosystems through the exploitation of wild living resources, expansion of agriculture, forestry and aquaculture, habitat loss and fragmentation, pollution of soil, water and atmosphere, and global climate change (McNeely *et al.* 1995). These human activities are changing the biotic structure and composition of ecological communities, through species extinction or the introduction of exotic species (Ehrlich & Wilson 1991; U.N.E.P. 1995; Chapin III *et al.* 2000; Hooper *et al.* 2005). Current extinction rates are estimated to be up to four orders of magnitude greater than any reasonable estimate of background (pre-human) rates (Lawton & May 1995; Pimm *et al.* 1995).

Biodiversity loss is tragic for its ethical and aesthetic value alone (Gaston & Spicer 1998). However, in addition, ecologists have emphasised that because the magnitude of these changes are so large (Pimm *et al.* 1995) and because biodiversity is linked to ecosystem processes (such as production, carbon storage, hydrological and nutrient cycling) (Chapin III *et al.* 1997; Tilman *et al.* 1997a) these kinds of alterations may in turn affect the provision of the goods and services that ecosystems provide to humanity (Daily 1997; Sala *et al.* 2000; Palmer *et al.* 2004; Worm *et al.* 2006). Examples of key ecosystem goods include: the provision of food, medicines, and industrial materials. Key ecosystem services include: nutrient cycling, soil formation and maintenance, atmospheric regulation, climatic regulation, hydrological regulation, pest control, photosynthesis, and pollination (Gaston & Spicer 1998). The estimated monetary value of the goods and services provided by ecosystems exceeds US $ 33 trillion per annum (Constanza *et al.* 1997). This alone provides a strong incentive to understand the nature of the relationship between biodiversity and ecosystem functioning.

In order to afford protection of biodiversity and the provision of key ecosystem goods and services we need to assess and monitor the impacts of human activities, and further understanding of the mechanisms and circumstances under which diversity influences ecosystem properties (Hooper *et al.* 2005). This information will be important in making future management and policy decisions (Sutherland *et al.* 2006).

The central goal of this study was to examine the effects of anthropogenic impacts ('stressors') on the relationship between biodiversity ('structure') and ecosystem processes ('function') in freshwater stream ecosystems. In the following sections I summarise current research on the relationship between structure and function (Sections 1.2. and 1.3.), and examine why it is important to extend this work to study of the effects of stressors on the relationship (Section 1.4). Subsequently, I introduce the freshwater ecosystem study system (Section 1.5), draw attention to the pure and applied research goals which need addressing (Section 1.6.), and finish by outlining the specific objectives of this study (Section 1.7.).

1.1.2. General definitions: stress, structure and function.

The three central terms discussed in this thesis merit clear definition from the outset. 'Stressors' are considered to be physical or chemical perturbations to a system that are either: a) foreign to that system or b) natural to the system but applied at an excessive, or deficient, level (Barrett *et al.* 1976), which may cause significant changes in the ecological components, patterns and processes of the system. Examples include anthropogenic acidification, drought events, water abstraction, land-use change, and water and air pollution (Vinebrook *et al.* 2004).

Biotic 'structure' refers to the composition of the taxonomic groups such as fish, algae, or macroinvertebrates, relating primarily to the kinds and number of organisms in the group. Much of the previous research that has examined the relationship between structure and function has used the term 'biodiversity' to describe the structural part of the relationship, e.g. the 'biodiversity - ecosystem function' debate (Loreau *et al.* 2002). 'Biodiversity' refers to the extent of genetic, taxonomic and ecological diversity over all spatial and temporal scales (Harper & Hawksworth 1994). For the purpose of this study 'structure' is used

interchangeably with 'biodiversity', since measurable parameters of both terms are the same, e.g. species richness, relative abundances and biomass. Often these different parameters are correlated, e.g. biomass and relative abundance. The most frequently used measure of biodiversity is 'species richness', which is the number of species present in a habitat.

'Ecosystem functioning' is, in the general sense, an aggregate property of the rate and stability of ecosystem-level processes (e.g. fluxes of materials and energy among compartments) and properties (e.g. pools of materials such as carbon and organic matter) (Hooper *et al.* 2005). Ecosystem functioning is commonly quantified through measurement of a process, such as primary productivity, rate of decomposition and nutrient leaching.

1.2. The relationship between structure and function.

Since ecosystem processes involve organisms, it is logical to ask whether there is any relationship between the structure of the community and the processes ('function') of an ecosystem. Clearly a minimum composition of organisms is required to maintain relationships between the primary producers, consumers and decomposers that mediate the flow of energy and cycling of nutrients in ecosystems (Folke *et al.* 1996). In the face of major threats to biodiversity, attention has been drawn to the more general problem of whether any loss of species has the potential to alter ecosystem function.

1.2.1. Species richness and ecosystem functioning.

1.2.1.1. History of academic interest.

Darwin (1859) is the first to have documented the suggestion that there might be a relationship between biodiversity and ecosystem functioning. Darwin was inspired by an early plot experiment that examined the agro-ecological effects of intercropping to improve the yield of terrestrial grassland systems (Hector & Hooper 2002): "*it has been experimentally proved that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera*

of grasses, a greater number of plants and a greater weight of dry herbage can thus be raised" (Darwin 1859).

It was not until the middle of the 20th century that interest in the relationship grew. Authors of this era postulated a variety of reasons why the rates of various community and ecosystem processes, and particularly the stability of the community and its function (Section 1.2.3.), might depend on biodiversity (Odum 1953; MacArthur 1955; Elton 1958). The concepts were mainly developed verbally, and field evidence in support of the concepts came from informal comparisons of habitats (e.g. of tropical *vs.* temperate habitats, of islands *vs.* mainland). In contrast, May (1972) used mathematical models to show that multi-species communities with a greater richness of species were expected to be less stable than less diverse, or less complex, systems (Section 1.2.3.). This work stimulated research on the relationship between community stability and complexity (McNaughton 1978; Pimm 1979; Ehrlich & Ehrlich 1981; King & Pimm 1983; Pimm 1984). However, the focus was on the stability of populations and communities, and rather little work was done on the effects on other ecosystem processes.

Major interest in the relationship between species richness and ecosystem processes began with a conference in 1991 (Schulze & Mooney 1993). Since this point there has been a dramatic increase in the number of research papers addressing the issue (Naeem *et al.* 2002; Balvanera *et al.* 2006), stimulated by concern for the loss of biodiversity and associated impairment of ecosystem function caused by human activities (Tilman 1999). The major question driving this work is whether species-rich ecosystems are more capable of maintaining ecosystem processes than species-poor ones.

1.2.1.2. Central hypotheses.

The central questions research in this area has focussed on addressing are: 1) is there a relationship between biodiversity and ecosystem processes? 2) Is the relationship positive or negative? 3) What is the shape of the relationship? (Schläpfer & Schmid 1999). Consideration of these questions has lead to the proposition of several hypothetical relationships between biodiversity and

ecosystem processes (Figure 1.1) (Naeem *et al.* 2002). Five central classes of hypotheses can be identified: a) the null hypothesis, b) the linear hypothesis, c) the redundancy hypothesis, d) the rivet hypothesis, e) the idiosyncratic hypothesis. On all graphs, the first black point (at the origin) is the point at or near zero biodiversity where there is no ecosystem functioning, because, as previously stated, there is a minimum required level for a process to occur at all. The second black point is the natural level of biodiversity in the absence of anthropogenic impacts.

The null hypothesis (Figure 1.1a) predicts that there is no effect of variation in biodiversity on ecosystem processes and therefore a slope of zero for the trajectory between low biodiversity and the natural level of biodiversity (Vitousek & Hooper 1993). The linear hypothesis (Figure 1.1b) assumes that all species contribute equally to ecosystem processes and therefore predicts that the addition or loss of species causes proportional changes in ecosystem processes. The 'redundancy' hypothesis (Figure 1.1c) assumes that species are a least partially substitutable in terms of their contribution to ecosystem processes, such that the loss of some species is compensated for by other species, or the addition of such species adds nothing new to the system. This predicts a positive asymptotic relationship between biodiversity and ecosystem function (Lawton & Brown 1993). The 'rivet' hypothesis (Figure 1.1d) reflects the notion that redundancy is important to the point where once so many species are lost, the system fails (Ehrlich & Ehrlich 1981). Finally, the 'idiosyncratic' (Figure 1.1e) hypothesis assumes that species impacts are context-dependent (for example, on the composition of the remaining community, local nutrient levels or disturbance regime) and therefore unpredictable in their contribution to ecosystem processes (Lawton 1994). This predicts no simple relationship between biodiversity and ecosystem processes, and the slope of the hypothetical trajectory changes along the biodiversity gradient.

The hypotheses outlined above have provided a useful conceptual framework from which to examine the relationship between biodiversity and ecosystem functioning, encompassing both the question of whether a relationship exists at all (Figure 1.1a & e *vs.* b, c & d) and what form any relationship takes (b, c or d).

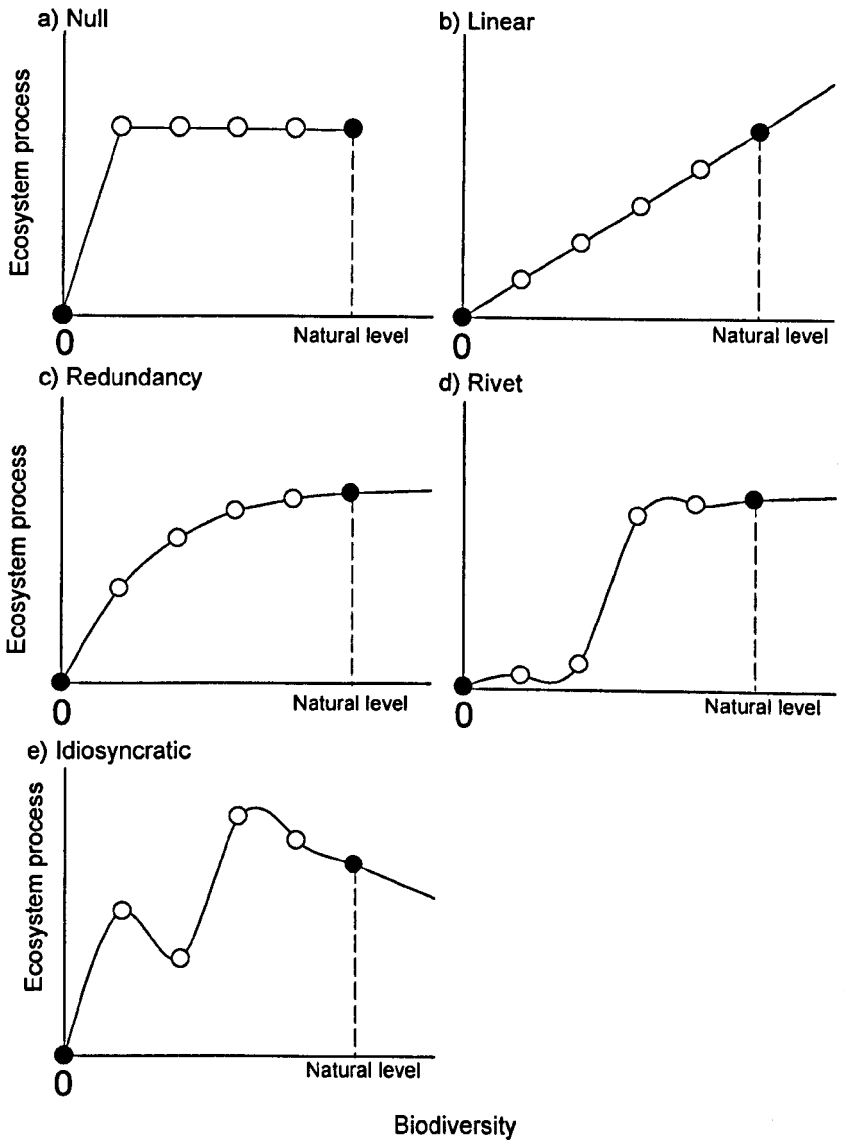

Figure 1.1: Graphical representation of the five central hypotheses of the relationship between biodiversity and ecosystem processes: (a) the null hypothesis; (b) the linear hypothesis; (c) the redundancy hypothesis; (d) the rivet hypothesis; (e) the idiosyncratic hypothesis (reviewed by Naeem *et al.* 2002) (see text). The black dot at the origin represents that where there are no species then there can be no process. The second black dot represents the rate of processing at the natural level of biodiversity (see dotted line). White dots represent different levels of species richness either as a result of extinction (moving to the left) or invasion (moving to the right).

1.2.1.3. Empirical studies.

Empirical studies (i.e. studies involving actual observation or manipulation, as opposed to theoretical or review studies) now constitute around 40 % of the studies published on biodiversity – ecosystem function relationship, considerably more than ten years ago (Balvanera *et al.* 2006). Extensive reviews of these studies already exist (Kinzig *et al.* 2001; Loreau *et al.* 2002; Hooper *et al.* 2005; Balvanera *et al.* 2006; Cardinale *et al.* 2006) and so here I simply summarise some important points from this work which are relevant to the ideas and experiments used in this study.

The commonest approach has been to create a gradient of biodiversity under homogeneous extrinsic conditions (e.g. fertility, climate, space, history) and monitor a variety of ecosystem function response variables. Other experiments have manipulated species richness indirectly through varying site environmental conditions (nutrient level, successional stage). A recent meta-analysis found no significant difference between those experiments where biodiversity was manipulated either directly or indirectly (Balvanera *et al.* 2006).

The majority of studies have been performed in terrestrial systems (predominantly grasslands systems) far outweighing those in aquatic ecosystems (Balvanera *et al.* 2006; Cardinale *et al.* 2006). Around 326 studies have been performed in terrestrial ecosystems, *versus* 32 in marine and 68 in freshwater (Balvanera *et al.* 2006). The relative paucity of studies performed in aquatic ecosystems is in spite of the huge genomic diversity in oceans and freshwaters, compared to land, and considerable value in the ecosystem goods and services provided (Hendriks *et al.* 2006), as well as their being the more extensive habitat on Earth (Covich *et al.* 2004).

Contention exists as to the most important measure of biodiversity to manipulate when designing an experiment. For example, species richness, evenness, or 'functional group'[1] richness (Loreau *et al.* 2001a; Hooper & Dukes 2004; Petchey & Gaston 2006; Wright *et al.* 2006). The predominant view is that functional

[1] A functional group is a group of species that play a similar functional role in a specific ecosystem process (Naeem *et al.* 2002).

group richness is more important than species richness (Balvanera *et al.* 2006). Species richness has been the most frequently used measure of biodiversity: species richness (393 studies), evenness (11 studies), diversity indices (19 studies) and functional group richness (23 studies) (Balvanera *et al.* 2006).

Of those studies which have manipulated biodiversity as species richness, the predominant taxonomic group has been primary producers (42 % of studies) as herbaceous plants (35 %). While other taxonomic groups have received less attention: animals (31 %), of which most are arthropods (arachnids, insects, crustaceans and gastropods: 30 %), bacteria (2 %), fungi (14 %) and protists (11 %) (Cardinale *et al.* 2006).

Most of the studies that have manipulated biodiversity have focussed on horizontal (i.e. competitive) interactions among terrestrial primary producers (i.e. within a trophic level) (Lehman & Tilman 2000; Balvanera *et al.* 2006). More recently aquatic ecologists have recognised the importance of considering vertical (i.e. predatory) as well as horizontal interactions (i.e. within trophic levels) (McGrady-Steed *et al.* 1997; Naeem & Li 1997; Petchey *et al.* 1999; Downing & Leibold 2002; Paine 2002; Petchey *et al.* 2004). Integrating horizontal and vertical effects of diversity will enable us to better understand the effects of biodiversity loss and implications for the functioning of complex ecosystems (Duffy *et al.* 2007). Of 446 records included in a recent quantitative review, only 5 were multi-trophic (Balvanera *et al.* 2006), and only one single study measured ecosystem functioning after manipulation of a primary consumer community mediated through a secondary consumer (Montoya *et al.* 2003; but see also Lecerf *et al.* 2005).

1.2.1.3.1. General patterns observed.

Recent quantitative reviews of the literature indicate significantly positive effects of biodiversity on ecosystem processes (Balvanera *et al.* 2006; Cardinale *et al.* 2006). There is clear indication that further biodiversity loss can be expected to compromise the provision of ecosystem service delivery (Balvanera *et al.* 2006). However, there are still strong arguments (Emmerson *et al.* 2001; Raffaelli *et al.*

2002; Duffy 2003; Covich *et al.* 2004) and evidence to suggest (Cardinale *et al.* 2006) that the consequences of biodiversity loss are idiosyncratic, differing quantitatively and qualitatively between trophic groups and ecosystems.

Reviews have highlighted the importance of species identity in its contribution to ecosystem functioning (Hooper *et al.* 2005), leading researchers to begin to address the importance of species identity effects on ecosystem functioning (Section 1.3). For example, an additional analysis has recently been performed on the data from the BIODEPTH project (Hector *et al.* 1999; Spehn *et al.* 2005), on the importance of single species to a multitude of ecosystem functions (Hector & Bagchi 2007).

There has been debate over the interpretation of much of the evidence (Grime 1997; Huston 1997; Tilman *et al.* 1997b; Wardle *et al.* 1997b; Allison 1999; Naeem 1999; Huston *et al.* 2000; Fukami *et al.* 2001; Wardle 2001; Hooper *et al.* 2005; Srivastava & Velland 2005). Issues that have arisen focus around some key areas relevant for this study. Firstly, the effect of experimental design on differences in the shape of the observed relationships (Allison 1999; Schmid *et al.* 2002). Secondly, the relevance of experiments to natural systems (see Section 1.2.1.3.2.) and to conservation (Srivastava 2002; Srivastava & Velland 2005). Thirdly, separation of, and the relative importance of, the mechanisms underpinning a positive biodiversity – function relationship (see Section 1.2.3.) (Balvanera *et al.* 2006).

1.2.1.3.2. The relevance of patterns observed for natural systems.

One of the biggest problems of interpretation of studies is that biodiversity has been manipulated through the random assembly of species, from systems subject to minimal environmental variability. In 'real' systems the effects of species on ecosystem processes are likely to depend upon individual species traits, such that the pattern of species loss is non-random (Raffaelli 2004). For example, in freshwater ecosystems, macroinvertebrate community structure responds predictably to environmental gradients (Hämäläinen & Huttunen 1996; Wright *et al.* 2000) (see Section 1.5.). The kinds of species traits which affect the pattern of species extinction reflects differences in body size, trophic position, habitat

specialization, physiology, morphology, life history, and how they respond to stressors and the environment (Tilman & Lehman 2001; Raffaelli 2004). Future research would benefit from generating a better understanding of the roles which certain species play in communities, and specifically whether the traits which determine vulnerability to extinction are related to functional dominance in communities (Cardinale *et al.* 2006).

In most studies with manipulated biodiversity, biodiversity has been reduced to a single number, for example species richness, whereas, natural communities are dominated by a few common species while the remainder remain rare. This has resulted in many of the experiments having higher species evenness than is encountered in natural systems (Schwartz *et al.* 2000; Wardle 2002). There have been a few studies which have examined the degree to which evenness has influenced ecosystem properties and these are restricted to terrestrial grassland ecosystems (Wilsey & Potvin 2000; Wilsey & Polley 2002; Polley *et al.* 2003), with the exception of one freshwater study (Dangles & Malmqvist 2004), which found that rates of ecosystem processing varied depending on the identity of the dominant processing species (see also Section 1.3.1.).

1.2.2. Mechanisms which underpin a positive relationship.

One of the most contentious issues emerging from research in this area has been whether widely observed patterns seen in experimental systems of increases in ecosystem processes with increases in the number of randomly assembled species (see previous discussions in Section 1.2.) are the result of positive interactions between species (i.e. the 'complementarity' effect: see Section 1.2.2.2.), or are the result of chance inclusion of dominant and highly productive species (i.e. the 'sampling' effect: see Section 1.2.2.1.) (Aarssen 1997; Grime 1997; Huston 1997; Tilman *et al.* 1997a), and the relevance of these patterns to natural systems (Loreau 2000; Fridley 2001; Lepš *et al.* 2001). In the following sections (Sections 1.2.2.1.-3.) I illustrate that the proposed mechanisms by which species richness can affect ecosystem functioning all relate strongly to the functional attributes of species (Giller *et al.* 2004). As such, species richness matters because species differ in their traits. This implies that species composition may be as important in the determination of rates of ecosystem processes as species richness (Aarssen

1997; Huston 1997; Tilman *et al.* 1997c; Tilman 1999). Despite this, few, if any, studies have manipulated community composition in the absence of manipulation of species richness, and measured changes in the rates of ecosystem processing.

1.2.2.1. Sampling effect.

The 'sampling' effect is the "*increasing probability of selecting species with a specific property (e.g. large maximum height, stress tolerance, nitrogen-fixing ability, high seed germination rate) in samples of increasing number that are randomly selected for any group of species*" (Huston 1997). The mechanism derives from the idea that one or a few species may have a large effect on any given ecosystem process, meaning that species rich communities are more likely to contain a single species with extreme traits which could become dominant and drive ecosystem functioning (Aarssen 1997; Huston 1997; Tilman *et al.* 1997a).

The sampling effect is likely to play a role in most experiments with manipulated species richness (Tilman *et al.* 1997a), although its existence in natural communities is debated (Fridley 2001; Cardinale *et al.* 2006). This is because it is considered by some to be a statistical artefact of random sampling (Huston 1997). Wardle (1999) pointed out that if sampling effects occur in natural communities we would need to assume that communities of species are randomly assembled with regard to their "*relative effect on the ecosystem function being investigated... and that whatever species were lost from an ecosystem were lost at random with respect to these effects*". In reality, communities are not usually random assemblages of species, and species are not lost at random (Lepš *et al.* 2001). Lepš *et al.* (2001) then went on to argue that "*complete randomness is* [not] *necessary for the sampling effect to manifest itself...* [and] *it is sufficient that the traits differ among the species in a community.*" In essence, this highlights the importance of considering differences in species traits, when deciding whether or not the sampling effect is a possible mechanism in a system; the greater the dissimilarity between species, the greater chance that the sampling effect will occur.

1.2.2.2. Complementarity effects.

'Complementarity effects' result when "*the inclusion of specific groups of two or more species that interact positively either through facilitation, or through complementarity among groups of two or more species (i.e. niche effects) to produce more biomass* [or higher rates of ecosystem processes] *than a smaller number of species could produce* (Huston 1997; Tilman *et al.* 1997c; Loreau 2000; Fridley 2001)" (Huston & McBride 2002). Complementarity is not in itself a mechanism, but rather a property of a set of species (Petchey 2003). The two kinds of mechanisms which underpin complementarity effects (i.e. facilitation and niche differentiation) are discussed in the following sections.

1.2.2.2.1. Facilitation.

'Facilitation' was first defined for the purpose of intercropping studies (Vandermeer 1989) as "*the circumstance where a species modifies the environment in a way favourable to a co-occurring species*" (Fridley 2001). In terms of resource-use between species, facilitation occurs when one species modifies a resource in a way favourable to another co-occurring species, such that overall resource-use is increased when certain combinations of species occur together. For example, the fertilization effect of a nitrogen-fixing legume on a grass in a nitrogen limited environment (Grime 1997; Hooper & Vitousek 1997; Huston 1997; Huston *et al.* 2000; Loreau 2000), or when the feeding action of a certain species of leaf eating detritivore increases the leaf surface area available for other species (Jonsson *et al.* 2002). Facilitation predicts that ecosystem function will increase asymptotically with species richness, as the strength of species facilitative interactions increases, and then saturates (Tilman *et al.* 1997a; Loreau 1998b; Tilman 1999; Loreau 2000).

Species interactions can be negative as well as positive. Negative interaction, such that one species modifies a resource in a way that is unfavourable to another co-occurring species, is called 'inhibition'. For example, certain species of fungi (aquatic hyphomycetes) can modify the surface of leaf material (through emission chemicals) inhibiting growth of bacterial, in addition to having anti-fungal properties (Gulis & Stephanovich 1999).

1.2.2.2.2. Niche differentiation.

Niche differentiation (otherwise known as 'resource-use complementarity') occurs because not all species are equal in their requirements for a resource. According to niche theory there must be some partitioning of resources between species in order for them to co-exist (Giller 1984). Examples of the kinds of resources which may be partitioned between species include time, space and food (Schoener 1974). Examples of resource partitioning include, distinct size classes of algae consumed by different cladoceran species (Norberg 2000) and interspecific variation in net sizes by net-spinning caddis larvae in streams (Hildrew & Edington 1979). Theory suggests that if species differ in their resource-use in at least one dimension, they may be complementary. As such, each single species can utilize a certain portion of a resource, but no single species can utilize the whole range of resources. Where this happens, the greater the number of species in an assemblage, the greater overall resource will be utilized (Tilman *et al.* 1997c; Loreau 1998b; Tilman 1999; Loreau 2000). Like facilitation, this also predicts a saturating relationship between biodiversity and ecosystem functioning.

1.2.2.3. Distinguishing between mechanisms.

It is very difficult to separate the relative contributions of facilitation and niche differentiation experimentally (Loreau & Hector 2001). However, methods have been developed to aid in the separation of sampling and complementarity effects. Both the sampling effect and complementarity effects produce a decelerating species richness-ecosystem functioning relationship under some conditions, so it is not possible to distinguish between the two based on the shape of the relationship alone (Petchey 2003). Distinguishing among the different mechanisms requires comparison of individual species' performances in monoculture with that of polycultures. The most unambiguous evidence for the existence of complementarity effects is the detection of 'transgressive overyielding' (Trenbath 1974; Loreau 1998a; Hector *et al.* 2002). Transgressive overyielding occurs when the observed response for a polyculture is greater than that for the monoculture with the greatest response, an effect that can only be brought about by complementarity between species and cannot be brought about through the sampling effect (Loreau 1998b). Empirical tests for transgressive overyielding include studies by Hector *et al.* (2002), Špaèková & Lepš (2001),

Wardle *et al.* (1997a), Dang *et al.* (2005) and Cardinale *et al.* (2006). It is also worth noting that sampling and complementarity effects are not mutually exclusive and may operate simultaneously (Lepš *et al.* 2001).

1.2.3. The relationship between biodiversity and ecosystem stability.

Theoretical work on the relationship between biodiversity and stability has outpaced experimental work, especially field research. May (1972) used theoretical models to examine the relationship between biodiversity and ecosystem stability. Since this, debate into the relationship has deepened and developed (Pimm 1979; 1984; McCann 2000). The focus has been around whether or not increasing species richness or 'complexity' begets greater community stability. Experimental studies have examined the relationship between species richness and various measurable properties of ecosystem stability, including invasion resistance, resistance of above-ground biomass or other ecosystem processes (Schläpfer & Schmid 1999; Schmid *et al.* 2001; Loreau *et al.* 2001b). More recent reviews indicate that increasing species richness should enhance ecosystem stability (Palmer *et al.* 2004; Hooper *et al.* 2005; Balvanera *et al.* 2006).

Interest has been in whether ecosystem properties become less predictable and more variable as species are lost from a system (Loreau *et al.* 2001b). The 'Insurance Hypothesis' (Yachi & Loreau 1999) proposes that "*biodiversity buffers ecosystem processes against environmental changes, because different species or phenotypes respond differently to the changes, leading to functional compensations among species or phenotypes, and hence more predictable aggregate community or ecosystem properties*" (Loreau *et al.* 2001b). As such, species that are functionally redundant for a given ecosystem process at a given time show temporal niche differentiation (see Section 1.2.2.2.2.). This hypothesis might explain how it is possible for the loss of species from ecosystems due to anthropogenic stressors to be associated with no change in the rate of an ecosystem process, because other species provide insurance against this loss, thus maintaining rates of processing.

1.3. Species identity and composition effects on ecosystem functioning.

1.3.1. Species identity.

Controversy exists as to whether the widely observed pattern of increasing ecosystem productivity with increases in species richness is due to species richness *per se* or due to the chance inclusion of important and dominant species in polyculture (i.e. the sampling effect: Section 1.2.2.1.). Theory suggests that individual species may have hugely important effects on ecosystem processes when there are differences between species in their competitive abilities for a resource (Tilman *et al.* 1997c). Resource competition theory predicts that "*of all the species initially present in a habitat, the one species with the lowest requirement for the resource would dominate at equilibrium, displacing all other species*" (Tilman *et al.* 1997c). As such, the pattern of resource-use of the community will largely reflect that of the dominant competitor. To translate this into the context of the present study, this suggests that the presence of particular dominant (i.e. best competitor) species should strongly influence the level of an ecosystem process. If this is the case, then at the point on the species richness gradient where this species occurs there should be a marked change in the overall rate of an ecosystem process, indicative of an idiosyncratic relationship between biodiversity and ecosystem processing (Figure 1.1e).

Some of the key contributing authors to the biodiversity – function debate strongly advocate the view that the functional traits of dominant species are more important for determining ecosystem processes than the number of species *per se* (Aarssen 1997; Grime 1997). This view is supported by Cardinale *et al.* (2006), who performed a qualitative review of 111 published experimental studies of the effects of biodiversity on ecosystem functioning, and concluded: "*collectively, our analyses suggest that the average species loss does indeed affect ecosystem functioning of a wide variety of organisms and ecosystems, but the magnitude of these effects is ultimately determined by the identity of species that are going extinct*".

The importance of species identity effects on ecosystem processes could be measured through either the addition or deletion of individual species from assemblages of species. Loreau *et al.* (2001b) reviewed 13 studies which found a

positive effect of species richness on invasion resistance. Of these, strong effects of species identity were found in four studies. Other studies have shown that the selective removal of functionally important and dominant species ('key-stone' species) have the power to causes dramatic changes in ecosystem processes (Power *et al.* 1996).

The only experimental design that truly allows differentiation of the effects of species richness from those of species identity requires true replication of species richness treatments with different species assemblages (Allison 1999). However, most studies have not been designed to satisfy this requirement. In many studies there has not been replication in species mixture composition at the highest species richness treatment and as such the relative importance of changes in species richness cannot be separated from those of species identity and composition.

Of those few studies that have evaluated the relative importance of species identity *versus* species richness for rates of ecosystem processing there is evidence in support of the argument that species identity can have large effects, above and beyond those of species richness (Symstad *et al.* 1998; Ruesink & Srivastava 2001; Stampe & Daehler 2003; Wardle *et al.* 2004; Bruno *et al.* 2005; Bruno *et al.* 2006; Straub & Snyder 2006). One study examined the effect of the loss of a single species on productivity in experimental grassland communities (Symstad *et al.* 1998) and found that although average productivity decreased as species richness decreased, the magnitude and direction of the change depended on the identity of the species lost and the composition of the community from which it was lost. Strong species identity effects were also revealed in below-ground mycorrhizal communities, through their ability to influence the structure of the plant community above-ground and their role in either facilitating or repelling invasion (Stampe & Daehler 2003).

1.3.2. Species composition.

Many experimental studies have manipulated the composition of assemblages within treatments of species richness, while testing for effects of changes in species richness. The results of these studies indicate that species composition can

be more important for determining rates of ecosystem processes than species richness *per se*. For example, one pond food web experiment assessed the relative impact of random compositional changes within three levels of species richness (Downing & Leibold 2002). The relationship between species richness and ecosystem productivity was idiosyncratic, and authors concluded that "*the composition of species within richness levels can have equally or more marked effects on ecosystems than average effects of richness* per se". Studies have been across a range of study systems, including: terrestrial plant systems (Hooper & Vitousek 1997; Tilman *et al.* 1997a), soil decomposer systems (Wardle *et al.* 1997a; Mikola & Setälä 1998), and aquatic systems (Norberg 2000; Downing & Leibold 2002; Jonsson *et al.* 2002; Jonsson & Malmqvist 2003b; 2005).

1.4. The direct and indirect effects of stressors on ecological structure and function.

So far I have introduced the relationship between biodiversity and ecosystem functioning. In the majority this has been considered in the absence of stressors. In the real world, anthropogenic stressors are a prevalent force acting upon ecosystems and there is the possibility that they can affect both structure and function (Figure 1.2). When considering the effects of stressors on ecosystems it is useful to consider that all ecosystems are subject to a natural disturbance regime (e.g. fires, storms, floods), which affects community structure and has the potential to affect ecosystem functioning (see Hughes *et al.* 2007). Disturbances, by their nature, have negative effects on assemblages, although their wider consequences on species richness and abundance are uncertain (Lepori & Hjerdt 2006). Several ecologists have emphasized that natural disturbances are important features of ecosystems rich in species (relative to the regional pool), promoting biodiversity by maintaining habitat heterogeneity (Ward & Stanford 1983; Ward *et al.* 2002) (reviewed in Lepori & Hjerdt 2006). Many types of disturbance promote species co-existence by precluding competitive dominance (Paine 1966; Armstrong 1976; Connell 1978; Huston 1979; Sousa 1979; Holt & Pickering 1985; Poff *et al.* 1997). Cardinale *et al.* (2000) use a theoretical argument to show how periodic disturbances (e.g. mortality induced by fires, droughts, floods,

herbivory, predation and parasitism) might modify the effects of species richness on ecosystem level processes by controlling how the relative abundances of competitively superior and inferior species change across levels of species richness. Evidence from stream mesocosms suggests that periodic disturbances prevent taxonomic dominance (Cardinale & Palmer 2002). Therefore, the role of species richness on ecosystem functioning may depend on how disturbances regulate community structure.

Anthropogenic stressors are likely to elicit strong effects on ecosystems, over and above that of the natural disturbance regime. What is more they are increasing in frequency and are likely to interact with each other, with unknown consequences for ecosystem functioning (Vinebrook *et al.* 2004). In summary, our understanding of how stressors affect ecological structure is relatively well developed, but our understanding of concomitant alterations to ecosystem processing remains poorly understood. A large unknown is the resilience of ecosystem processes to novel anthropogenic stressors.

In 1976, Barrett *et al.* stated that in order to understand the effects of stress on ecosystems "*both structure and functional ecosystem parameters should be employed*", given that "*often much information is collected regarding structural parameters (e.g. density, diversity, life history or biomass), with only limited information gathered concerning functional ecosystem parameters (e.g. energy flow pathways, resource recycling, or regulatory processes operating in the system*" (Barrett *et al.* 1976). Since this statement, responses of ecosystem functions to a variety of stressors have been documented. The most contemporary example being changes in climate, especially elevated CO_2 levels as a result of human activities (Diaz *et al.* 1993; Mooney & Koch 1994; Penuelas & Estiarte 1998; Niklaus *et al.* 2001a; Niklaus *et al.* 2001b).

I hypothesize that stressors may affect structure and function directly (Figure 1.2: Arrows A and B), with the additional possibility that changes in one may elicit indirect effects on the other (Figure 1.2: via Arrows A and C or Arrows B and D). The direct effects of stressors on ecosystem functioning may be quantitatively more important than those indirect effects mediated through changes in ecological structure.

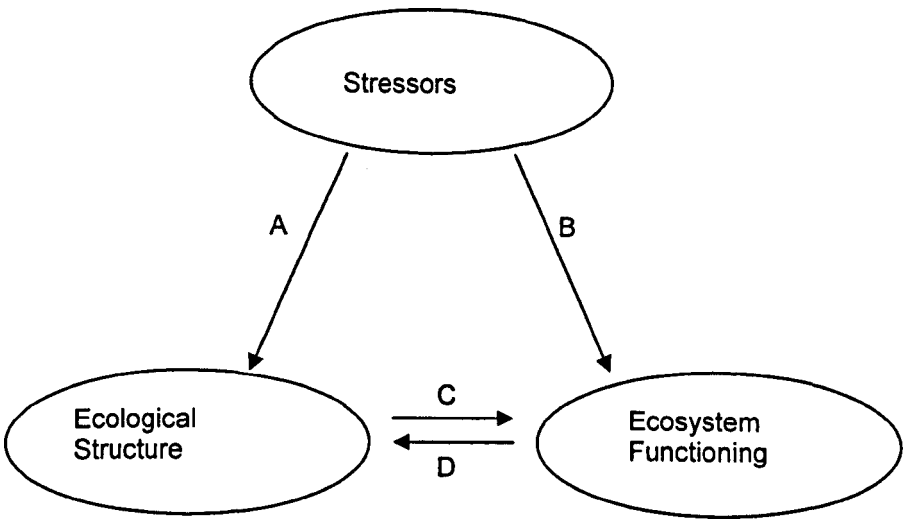

Figure 1.2.: Schematic of the direct (Arrow B) and indirect effects, via ecological structure (Arrows A and C), of stressors on ecosystem functioning. There is also the potential for indirect effects of stressors on ecological structure, via changes in ecosystem functioning (Arrow D).

1.5. Freshwater ecosystems.

1.5.1. The importance of examining the effects of stressors on the relationship between structure and function in freshwater systems.

Freshwater ecosystems warrant study, not only because of the importance of their ecosystem goods and services (Covich *et al.* 2004), but also because they present ecologists with an opportune study system with which to address questions about the relationship between ecosystem structure and function. In comparison with terrestrial ecosystems, where a wealth of evidence indicates the functional importance of biodiversity for ecosystem properties (see Section 1.2.1.3.), little attention has been paid to the relationship between biodiversity – ecosystem functioning in freshwater ecosystems. This is in spite of suggestion that freshwater ecosystems are some of the most impaired in the biosphere, with some of the highest rates of species extinctions (Wall *et al.* 2001; Malmqvist & Rundle 2002). The scenario for biodiversity loss in freshwater systems is perhaps far greater than for any terrestrial system (Riccardi & Rasmusen 1999).

Freshwater ecosystems have several unique features which provide a compelling argument against directly extrapolating evidence and lessons learned with regard to biodiversity and ecosystem function from terrestrial ecosystems. Aquatic ecosystems "*are characterised by greater propagule and material exchange, often steeper physical and chemical gradients,* [and] *more rapid biological processes*" (e.g. nutrient cycling) (Giller *et al.* 2004). These differences may provide a unique perspective on the relationship between biodiversity and ecosystem processes.

Of those few studies to have examined the relationship, the primary functional response variable has been the rate of leaf breakdown (Jonsson & Malmqvist 2000; Jonsson *et al.* 2001; Hieber & Gessner 2002; Jonsson *et al.* 2002), although some studies have measured predation and filtration rates (Cardinale & Palmer 2002; Cardinale *et al.* 2002). Studies suggest either positive effects of invertebrate species richness, or no effect on ecosystem function (reviewed by Covich *et al.* 2004). There is indication from some studies that species identity, rather than species richness *per se*, is an important driver of function (Jonsson *et al.* 2001; Jonsson *et al.* 2002) (Section 1.5.3.2.).

The recent Water Framework Directive (WFD) (E.C. 2000/60/E) is aimed at establishing a framework for Community action in the field of water policy. It recognises that "*waters in the* [European] *Community are under increasing pressure from the continuous growth in demand for sufficient quantities of good quality water for all purposes*" (e.g. domestic, industrial and agricultural use). The WFD acknowledges not only the intrinsic value of water: "*water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such*", but also the important natural resources it provides to human society e.g. potable drinking water.

The implementation of the WFD has implications for both current and future ecological assessment of freshwater ecosystems. It aims for all European waters to be maintained at "*good ecological quality*". Within the directive, 'ecological status' is defined as "*an expression of the quality of the structure and functioning of aquatic ecosystems*" (Article 2). At present, an appropriate methodology for assessing the functional status of freshwaters does not exist. All current freshwater ecological assessment methods are structural (Section 1.5.2.1.). This raises the

important question: what is the extent to which structure reveals function in freshwater systems? If structure and function are closely linked and one reveals the other, then monitoring good ecological quality through structural assessment will be straightforward. If structure does not simply reveal function, then monitoring ecological status may require the development of new functional assessment tools for freshwaters.

Some freshwater ecologists have proposed that when considering the impacts of anthropogenic stressors on freshwater ecosystems, the concept of 'ecological integrity' provides a useful framework (Gessner & Chauvet 2002). Though its formal definition remains subject to debate (Karr 1991), ecological integrity is considered to be a measure of deviation from a desired ecosystem condition. It has been defined as "*The capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having species composition, diversity, and functional organization comparable to that of natural habitats of the region*" (Karr 1999). Currently few ecosystems remain in a pristine condition, and because so much natural variability exists in freshwater ecosystems a more useful comparison can be made to a nearby 'reference' condition (i.e. a nearby stream in close proximity, with broadly similar abiotic conditions, which can be sampled to minimise variability in space, time and sampling technique, but which is not impacted). Streams with minimal deviation from the reference condition may be considered as having high ecological integrity (Bunn & Davies 2000) because reference conditions are chosen to reflect the ecosystem state when free from anthropogenic stressors.

1.5.2. Lotic freshwater ecosystems.

1.5.2.1. Macroinvertebrate communities.

Macroinvertebrates are ubiquitous and abundant in freshwaters. As a taxonomically diverse group, they include members of the following phyla: Annelida (segmented worms and leeches), Nematoda (roundworms), Turbellaria (flatworms), Mollusca (snails and mussels), and Arthropoda (insects: beetles, mayflies, stoneflies, dipterans, caddisflies, dragonflies etc. and crustaceans). Most species are benthic (i.e. are associated with the stream bottom).

Macroinvertebrates have been extensively studied in stream ecology, because of the crucial roles which they play in the aquatic food web, in the transfer of energy and nutrients from organic matter resources (e.g. leaf material and algae), through to fishes (Vannote *et al.* 1980).

One of the most useful things about them is that they are predictably sensitive to the effects of stressors (Allan 1995). In addition, they are relatively easy sample and identify. In the United Kingdom, and elsewhere, ecological assessment of lotic freshwater ecosystems is through assessment of macroinvertebrate community structure (Rosenberg & Resh 1993; Wright *et al.* 2000). There are several good reasons why macroinvertebrates are preferable to other taxonomic groups for this purpose (Metcalfe 1989), although it remains to be seen whether assessment of macroinvertebrate community structure is indicative of the status of other taxonomic groups (e.g. fish or diatoms).

Macroinvertebrates can be divided into six functional groups based on feeding mechanism: 'shredders' (feed on living or decomposing plant tissue or wood), 'collectors' (feed on decomposing fine particulate organic matter), 'scrapers' (feed on periphyton-attached algae and associated material), 'macrophyte piercers' (feed on living vascular hydrophyte cells and tissue fluids or macroscopic algal cell fluids), 'predators' and 'parasites' (both feed on living animal tissue) (Merritt & Cummins 1996). However, this view is over-simplistic as many studies have shown that many macroinvertebrates are actually omnivorous. For example, macroinvertebrate shredders actually ingest not only decomposing detritus, but also its associated microbes (e.g. fungi, bacteria and protists) and other diatoms, algae and other small invertebrates attached to the leaf surface, for example, first instar chironomid larvae (Diptera: Chironomidae) (Merritt & Cummins 2006).

1.5.2.2. Ecosystem functioning.

In temperate headwater streams, the primary source of energy entering into the system is in the form of autumn shed leaves from the surrounding riparian vegetation (Maltby 1996; Wallace *et al.* 1997; Gessner *et al.* 1999). In these low order streams, secondary production is dependent upon the input of leaf material

and other allochthonous detrital matter, rather than on *in situ* primary production, which is more important in lowland 'autotrophic' streams. The energy base of a stream will influence both the structure and function of its invertebrate community, and as such, streams dependent on allochthonous detrital inputs are usually dominated by detritivorous macroinvertebrates (shredders) (Vannote *et al.* 1980).

The process of leaf breakdown *in situ* is considered to be a cumulative product of several non-independent processes: physical breakdown involving leaching of soluble compounds and physical abrasion (Bärlocher 2005), breakdown by microbes such as bacteria and fungi (Hieber & Gessner 2002; Pascoal & Cassio 2004) and fragmentation by macroinvertebrate shredders (Gessner *et al.* 1999; Graça 2001). Macroinvertebrate shredders include members of several different taxonomic orders e.g. Amphipoda, Isopoda, Plecoptera, Trichoptera, Ephemeroptera, Diptera (Andersen & Sedell 1979).

The rate of leaf processing has been measured in numerous studies as an indicator of the functional status of freshwater ecosystems (see above, Section 1.5.1.). The RIVFUNCTION project has been focussed on "*developing and disseminating a methodology for assessing the functional component of ecological river status... by determining the performance of...* [leaf] *litter decomposition... in response to two types of serious and widespread anthropogenic impacts on European rivers... excessive nutrient loading (eutrophication) and modification of the riparian vegetation*" (RIVFUNCTION). The rate of leaf processing is measured through deployment of leaf bags containing known weights of leaf material into streams for periods of weeks to months (Petersen & Cummins 1974; Benfield *et al.* 1977; Webster & Benfield 1986; Boulton & Boon 1991; Graça 1993b; Gessner & Chauvet 2002). Through variation in the size of the mesh used to construct leaf bags, shredders can either be included or excluded from bags, permitting separation of the relative proportion of leaf mass loss caused by shredders and that of other associated processes (e.g. microbial processes & physical abrasion). This method reveals that shredders are often the dominant processors in temperate streams, accounting for up to 75 % of the mass loss of coarse particles (Hieber & Gessner 2002), although this may not be the case in tropical streams, where low

densities of macroinvertebrate shredders have been recorded (Dobson *et al.* 2002; Gonçalves Jr *et al.* 2007).

After entering streams, and before being fragmented by shredders, leaves are rapidly colonised by microbes, e.g. bacteria and fungi. A group of widely and hugely abundant fungi, called aquatic hyphomycetes (the largest order of Fungi Imperfecti) (Ingold 1975) are dominant (Gessner & Chauvet 1994), probably due to their mycelial nature which enables them to penetrate the leaves (Harley 1971). Fungal biomass contributes 95-99 % of total biomass on the surface of leaf material associated with the breakdown of leaves (Hieber & Gessner 2002; Gulis & Suberkropp 2003). Bacteria have in the past been considered not to be as important, owing to their dependency upon fungal breakdown to increase the surface area of the leaves available for colonisation (Kaushik & Hynes 1971; Suberkropp & Klug 1976). However, the more recent view is that the role of bacteria may have been underestimated (Hall Jr & Meyer 1998), and shredders in tropical streams have shown no preference for leaves colonised by fungi over those colonised with bacteria (Wright & Covich 2005). More recently, protists have also been implicated for their role in leaf breakdown (Ribblett *et al.* 2004), though the relative size of their contribution in comparison with aquatic fungi remains to be assessed.

Aquatic hyphomycetes are important, not only for their ability to breakdown leaf material (Suberkropp *et al.* 1983) but also because they improve the palatability and nutritional value of the leaf material as a food source for macroinvertebrate shredders, a process known as 'conditioning' (Bärlocher & Kendrick 1981; Suberkropp 1992; 2003). Various studies have shown that shredders exhibit preference for, and grow better when fed on, leaf material conditioned with fungi than on unconditioned leaves (reviewed by Suberkropp 2003). Therefore, in addition to playing an important role in the decomposition of organic material, fungi also play a role in mediating the transfer of energy and nutrients to higher trophic levels.

1.5.2.3. Anthropogenic Stressors.

Freshwater ecosystems are hugely threatened by anthropogenic stressors (Abell 2002). Some of the major threats include: changes in land-use (e.g. deforestation or afforestation), species introductions and invasions (e.g. translocation of fish for sport fishing), overexploitation, flow modification and larger-scale environmental impacts such as pollution and climate change (Dudgeon *et al.* 2006). All of these kinds of stressors have the potential to alter the structure and function of streams, but probably the most widely studied are pollutant stressors. In this study I will only consider pollutant stressors, because they are relatively easy to quantify and detect from analysis of water chemistry, and are widespread. Three major types of freshwater pollutant stressors include (Mason 2002):

1. Metals, such as lead, nickel, cadmium, zinc, copper, mercury; originating from many industrial processes (especially mining) and some agricultural uses.
2. Acids and alkalis.
3. Organic compounds, such as organochlorine pesticides, herbicides, PCBs, chlorinated hydrocarbons, polynuclear aromatics, etc.; arising from a variety of industrial, agricultural and domestic sources.

There are some notable complexities with regard to studying the effects of pollutant stressors on freshwater ecosystems. Firstly, it is rare that only a single pollutant be present in a watercourse. Most effluent discharges contain a variety of potentially harmful substances and most watercourses will receive a number of different effluents. Secondly, "*the effects of pollutants may be additive, or antagonistic (in which the combined effects on the target organisms is less than predicted by each pollutant's effect when alone) or synergistic (when the combined effect is greater than predicted from their effects when alone)*" (Mason 2002). This indicates that when studying the effects of anthropogenic stressors, it is useful to consider stressors in isolation in order to be able to determine the relative effect or mechanism by which they operate, but in practice this may present a challenge to the researcher to find study sites where a stressor is acting in isolation.

1.6. Relationships between stress, structure and function in streams.

Combining the specific elements of lotic freshwater systems (Section 1.5.2.), with the general scheme for describing the interaction of stress, structure and function outlined earlier (Section 1.4.), we can hypothesize that there are various pathways through which stressors could possibly affect the rates of leaf processing (Figure 1.3). Stressors may affect the rate of leaf processing directly (Arrow B) or indirectly through changes in macroinvertebrate community structure (Arrows A and C). Direct effects may operate through changes in the fungal community present on leaf disks, which may in turn affect either the rate of leaf processing directly (Arrows E and G) or the macroinvertebrates (Arrow H) and their rate of leaf processing. Other arrows might also operate, including feedbacks from changes in leaf material available to macroinvertebrates (Arrow D) and to fungi (Arrow F). Macroinvertebrates may also affect the fungal community (Arrow I). In the remainder of this section, I evaluate evidence for each arrow and identify knowledge gaps.

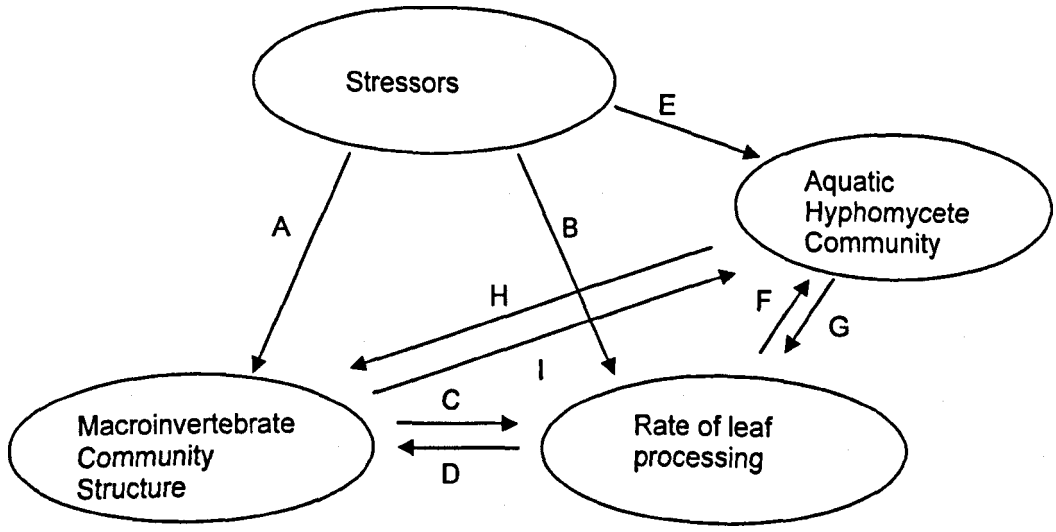

Figure 1.3.: Schematic of the central concepts for investigation in this study. The effects of stressors on stream ecosystem function, measured as the rate of leaf processing: the direct (Arrow B) and indirect effects, via macroinvertebrate community structure (Arrows A and C). Stressors may also affect the aquatic hyphomycete community (Arrow E), with implications for the macroinvertebrate community (Arrow H) and for leaf breakdown (Arrow G). It is possible that feedbacks also occur (Arrows D, F and I).

1.6.1. Effects of stressors on macroinvertebrate community structure (Arrow A).

Much of the work on effects of pollutant stressors on aquatic systems has focussed on the individual or population level, and has ascertained lethal and sub-lethal doses for invertebrate and fish taxa. However, numerous field studies have also reported changes in macroinvertebrate community structure, including reductions in species richness (e.g. Winner *et al.* 1980) downstream of pollution discharges. Some structural measures may be relatively more sensitive in their ability to detect an effect than others. For example, whole community density may not be sensitive to the effects of stressors, because some taxa are able to tolerate higher levels of contamination and are able to increase in abundance as sensitive taxa are lost (as shown by Richardson & Kiffney 2000).

Early field studies to have documented the effects of stressors on macroinvertebrate community structure include the effects of heavy metal contamination (Carpenter 1924; Jones 1948) and organic pollution (Hynes 1960; 1969). A more recent focus (c. last 20 years) has been acidification (Hall *et al.* 1980; Zischke *et al.* 1983; Ormerod & Wade 1990; Weatherley *et al.* 1990; Reynolds *et al.* 1999; Monteith & Evans 2000; Shilland *et al.* 2004). In the following three subsections I discuss these three pollutant stressors and provide an overview of the kinds of effects to be expected.

1.6.1.1. The effect of heavy metal contamination on macroinvertebrate community structure.

Some metals exist naturally in streams, released from natural processes such as weathering of rocks. At low levels they are not toxic, but rather, essential to life (e.g. zinc and copper). However, industrial processes (e.g. mining) have massively increased loadings of heavy metals in aquatic ecosystems (Mason 2002). Heavy metal contamination is an important stressor of stream organisms (Pollard & Yuan 2006), owing to the fact that heavy metals are conservative pollutants which are not broken down, they can accumulate in organisms and some may biomagnify through the food chain (Peakall 1992). The effects of heavy metal contamination can be seen at several levels of biological organisation, i.e. at the biochemical, physiological, population and community level (Luoma & Carter 1991).

Heavy metal contamination has the potential to reduce the species richness of communities, and reductions in macroinvertebrate species richness have been seen in numerous studies (e.g. Armitage 1980; Winner *et al.* 1980; Chadwick *et al.* 1986; Clements *et al.* 1988; Hoiland *et al.* 1994; Kiffney & Clements 1994a; 1994b; 1996; 2003). The effects can operate through three pathways: 1) direct toxic effects (e.g. Brown 1977); 2) indirect effects, via increases or decreases in predation, or via contaminated food sources (Namminga *et al.* 1974; Brown 1977; Burrows & Whitton 1983); 3) altered physical habitat, e.g. ochre (hydrous iron oxides) deposits from metal-rich discharge.

Some metals are more toxic at higher concentrations than others. Cadmium is highly toxic to some animals (Mason 2002). Zinc (Jones 1958; Namminga *et al.* 1974; Brown 1977; Solbé 1977; Hoiland & Rabe 1992; Kiffney & Clements 1994a), copper (Namminga *et al.* 1974; Brown 1977; Leland *et al.* 1989; Schultheis *et al.* 1997; Millward & Grant 2000; Mebane 2003), lead (Richardson & Kiffney 2000), cadmium (Carpenter 1924; Namminga *et al.* 1974) and aluminium (Burton & Allan 1986) have all been implicated for their effects on macroinvertebrate community composition. Field studies show that species richness in heavy metal polluted water is reduced, but tolerant taxa can be very abundant (Wood 1995 unpublished; Herrmann 2001). For example, in Richardson & Kiffney (2000) chironomids accounted for over 80 % of the taxa in heavy metal contaminated waters, thus compensating numerically for the loss of other taxa.

Particularly toxic metals may exert differential mortality on populations within aquatic communities, as they exterminate some species and have little direct effect on others, although there may be indirect effects and there is the possibility of cascades. For example, Carlisle & Clements (2003) detected indirect effects of zinc contamination on production of predatory stoneflies, caused by decreases in total production attributable to algal and animal prey in contaminated streams.

Evidence from Rocky Mountain streams in Colorado, suggest that insect taxa vary widely in their range of metal sensitivity (Clements *et al.* 2000; Griffith *et al.* 2001; Clements *et al.* 2002). Richardson & Kiffney (2000) used experimental mesocosms to determine the responses of stream macroinvertebrate communities to mixtures of heavy metals: copper, zinc, manganese and lead. They found that

the most sensitive taxa were mayflies of the following genera: *Baetis, Ameletus* and *Paraleptophlebia* (Ephemeroptera); while certain stonefly taxa (Plecoptera: Nemouridae) and oligochaetes were only mildly affected. What is more, closely related species within the same functional feeding groups may be differentially sensitive to metal contamination (Clements *et al.* 2002).

Heavy metals also have the potential to reduce population and community density of individuals and biomass through reduced growth rates and fecundity. Hrusks & Dube (2004) used artificial stream mesocosms to assess the effect of heavy metal mine effluent on the life cycle of the freshwater midge, *Chironomus tentans* (Diptera: Chironomidae). They found that the mine effluent caused reduced survival, reduced emergence, increased time-to-emergence, and reduced hatchling success. However, there was no significant affect on growth, sex ratio, number of egg cases/female or number of eggs/egg case. These kinds of effects indicate that heavy metal contamination might have repercussions on the energy available for shredder growth and reproduction. Evidence for this was presented by Maltby & Naylor (1990), who performed a seven day assay that measured the effect of zinc stress on brooding female *Gammarus pulex* L. (Amphipoda: Gammaridae) individuals. Effects were measured on feeding rate and reproductive parameters (e.g. size, number of offspring from one brood and a second brood provisioned under zinc stress). They observed that the amount of energy individual females had available for either growth or reproduction was reduced as a result of them consuming less leaf material. There was no effect on either the size or the number of offspring, but there was an increase in the number of broods aborted.

1.6.1.2. The effects of acidification on macroinvertebrate community structure.

Stream acidifying compounds derive from air-borne pollutants (e.g. sulphur dioxide SO_2 and the oxides of nitrogen NO_x) which form acids when they undergo oxidation in the atmosphere. They deposit and affect streams across large parts of the globe, and the degree of impact depends on the geology and soils surrounding a particular stream (Hall *et al.* 1980; Zischke *et al.* 1983). When stream ecosystems become acidified this can mobilise aluminium and other metals, increasing toxicity to biota (Hildrew & Ormerod 1995). Weak acids do naturally

occur in soil, in the form of organic and carbonic acids. However, these compounds do not have such severe affects on biota because they do not dissociate into their respective anions and cations to the same extent as the strongly acidifying compounds found in acid rain (Hildrew & Ormerod 1995).

Studies have documented reductions in macroinvertebrate species richness in acidified streams (Mason 2002). In upland streams in Wales, species such as *Gammarus pulex* (Amphipoda), *Ancylus fluviatilis* (Pulmonata) and *Hydropsyche* spp. (Trichoptera) were absent, despite their food sources being present (Stoner *et al.* 1984; Wade *et al.* 1989). Changes in macroinvertebrate community structure could result from a range of different factors, a combination of physiological stress, a change in food supply and a reduction in predators. Many fish species are intolerant of low pH and their absence from acid streams allows large, generalist predators to proliferate (Ormerod *et al.* 1987a). Switches in macroinvertebrate community structure, from an algivore-dominated to a detritivore-dominated community, have been associated with low pH (Hildrew *et al.* 1984). Specialist invertebrate grazers (e.g. mayflies and snails) are usually missing from acid streams, which are generally less productive than circumneutral streams (Sutcliffe & Hildrew 1989).

1.6.1.3. The effects of organic pollution on macroinvertebrate community structure.

Organic pollution occurs when large quantities of organic compounds, acting as substrates for micro-organisms, are released into watercourses from domestic sewage, urban run-off, industrial and agricultural wastes. During the decomposition process of the substrate, available oxygen in the water is lowered. This has consequences for macroinvertebrate physiology, causing reduced fitness and, when severe, asphyxiation. Organic effluents often also contain ammonia which increases the toxicity to macroinvertebrates (Abel & Bärlocher 1988; Timmermans *et al.* 1992).

There has been much documented on the effects of organic pollution on macroinvertebrate community structure (Mason 2002). Researchers have highlighted that effects of heavy pollution affect whole taxonomic groups of

macroinvertebrates, rather than just individual species (Mason 2002). Different taxa characterise different levels of organic pollution (Hynes 1960), with tubificid worms being typically massively abundant in heavily polluted water. To illustrate, some example of species, in order of their increasing tolerance to organic pollution are: *Dinocras cephalotes* (Plecoptera), *Ecdyonurus venosus* (Ephemeroptera), *Gammarus pulex* (Amphipoda), *Asellus aquaticus* (Isopoda), *Chironomus riparius* (Diptera), *Tubifex tubifex* (Oligochaeta).

1.6.2. The relationship between macroinvertebrate community structure and the rate of leaf litter processing (Arrow C).

The component of the macroinvertebrate community most closely associated with the process of leaf breakdown is the shredder community (Section 1.5.2.2.). A recent study reviewed five empirical studies of the relationship between macroinvertebrate shredder community and the rate of leaf processing in freshwater systems (Covich *et al.* 2004). The studies ranged from observational approaches performed in natural stream reaches (Jonsson *et al.* 2001; Huryn *et al.* 2002) to experimental laboratory mesocosm systems (Jonsson & Malmqvist 2000; Jonsson *et al.* 2002; Jonsson & Malmqvist 2003a). Between the studies the range of the number of species varied from either one to three species (Jonsson & Malmqvist 2000; Jonsson *et al.* 2002), one to six (Jonsson & Malmqvist 2003a), one to seven species (Huryn *et al.* 2002), or one to eleven species (Jonsson *et al.* 2001). The results of all five studies indicate a positive relationship between shredder species richness and leaf breakdown.

As expected (see Section 1.3.1.), studies suggest that shredder species identity also is important in determining rates of leaf processing in freshwater ecosystems (Ruesink & Srivastava 2001; Jonsson & Malmqvist 2003a; Dangles & Malmqvist 2004; Carlisle & Clements 2005). In the laboratory study by Jonsson & Malmqvist (2003a), the addition of one shredding stonefly species had large effects on the rate of leaf processing, while the addition of a second stonefly species did not alter rates of leaf processing. In the study by Ruesink & Srivastava (2001), two dominant leaf eating species (one stonefly and one caddisfly) were removed separately from field enclosures. The resulting changes in ecosystem functioning depended upon the identity of the species lost. To my knowledge, a

single other study has documented the effect of shredder community composition on leaf processing rates (Dangles & Malmqvist 2004). The results of this field study indicate that some freshwater species, whose traits include strong interspecific interactions, high densities, year-around presence in the system and high mobility, might be expected to have particularly strong effects on benthic communities and processes.

There has been some examination of the relative importance of top-down and bottom-up controls (e.g. from other functional feeding groups) in determining the rate of leaf processing (Hawkes 1979). Leaf breakdown in temperate zones is often dominated by the larger macroinvertebrate shredders, such as crayfish and amphipods (Rosemond *et al.* 2001). These larger species may not only dominate rates of processing in streams, but also be the main drivers of species interactions (Usio & Townsend 2001; Dangles *et al.* 2004b). For example, fish may induce trophic cascades that alter both decomposition and primary production (Woodward & Hildrew 2002).

1.6.3. The direct and indirect effects of stressors on the rate of leaf processing.

In general, very little is known about the impacts of stressors on the rate of leaf processing. I hypothesise that stressors may affect the rate of leaf processing through three separate pathways: 1) direct effect; 2) indirect effect via changes in macroinvertebrate community; 3) indirect effect via changes in microbial community. These are discussed in the following subsections.

1.6.3.1. Direct effects (Arrow B).

Not much is known about how stressors could elicit direct effects on leaf processing. All such mechanisms will be ones that either prevent the process, or change the input of leaves into the system. Physical prevention of the process might occur when, for example, heavy metal contamination containing iron, is released into streams and coats leaves in a layer of ochre (Fe(III) oxide) rendering the leaves unpalatable to macroinvertebrate shredders and uncolonizable by microbes. If this happens it is unlikely that leaf breakdown will occur at all, or at a

much reduced rate. Alternatively, changes in leaf inputs into the system might results from the exclusion of leaves entering streams as a result of deforestation (Sweeney *et al.* 2004) or from changes in the composition of the riparian vegetation (Lecerf *et al.* 2005). However, mechanisms of the effects of stressors are more commonly reported through either macroinvertebrate (Arrow C) or microbial or fungal pathways (Arrow H).

1.6.3.2. Indirect effects via the macroinvertebrate community (Arrows A and C).

If stressors affect the rate of leaf processing indirectly through changes in macroinvertebrate community structure (i.e. through arrows A and C) (Figure 1.3) then we might expect to see strong associations between macroinvertebrate community structure and the rate of leaf processing. A recent study (Dangles *et al.* 2004b) tested for associations between responses of structure and function in response to acidification. Dangles *et al.* used simple linear regression analyses to test for associations between macroinvertebrate community structure (measured as shredder biomass, abundance and richness, and *Gammarus* spp. abundance and biomass) *vs.* function (measured as the leaf breakdown coefficient (k)). The study surveyed 25 streams in the Vosgues Mountain range, France. The results suggest that the relative abundance and identity of certain key taxa might be more important in determining rates of leaf processing than the number of species *per se.*

The Insurance Hypothesis (Section 1.2.3.) predicts that it might be possible for ecosystem function to be maintained in spite of species losses, because functionally redundant species are able to maintain function, in spite of losses of more sensitive taxa. Indeed, there is a lot of feeding redundancy in stream food webs (Power 1990; Flecker 1996), and evidence exists to suggest that ecosystem processes may be maintained despite species losses (Woodward & Hildrew 2002).

1.6.3.3. Indirect effects via the microbial community (Arrows E and G).

There is evidence to suggest that the fungal community is sensitive to the effects of pollutant stressors (Arrow E) and that there may associated affects on the rate

of leaf processing. It has been fairly well established that heavy metal contamination affects fungal communities, by reducing species richness and selecting for tolerant or resistant species (Gadd 1993; Raviraja *et al.* 1998; Pascoal *et al.* 2005a). Elevated levels of aluminium have been shown to affect the growth and sporulation rate of aquatic hyphomycetes (Krauss *et al.* 2003; Sridhar *et al.* 2005). The effects of cadmium have directly inhibited microbial colonisation of leaf material and reduced rates of leaf decomposition (Chamier & Tipping 1997). The effects of zinc have been shown to affect the composition of the aquatic hyphomycete community and the amount of leaf processing, without severely affecting the number of fungal species or fungal biomass (Giesy 1978). Finally, the effects of coal mine effluent on aquatic hyphomycetes (Bermingham *et al.* 1996b) have had inhibitive effects on the enzyme activity of the fungi and in the longer term resulted in decreased rates of sporulation, decreased abundances of fungi, decreased fungal biomass and decreased species richness, all of which resulted in a decrease in the rate of leaf processing overall.

Several studies have demonstrated that microbial numbers and/or activities are reduced under acid conditions, especially at pH <5 (Mackay & Kersey 1985; Allard & Moreau 1986; Chamier 1987; Mulholland *et al.* 1987; Palumbo *et al.* 1987; Duarte *et al.* 2004).

Aquatic hyphomycetes are most common in clean, well-aerated waters (Suberkropp *et al.* 1988; Au *et al.* 1992a). This suggests that they require relatively high oxygen concentrations, which are rare in organically polluted waters (Bärlocher 1992). Some field studies show that organic pollution can reduce the number of fungal species present at sites (Burton *et al.* 1985), while other studies show no effect (Greathead 1961; Conway 1970; Kreisel & Manoharachary 1983; Burgos & Castillo 1986; Au *et al.* 1992b; Raviraja *et al.* 1998). In a study at two polluted sites and two unpolluted sites on the Ave River in Portugal, nutrient enrichment and low dissolved oxygen concentration were associated with a reduction in fungal production, biomass and sporulation rates (Raviraja *et al.* 1998). However, despite reductions in fungal species richness and sporulation, rates of leaf decomposition may remain unaffected by organic pollution (Pascoal & Cassio 2004).

1.6.4. Other effects.

Studies discussed in the previous section show that stressors can elicit changes in microbial community structure. These changes may affect not only the rate at which microbes decompose leaves (Arrow G), as seen in several studies (Eggenschwiler & Bärlocher 1983; Bärlocher 1987), but also might result in poorer quality leaf material downstream of pollution sources, with implications for rates of shredder leaf processing (Arrow H) and their predators (Gulis *et al.* 2004). This is because fungal species have different abilities to increase leaf palatability for macroinvertebrate shredders (Gray & Ward 1983; Maltby & Booth 1991). The extent to which changes in microbial community structure affects rates of shredder leaf processing has received some attention (Bärlocher & Kendrick 1973a; Rossi & Fano 1979; Lecerf *et al.* 2005), however, more attention is needed (Suberkropp 1992; 2003).

There is some evidence to suggest that changes in macroinvertebrate community structure may affect the structure of the microbial decomposer assemblages (Arrow I) (Howe & Suberkropp 1994). However, a more recent study by Ferreira & Graça (2006) tested for and found no effect of shredder activity on microbial community structure.

There is also some evidence to suggest that effects of stressors may operate indirectly via leaf material (Arrows B and D). Snyder & Hendricks (1995) found that concentrations of mercury (Hg) in *Hydropsyche morosa* (Trichoptera: Hydropsychidae), a detritivorous net-spinning caddis larvae, were significantly higher during the summer months when it collects detritus from within nets, than in the winter when they graze on algae. In other words the metal was being accumulated from the contaminated detritus from within the nets.

1.7. Aims and objectives.

An extensive body of literature has documented the effect of manipulating species richness on rates of ecosystem process rates, through random assembly of communities of species (Section 1.2). However, theoretical considerations have illustrated that the effects of species richness are mostly underpinned by the

effects of individual species' traits (Sections 1.2.2. and 1.3.) of which responses to stressors are an important consideration. Very few studies have considered the effects of stressors on the relationship between structure and function.

Another body of literature has documented the responses of macroinvertebrate community structure to anthropogenic stressors in freshwater ecosystems (Section 1.5.3.1.). However, uncertainty exists in the extent to which altered macroinvertebrate community structure may be associated with impaired ecosystem functioning.

The central aim of this study was to investigate the direct and indirect effects of stressors on the relationship between macroinvertebrate community structure and the rate of leaf processing in stream ecosystems. The study took a variety of approaches to address three specific knowledge gaps: firstly, to document patterns of the effects of stressors on the structure and function of stream ecosystems, and to evaluate evidence for associations between structure and function in their responses to stressors (Objectives 1 and 2); secondly, to evaluate of the importance of macroinvertebrate community composition for determining rates of leaf processing (Objective 3); and thirdly to examine the relationship between fungal species richness and macroinvertebrate shredder leaf processing rates (Objective 4).

The four objectives were:

1. To perform a meta-analysis of published experimental and field studies to quantify the effects of anthropogenic stressors on macroinvertebrate community structure and ecosystem function across streams, and to examine whether structure and function are associated (Chapter 2).

2. To conduct field studies to document the effects of heavy metal contamination on the relationship between macroinvertebrate community structure and ecosystem function in streams (Chapter 3).

3. To use artificial stream mesocosms to test whether rates of leaf processing by mixed-species assemblages are predictable from the sum of their constituent parts (Chapter 4).

4. To assess the affect of fungal species richness on the rate of leaf processing mediated through macroinvertebrate shredders (Chapter 5).

Previous theoretical and experimental work has examined the fundamentally important relationship between the structure and function of ecosystems (Section 1.2). What is especially novel about this study is that it considers structure - function relationships in ecosystems subjected to anthropogenic stressors (Section 1.4 and 1.5). This has relevance for both pure and applied research goals (Section 1.5.1.).

2. A meta-analysis of the effects of anthropogenic stressors on the relationship between macroinvertebrate community structure and function.

2.1. Introduction.

Freshwater ecosystems are hugely threatened by anthropogenic stressors (Abell 2002). Major threats include: overexploitation, water pollution, flow modification, destruction or degradation of habitat, and invasion by exotic species (Dudgeon *et al.* 2006) (Section 1.5.2.3.). Ecologists and ecosystem managers must understand how stressors affect ecosystems in order to be able to minimise impacts and protect against the loss of essential goods and services (Sections 1.1.1. and 1.5.1.). Benthic invertebrate species are considered to be of particular importance globally because of their high biodiversity and association with the processes of storage and cycling of materials, nutrients and energy flow (Covich *et al.* 1999).

Most assessment of anthropogenic impacts focuses on structure (species diversity, composition, etc.) and increasingly it is recognized that conservation of the functional characteristics of ecosystems is a critical element in ensuring their continued integrity and provision of ecosystem services. Given that many assessments of ecosystem status are, of necessity, focussed on structural measures, it is important to understand whether impacts on function mirror those on structure. However, most studies of anthropogenic impacts have been carried out on a few sites, within one particular type of system, and this disparity makes it difficult to see if generalizations about the covariance of structure and function exist. One way to address this issue is to combine the result of many studies and assess the trends in both structure and function across these data. Here, I use data compiled from a systematic search of studies in the literature to attempt to quantify the effects of three distinct stressors on ecological structure and function in benthic freshwater ecosystems. I ask whether the effects of anthropogenic stressors on macroinvertebrate community structure are indicative of changes in ecosystem function.

2.1.1. Assessing freshwater ecosystem structure and function, and the relationship between the two.

Researchers quantify community structure in a variety of ways, for example as indices, aggregate numbers and measurements of either density or biomass (Section 2.2.4.), with the aim of characterising community composition in space and time. Recent understanding from consideration of the effects of species loss, is that assessing and monitoring the functional status of ecosystems is potentially extremely important. It is not clear whether many of the structural measures used to characterise communities are indicative of functional status.

Experimental evidence for the relationship between structure and ecosystem function is compelling (Hooper *et al.* 2005) (Section 1.2.1.3.1.). Previous studies in freshwater systems suggest that there is a relationship between structure and function (Section 1.5.3.2.). For example, in small scale laboratory experiments, where the species richness of benthic consumers had been reduced, there was a reduced probability of positive species interactions, which lead to non-additive decreases in carbon cycling (Cardinale *et al.* 2000; Cardinale *et al.* 2002; Jonsson & Malmqvist 2003b). Evidence from the field has not currently been summarised, and it is unclear whether the results from small scale manipulation experiments scale up to produce similar effects in the field, and therefore how the impacts of stressors on structure might actually impact on function in natural systems.

Although at present there is no standardised assessment of freshwater ecosystem function there are many candidate ecosystem processes which could be used to assess the effects of stressors on freshwater ecosystems (see Gessner & Chauvet 2002, pp 500). Of these, the rate of leaf processing is the best documented, and is a centrally important process in aquatic food webs (Moore *et al.* 2004) (Section 1.5.2.2.). Several studies have suggested using leaf breakdown rates to develop a diagnostic tool to assess the functional status of freshwater ecosystems in response to anthropogenic stressors (Webster & Benfield 1986; Gessner *et al.* 1999; Gessner & Chauvet 2002; Hagen *et al.* 2006; Lecerf *et al.* 2006). Previous studies have measured the rate of leaf processing in streams in response to pollutant stressors, such as heavy metal

contamination, acidification and organic pollution, which are important stressors of freshwater ecosystems (Section 1.5.2.3.).

2.1.2. The effects of pollutant stressors on freshwater ecosystems.

The effects of pollutant stressors are likely to impact on ecosystems at many levels, primarily at the level of the physiology of individual organisms but scaling to the population level (Maltby 1999), indirectly between trophic levels to the community level, and between communities and their respective process rates to the ecosystem level. It is unknown how sensitive the various (structural) metrics are to stressors, and whether they respond consistently across ecosystems.

If the effects of stressors on structure and function are consistent across distinct stressors, then ecosystem managers are in a strong position and monitoring the status of ecosystems will be a relatively simple task, because at the simplest level ecosystems will respond in a uniform way to any given stressor, providing ecosystem managers with a common signal with which to detect stress. However, if structure and function respond differently to different stressors then the task is less simple. The mechanisms through which heavy metal contamination, acidification and organic pollution affect biota are different (Sections 1.6.1.1.-3.), and therefore responses of structure and function to different stressors are expected to differ. For example, some species of macroinvertebrates which are very sensitive to organic pollution are relatively tolerant of heavy metal contamination (e.g. some stonefly and case-less caddis larvae).

Published information includes a range of studies reporting responses of rates of leaf processing to anthropogenic stressors (Andersen & Sedell 1979; Webster & Benfield 1986; Gessner *et al.* 1999). Gessner & Chauvet (2002) compared rates of leaf breakdown from published studies of the effects of stressors. From each study they calculated the ratios of leaf breakdown coefficients at impacted (k_i) and reference (k_r) stream sites. For each study they reported: the type of stressor, the ratio (k_i:k_r), plant species, stream order, number of study sites, and geographic location (Table 2.1). The

Table 2.1: Range of ratios of leaf breakdown coefficients at impacted (k_i) and reference (k_r) stream sites for different types of anthropogenic stressors (a sub-sample of the information in Table 1 of Gessner & Chauvet 2002) (see text).

Type of stress	k_i:k_r (%)	Plant species	Stream order	Study sites	Location	Reference
1) Mine drainage effluent						
Copper	45-55	*Acer rubrum*	1/2	3 impacted downstream sites *vs.* 1 upstream reference site	VA, USA	Schultheis *et al.* 1997
Zinc	18-125	*Salix* spp.		18 impacted *vs.* 9 reference sites	CO, USA	Niyogi *et al.* 2001
Metals	22-34	*Alnus tenuifolia*	3	1 untreated and 1 treated downstream site *vs.* corresponding reference sites upstream	CO, USA	Gray & Ward 1983
2) Acidic precipitation	11	*Fagus sylvatica*	2	1 acidified *vs.* 1 adjacent reference stream.	France	Dangles & Guerold 1998
	68	*Fraxinus americana*	1	1 acidified *vs.* 1 reference stream	PA, USA	Kimmel *et al.* 1985
4) Nutrients						
Nitrate	85-190	*Acer circinatum, A. macrophyllum, Alnus rubra, Pseudotsuga menziesii*		2 manipulated *vs.* 1 reference experimental stream channel	WA, USA	Triska & Sedell 1976
	278-289	*Betula lenta, Robinia pseudoacacia*	1	1 impacted *vs.* adjacent reference stream	NC, USA	Meyer & Johnson 1983
	164-760	*Liriodendron tulipifera*	1/2	7 high-nutrient *vs.* 3 adjacent low-nutrient streams.	AL, USA	Suberkropp & Chauvet 1995
Phosphate	120-127	*Quercus rubra*	2	1 manipulated *vs.* 1 reference site in each of 2 streams	TN, USA	Elwood *et al.* 1981
	207	*Alnus viridis*	1	Fertilized *vs.* unfertilized leaf packs in a single stream	Switzerland	Robinson & Gessner 2000

patterns across these studies indicate that the effect of heavy metal contamination and acidification is to reduce the rate of leaf processing ($k_i:k_r$ < 100 % indicating a reduction in the rate of leaf processing at impacted sites, relative to reference sites). Whereas the effect of nutrient addition is to increase it ($k_i:k_r$ > 100 % indicating an increase in the rate of leaf processing at impacted sites, relative to reference sites) (Table 2.1). In the current study I refine the comparison made by Gessner & Chauvet (2002) by also considering structural effects and drawing on meta-analytical techniques to test for statistical significance of any patterns seen.

2.1.3. Meta-analytic techniques.

Meta-analytic techniques allow us to summarise information taken from a variety of sources, while also providing the advantage of scientific rigour over previously more conventional narrative or 'vote-counting' reviews (Gates 2002). Meta-analytic techniques usually involve combining studies by standardising the outcomes using some metric of the 'effect size'. Effect size is "*the degree to which the phenomenon is present in a population*" or "*the degree to which the null hypothesis is false*" (Cohen 1988, pp 9 - 10).

In the past, researchers have employed various metrics for calculation of the treatment effect size (reviewed in Gurevitch *et al.* 2001). Example metrics include: the log response ratio *lr* (Hedges *et al.* 1999; as used in studies by Shurin *et al.* 2002; Cardinale *et al.* 2006; Worm *et al.* 2006); the standardised mean difference, Hedge's *d* (as used by Brett & Goldman 1996; Curtis 1996; Rustad *et al.* 2001; Maestre *et al.* 2005; McCarthy *et al.* 2006; Bancroft *et al.* 2007; Frampton & Dorne 2007); Fisher's *Z* transform of *r* (Cooper & Hedges 1994; as used by Arnqvist *et al.* 1996); the Odd's ratio (Maestre *et al.* 2005). Choosing the most appropriate metric requires careful consideration (see Gurevitch & Hedges 1999; Osenberg *et al.* 1999), because the metrics have different statistical properties. For example, different metrics suit some data types more so than others (e.g. categorical *vs.* continuous data types). In this study the log response ratio *lr* was used (Section 2.2.5).

2.1.4. Aim.

The overall aim of this chapter was to perform a meta-analysis of published experimental and field studies to quantify the effects of anthropogenic stressors on macroinvertebrate community structure and ecosystem function across streams, and to observe whether responses of structure and function were associated.

2.2. Methods.

2.2.1. Study design.

I compared responses of structure and function across pairs of sites (contaminated *vs.* reference) in different locations, using data from studies published in the academic literature, and made individual comparisons of this sort. A meta-analysis of data compiled from these studies was performed, and specifically, tests were performed to assess whether differences in responses of ecological structure and function between individual site pairs in independent studies were consistently different from zero across site pairs. Subsequently, correlation analyses were performed to test for associations in the responses of structure and function to stress. Selection of well-matched site pairs should ensure that any differences seen across studies were primarily attributable to stress. This study design enabled me to compare streams from very different biomes, which were sampled across different time scales and in different kinds of study designs.

For the purpose of this study three common and relatively well-reported stressors of freshwater ecosystems were examined: acidification, heavy metal contamination and organic pollution. The effects of these stressors were examined exclusively (i.e. individual studies were selected which reported the effects of any one of these three stressors, in the absence of any other confounding stressors), although, in natural ecosystems, multiple stressors often act on the same watercourse, and their effects may be synergistic (e.g. Bowman *et al.* 2006) (Section 1.5.2.3.).

2.2.2. Literature search strategy and study inclusion criteria.

Searches were made of ISI Web of Knowledge (1981 – March 2007) and BIOSIS Biological Abstracts (1985 – March 2007) databases, and Google™ Scholar online, using the following core keyword sequence: *(freshwater OR aquatic) AND (lotic OR river OR stream) AND (acid OR metal OR organic pollution OR nutrient enrichment NOT channelisation NOT siltation NOT drought)*. The search was made three times, and each time one of the following strings was added to the core sequence:

1. *AND macroinvertebrate AND species AND (structure OR richness OR diversity OR biomass OR productivity OR evenness OR community OR assemblage OR density OR abundance);*
2. *AND (function OR decomposition OR process OR processing);*
3. both 1 and 2.

After a list of references had been obtained, studies were systematically reviewed. To be included in the meta-analysis, a study had to meet the following criteria:

1. Study must report results from a novel field or experimental study, and not be a review or secondary presentation of results.
2. Study must have at least one 'suitable' site pair (see Section 2.2.3) (i.e. a contaminated site or treatment group, and a suitable reference site or control treatment group). Sites affected by acid mine drainage were not included, because "*acid mine drainage is a complex agent of stress in that it incorporates several distinct mechanisms of stress, any one of which can affect aquatic ecosystems: 1) acidity, 2) high concentrations of dissolved metals, 3) deposition of precipitated metal oxides e.g. iron hydroxides*" (Niyogi *et al.* 2001).
3. Study must report the impacts of a single stressor on individual sites (i.e. acidification, heavy metal contamination or organic pollution, and no other stressor).
4. Where a study reported responses of structure, it must have been at the level of the whole community, or part of it (see Section 2.2.4). Where information on a single taxonomic group had been reported (e.g. exclusively responses of the order Ephemeroptera: Clements *et al.* 2002) the study was eliminated.

2.2.3. Site pair selection.

Site pairs each comprised one contaminated site and one reference site. In natural streams, upstream – downstream comparisons were accepted (Figure 2.1a), as were independent stream comparisons (Figure 2.1b). In the ideal (hypothetical) situation, differences in abiotic conditions and influences acting within site pairs should be minimal, with the exception of the presence of a stressor at the contaminated site. In reality, there exist a range of abiotic differences within site pairs (no two stream sites will ever be identical). Site pairs were selected to minimise these differences as much as possible.

In the majority of cases, sites had already been designated by the primary researcher as either a 'contaminated' site, or a 'reference' site. In addition, where possible, abiotic data were consulted and selection was made of either the single most contaminated site (preferred), or at 'random' from a pool of several contaminated sites (i.e. by assigning numbers to the pool of sites and then using random numbers to select a single site). The reference site was selected to be either the site most similar in terms of abiotic characteristics to the contaminated site (preferred), or at 'random' from a pool of several uncontaminated sites. It was important that the sites were not only located in close proximity to each other, but had also been sampled at roughly the same point in time (i.e. within a few days of each other), in order to minimise spatial and temporal variability, which may have affected structure and function.

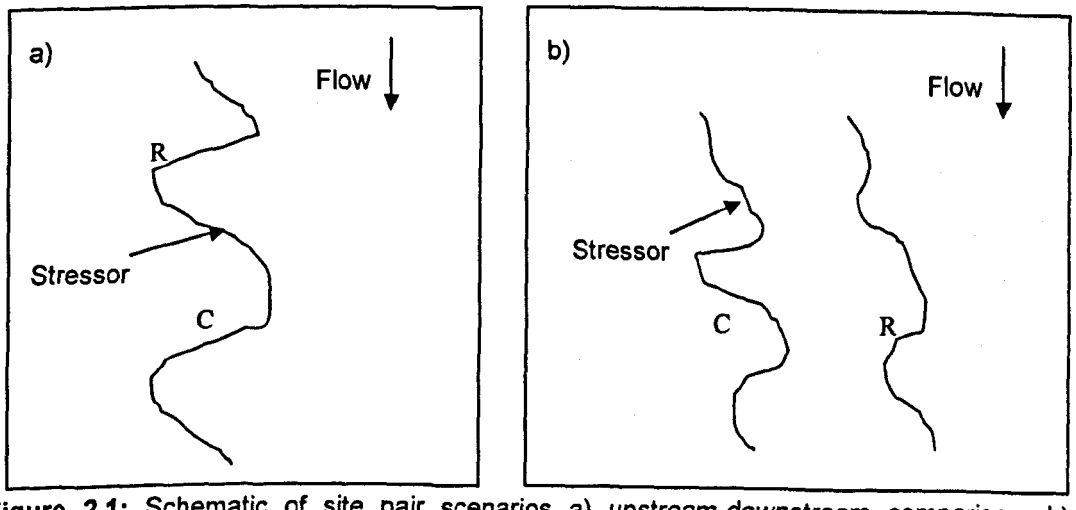

Figure 2.1: Schematic of site pair scenarios a) upstream-downstream comparison, b) independent stream comparison. C = contaminated site, R = reference site.

There were very few studies with many independent site pairs (i.e. multiple replicates) and many studies with a single independent site pair (i.e. a single replicate). It is theoretically possible to use a weighted analysis, where studies with many replicate site pairs are given greater weight than studies with just a single replicate pair (as seen in Arnqvist *et al.* 1996; Curtis 1996; Curtis & Wang 1998; Rustad *et al.* 2001). In practice, however, it is very difficult to calculate confidence intervals for those studies with just a single site pair (i.e. most of the studies). To circumvent this problem, a single replicate site pair from each study was selected for inclusion in the analysis, making studies with multiple replicate site pairs directly comparable with those with only a single replicate site pair.

2.2.4. Data extraction and the kinds of responses reported in the literature.

From each study, responses of structure and function were recorded for one contaminated site and one reference site. Responses of freshwater ecosystem function were reported as either a) the percentage leaf mass loss (L) (Section 3.2.5: Equation 3.2), or b) the leaf breakdown coefficient (k) (Equation 2.1) both calculated after deployment of leaf bags *in situ* (Petersen & Cummins 1974; Boulton & Boon 1991; Gessner 1991) (Section 1.5.2.2.). The leaf breakdown coefficient (k) represents the amount of leaf mass loss we would predict over time, where leaf breakdown is a non-linear (exponential) function of the number of days leaves are deployed into streams for. It is calculated as (Wieder & Lang 1982; Gessner & Chauvet 1994):

$$k = -\frac{\ln\left(\frac{W_z}{W_i}\right)}{t} \qquad \text{Equation 2.1.}$$

where t is the number of days leaf bags are deployed in streams. W_i is the initial mass of leaf material (mg, dried) and W_z is the final mass of leaf material (mg, dried).

Responses of macroinvertebrate community structure were reported at four non-

independent levels of taxonomic resolution (as follows, with reasons for their inclusion):

1) the whole macroinvertebrate community;

   Macroinvertebrate community structure has been extensively studied at this level (Section 1.5.2.1.), is known to be affected by stressors, and is also regularly monitored.

2) the Ephemeroptera - Plecoptera - Trichoptera (EPT) community;

   Some taxa of these orders are known to be particularly sensitive to stressors and are used as part of an EPT index by the US Environmental Protection Agency. The EPT index = number of EPT individuals/number of chironomid individuals (Diptera: Chironomidae). In studies where lists with species presence/absence and/or abundance data were reported, any additional information which could be extracted for the EPT community was also recorded (e.g. number of EPT taxa).

3) the shredder community;

   If a relationship between structure and function does exist, then it is most likely to be between the structure of this community and rates of leaf processing. Members of this community were classified by the primary researcher. This raises the possibility that classification of taxa into this group may be incongruous across studies, because researchers have used different techniques and sources of information. However, the classification should remain consistent within site pairs. In studies where lists with species presence/absence and/or abundance data were reported, additional structural information which could be extracted for the shredder community was not recorded because of the length of time it would have taken to assign individual taxa to functional feeding groups for each individual study, and because this method would perhaps have varied from that used by primary researchers.

4) the community of invertebrates found within leaf bags deployed in streams;

   These were considered separately from the shredder community because many animals from non-shredder feeding groups inhabit leaf bags without actually being involved in the process of leaf fragmentation (e.g. animals

seeking refuge from predators). For example, chironomid larvae are often found in leaf bags, but are not directly associated with leaf processing. That said, many of the taxa found within leaf bags are still likely to be shredders and are therefore likely to be related to rates of leaf processing, if such a relationship exists.

Studies reporting structure quantified responses in different ways, i.e. as density, biomass, number of taxa, or derivatives of these, and were not fully reported for all taxonomic groups. The following is a list of structural measures reported across the literature for which there was more than one replicate study reporting the same information:

1) for the whole macroinvertebrate community:
    - density per unit area,
    - drift density,
    - biomass,
    - number of taxa,
    - Simpson's Index *D*,
    - Shannon Index *H'*;
2) for the EPT community:
    - density per unit area,
    - percentage of total density,
    - number of taxa,
    - percentage of total number of taxa;
3) for the macroinvertebrate shredder community:
    - density per unit area,
    - percentage of total density,
    - percentage of total number of taxa;
4) for the macroinvertebrate community found within leaf bags:
    - density per unit area,
    - biomass,
    - number of taxa.

Data were summarised and tested for all of the above. In the event that a site had been sampled repeatedly over time, the data included in the meta-analysis were either the mean response, or a single date selected at random when both sites had been sampled on the same day. The preferred date selected for extracting the rate of leaf processing from a study was the longest possible after leaf deployment *in situ*. Where information had been presented in graphical form, either authors were contacted to obtain numerical values (preferred), or data were estimated from graphs.

2.2.5. Quantifying the effect size of stressors.

As previously discussed (Section 2.1.5.), it was necessary to calculate some estimate of treatment effect size, commonly the magnitude of an experimental treatment mean (in this case, the mean at a contaminated site $\overline{X}_C$), relative to the control treatment mean (in this case, the mean at the reference site $\overline{X}_R$). In this study, the log response ratio *lr* was used. This metric was appropriate to my data type, and has clear biological meaning (i.e. the proportional change in a response variable between treatment and control), thus making results easy to interpret. It also has good statistical properties, in that it shows the least bias of several metrics and its sampling distribution is approximately normal (Hedges *et al.* 1999), lending itself easily to parametric statistical tests. For each study, the log response ratio was calculated from responses of structure and function, using the following equation (Gurevitch *et al.* 2001):

$$lr = \ln\left(\frac{\overline{X}_C}{\overline{X}_R}\right) \qquad \text{Equation 2.2.}$$

The log response ratio, rather than just the response ratio (for example, as used by Gessner & Chauvet 2002) was used because differences (i.e. $\ln\overline{X}_C - \ln\overline{X}_R$) have better statistical properties than do ratios ($\overline{X}_C : \overline{X}_R$). Basically, if $\overline{X}_C$ and $\overline{X}_R$ are approximately normally distributed, and $\overline{X}_R$ is unlikely to be negative, then *lr* is approximately normally distributed (Curtis & Wang 1998) making data suitable for

using parametric statistical tests (Gurevitch *et al.* 2001).

2.2.6. Statistical analyses.

Two-tailed one-sample *t*-tests were used to test whether mean log response ratios were significantly different from zero. If they were, it was possible to reject the null hypothesis that stressors had no effect on structure and function across site pairs.

Correlation analyses were used to test for associations between structure and function. Data included in these analyses were only from those studies where structure and function had been measured and reported simultaneously. For comparability of results across studies, and to increase the number of studies included in the analyses, percentage leaf mass loss data (L) were converted to k using the following equation:

$$k = -\frac{\ln\left(1+\frac{L}{100}\right)}{t} \qquad \text{Equation 2.3.}$$

2.3. Results.

2.3.1. The kinds of studies included in the meta-analysis.

In total about 500 studies were reviewed, of which 97 met the criteria stated above, including two theses (Hirst 1983 unpublished; Green 1984 unpublished). Of the 97 studies, 28 reported the effects of heavy metals, 38 the effects of acidification, and 31 the effects of organic pollution (Appendix A). Studies were undertaken at various locations around the world (numbers in parentheses indicate the number of studies from each country): USA (41), UK (16), Spain (8), Canada (7), France (6), Portugal (4), Japan (2), New Zealand (2), Sweden (2), Switzerland (2), Australia (1), Ecuador

(1), Italy (1), Germany (1), Netherlands (1), South Africa (1), and Thailand (1). Of these, 18 were studies of experimental treatments which had been applied to either natural streams or artificial stream mesocosms, 78 were field surveys of existing contaminated sites and one was both. Sixty-nine studies reported the effects of stressors on structure, eight reported effects on function and 20 reported effects on both structure and function.

2.3.2. The direction and magnitude of the effect size of stressors and frequency of responses reported.

From estimation of the 'effect size' of stressors, using the log response ratio, responses of structure and function were sometimes significantly different from zero (i.e. there was a consistent effect of stress across streams). A positive response of structure and function indicates that the effect of stress was to increase the value of the metric at the contaminated relative to the reference site, and negative values indicate that the effect of stress was to decrease the value of the metric at the contaminated relative to the reference site. For example, Figure 2.2D shows that there was a consistent increase in the density of individuals per leaf bag at organically polluted sites relative to reference sites (indicated by the green coloured bar and asterisk) across streams.

Enough data were obtained to enable analysis of 25 combinations of structure and stressors (Table 2.2; Figure 2.2) and five combinations of function and stressors (Table 2.2; Figure 2.3). Of the structural responses (Figure 2.2), seven responses were significantly less than zero, two responses were significantly greater than zero, and eighteen responses were not significantly different from zero. Most studies reported structural responses at the level of the whole community. There were very few studies reporting responses at the level of the shredder community or of the community of invertebrates inhabiting leaf bags. The most frequently reported structural information was of differences in either the number of taxa, or the density of individuals per unit area across the whole community, and this was true for all three stressors. There were few studies reporting information on biomass.

Table 2.2: Statistical comparison of the effect size of stressors on ecological structure and function. Data were log response ratios (i.e. $\ln \overline{X}_C - \ln \overline{X}_R$) (see text) between contaminated and reference sites. Mean log response ratios were tested to see if they were significantly different from zero (one-sample *t*-tests). *k* is the leaf breakdown coefficient (see text). ↑ indicates that the effect of stress was to increase the value of the metric of structure or function at the contaminated site relative to reference. ↓ indicates that the effect of stress was to decrease it. Grey text = where n ≤ 5. **Bold text** = where p < 0.05.

	Taxa	Response	Heavy metal contamination				Acidification				Organic Pollution			
			n	t	p		n	t	p		n	t	p	
Structure	WC	Density per unit area	23	2.79	**0.011**	↓	15	2.05	0.059	↓	16	1.36	0.194	↑
		Drift density	-	-	-	-	6	2.48	0.056	↓	-	-	-	
		Biomass	4	4.29	**0.023**	↓	-	-	-	-	3	1.66	0.240	↑
		No. taxa	27	4.46	**<0.001**	↓	22	5.28	**<0.001**	↓	22	3.06	**0.006**	↓
		Simpson's Index *D*	-	-	-	-	-	-	-	-	3	1.55	0.261	↓
		Shannon Index *H'*	-	-	-	-	-	-	-	-	10	1.87	0.095	↓
	EPT	Density per unit area	7	0.80	0.454	↓	2	4.85	0.130	↓	-	-	-	
		% of total density	2	0.52	0.696	↓	-	-	-		3	0.97	0.433	↓
		No. taxa	15	1.59	0.134	↓	13	7.62	**<0.001**	↓	7	2.91	**0.027**	↓
		% of total No. taxa	-	-	-	-	-	-	-	-	7	0.98	0.366	↓
	SC	Density per unit area	2	1.40	0.395	↓	-	-	-	-	-	-	-	
		% of total density	-	-	-	-	-	-	-	-	3	1.84	0.207	↓
		% of total taxa	-	-	-	-	5	3.25	**0.031**	↑	-	-	-	
	LBC	Density	-	-	-	-	5	0.79	0.473	↑	5	3.39	0.028	↑
		Biomass	-	-	-	-	3	0.18	0.877	↓	3	1.93	0.193	↑
		No. taxa	-	-	-	-	-	-	-		2	20.91	0.531	↑
Function		% leaf mass loss (*L*)	-	-	-	-	9	1.81	0.107	↓	6	1.89	0.117	↑
		k	7	2.05	0.086	↓	7	2.34	0.058	↓	8	5.06	**0.001**	↑

Footnotes:

WC = whole community
EPT = Ephemeroptera - Plecoptera – Trichoptera community
SC = shredder community
LBC = leaf bag community
n = number of studies.
- = no data.

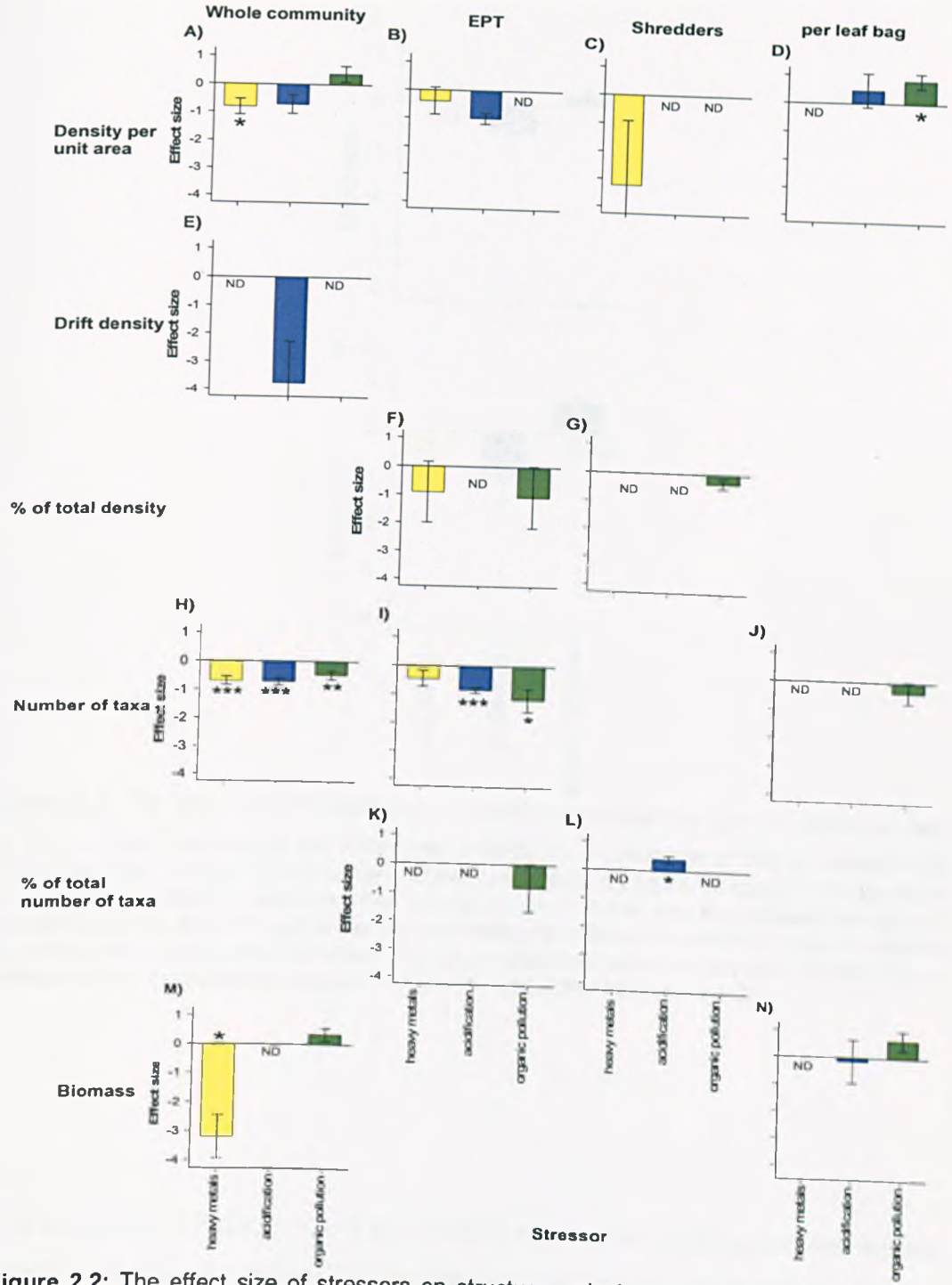

Figure 2.2: The effect size of stressors on structure, calculated as the log response ratio ($\ln \overline{X}_C - \ln \overline{X}_R$) (see text). Colour indicates stressor: yellow = heavy metal contamination; blue = acidification; green = organic pollution. Graphs are laid out in columns and rows, columns reflect different taxonomic groups: far left = whole community; middle left = EPT community = Ephemeroptera - Plecoptera – Trichoptera; middle right = shredders; far right = leaf bag taxa. Rows reflect different structural measures, ranging from: top = density per unit area, to bottom = biomass. ND = no data. * = significant at $p<0.05$, *** = significant at $p<0.001$. A positive trend indicates that the effect of stress was to increase the value of the metric of structure or function at the contaminated relative to reference site. A negative trend indicates that the effect of stress was to decrease the value of the metric of structure or function at the contaminated relative to reference site. Error bars are ± 1 SE.

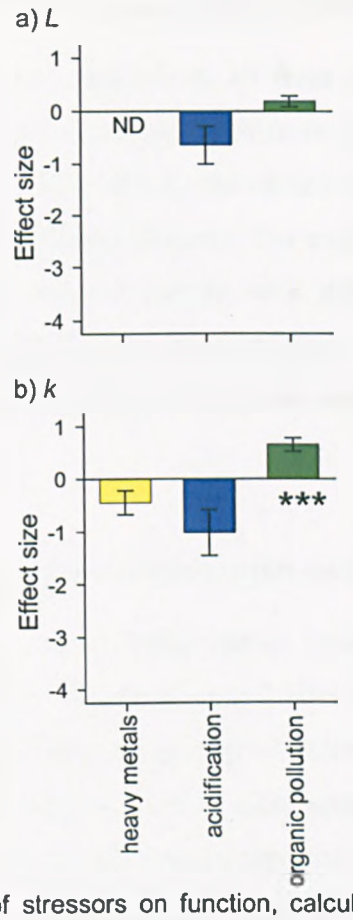

Figure 2.3: The effect size of stressors on function, calculated as the log response ratio ($\ln \overline{X}_C - \ln \overline{X}_R$) (see text) of two measures of function; a) measures of the percentage leaf mass loss over time (*L*); b) the leaf breakdown coefficient (*k*). ND = no data. *** = significant at $p < 0.001$. A positive trend indicates that the effect of stress was to increase the value of the metric of structure or function at the contaminated relative to reference site. A negative trend indicates that the effect of stress was to decrease the value of the metric of structure or function at the contaminated relative to reference site. Error bars are ± 1 SE.

Both responses of function (i.e. *L* and *k*) were reported for acidification and organic pollution, whereas the response of function to heavy metal contamination was only reported as *k* (Table 2.2). The effect size of one out of five responses of function to stress was significantly different from zero (Table 2.2). This was a positive response in the amount of leaf processing (measured as *k*) at organically polluted sites relative to reference sites (Figure 2.3b). Both heavy metal contamination and acidification had no significant effect on the rate of leaf processing across streams.

2.3.3. The consistency of responses to different stressors.

There was only one consistent response to all three stressors; the number of taxa present in a community was, on average, significantly reduced at all contaminated sites relative to reference sites (Table 2.2). No other response of structure or function was consistently sensitive to all three stressors. For example, in streams contaminated by heavy metals, total community density was significantly lower than at the reference, whereas in streams affected by acidification and organic pollution there was no difference in total community density across streams (Table 2.2; Figure 2.2).

2.3.4. The effect of heavy metal contamination on structure and function.

The effect of heavy metal contamination across streams was to reduce the whole macroinvertebrate community at contaminated sites relative to reference sites, in terms of number of taxa, biomass and density of individuals (Table 2.2; Figure 2.2). There was no significant effect on the EPT community, in terms of the number of taxa or density of individuals. Studies rarely reported the effects of heavy metals on the shredder community or animals found in leaf bags. In addition, neither measure of leaf processing was significantly affected by the heavy metal contamination (Table 2.2; Figure 2.3).

2.3.5. The effect of acidification on structure and function.

The effect of acidification across streams was to reduce the number of taxa of the whole macroinvertebrate community, at contaminated sites relative to reference sites (Table 2.2; Figure 2.2). Some of the taxa lost are likely to have been members of the EPT community, because the number of EPT taxa was also reduced at acidified sites. Total community biomass and density of individuals were not significantly affected by acidification. Little or no data exists for the density of EPT taxa. Once again, there was very little information reported on responses of shredder taxa, and the small number of replicates limits any confidence in the results. The rate of leaf processing was not consistently affected by acidification across studies (Table 2.2; Figure 2.3),

although a negative effect of acidification on k was indicated ($p = 0.058$).

2.3.6. The effect of organic pollution on structure and function.

The effect of organic pollution across streams was to reduce the whole macroinvertebrate community in terms of the number of taxa and density of individuals per unit area (Table 2.2; Figure 2.2). It also had the effect of reducing the Shannon index (H'). Organic pollution also reduced the number of EPT taxa present, at contaminated sites relative to reference. k was significantly increased by the presence of organic pollution at a site, whereas % leaf mass loss was not significantly affected (Table 2.2; Figure 2.3).

2.3.7. Does structure predict function?

There were enough data available to perform correlation analyses of the relationship of five different structural metrics *vs.* function. Structural metrics were at the level of either: the whole community a) density, and c) number of taxa; the EPT community d) number of taxa; or the leaf bag community b) density and e) biomass) (Figure 2.4). All functional responses were converted to k using Equation 2.3. Sample sizes were low: a) $n = 11$, b) $n = 6$, c) $n = 11$, d) $n = 5$, e) $n = 5$. There were only 20 independent studies. Of these, many studies reported information on more than one structural response. Thus, across the graphs, many of the points were not independent of each other. For example, study number 74 occurred in four separate analyses. Across the graphs/analyses the value of the structural metric for repeated studies differed, but function, the value of k, remained the same. Studies numbered 66 and 107 occur on three graphs, studies 12, 14, 23, 77, 145, 147, 148, 435, 444 and 447 all occur on two graphs, and all other studies on just one graph: 8, 24, 51, 75, 198, 199, 443. Generally, the low sample size and non-independence of the data were not ideal for meeting the assumptions of statistic tests. Ideally I would have had enough data to test for a relationship between structure and function for each of the three different types of stressors, but in the end I had to combine all three stressors into single

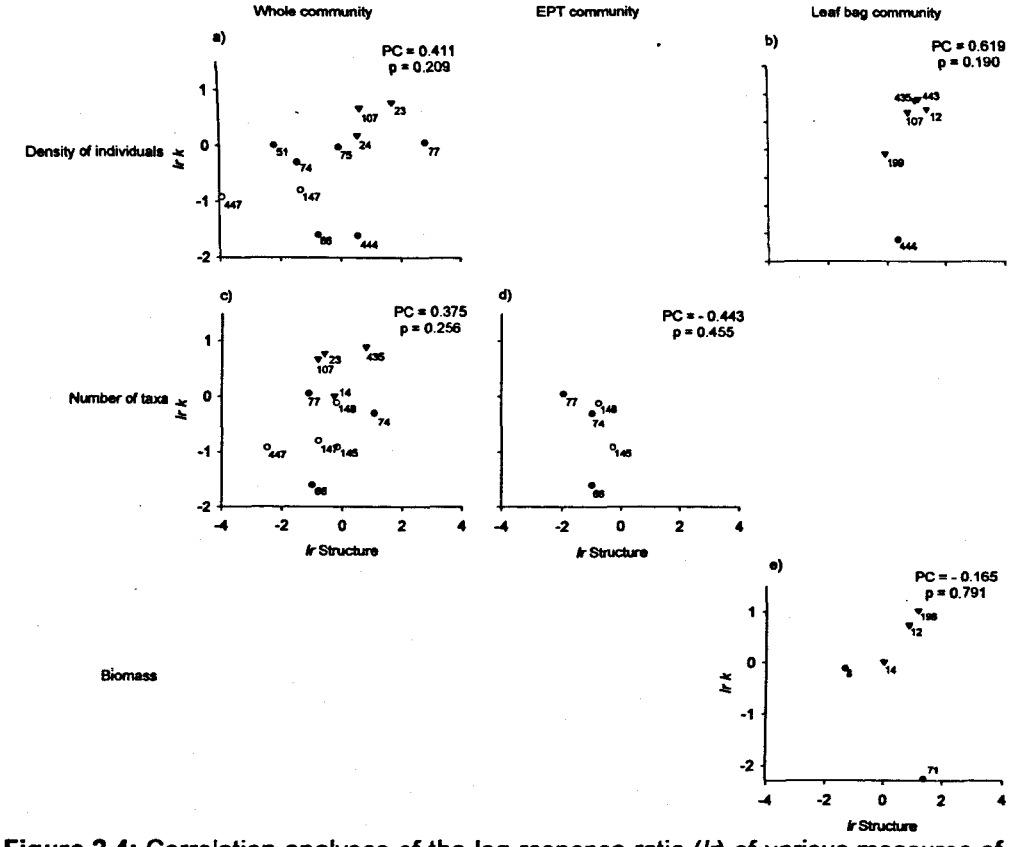

Figure 2.4: Correlation analyses of the log response ratio (*lr*) of various measures of structure *vs.* function (measured as the leaf breakdown coefficient (*k*) see text). From top to bottom are different structural response measures: Density of individuals (a and b); number of taxa (c and d); biomass (e). From left to right are different taxonomic groups: whole community (a and c); EPT community (d); leaf bag community (b and e). EPT = Ephemeroptera - Plecoptera – Trichoptera. PC = Pearson Correlation coefficient. A positive value for *lr* indicates that the effect of stress was to increase the value of the metric at the contaminated site relative to reference site. A negative value for *lr* indicates that the effect of stress was to decrease the value of the metric at the contaminated site relative to reference site. Symbols indicate different stressors: black circles = acidification, white circles = heavy metals, black triangles = organic pollution. Numbers to the right of each data point identify each study.

analyses (see Figure 2.4: circles and triangles).

If the way that stressors affect function is indirectly through structure then we may expect to see a strong positive relationship between the log response ratio (i.e. $\ln \overline{X}_C$ - $\ln \overline{X}_R$) of structure *vs.* function. The more strongly positive (or negative) the *lr* value, the stronger the effect that the stressor was having. A positive value indicates that the effect of the stressor was to decrease the value of the metric at the contaminated site relative to reference and negative *vice versa.* Overall, there were no significant associations between the log responses ratio (*lr*) of structure *vs.* function (Figure 2.4).

2.4. Discussion.

This study addressed a need to understand the relationship between the responses of structure and function to anthropogenic stressors. I achieved this by summarising the effects of anthropogenic stressors on macroinvertebrate community structure and function across streams. After compiling data from studies published in the academic literature, I quantified the effect of stressors on the direction of and consistency of responses of structure and function to three distinct stressors. Finally, I tested for a relationship between structure and function, in order to ascertain whether structure is indicative of function.

The results indicate that stressors had consistent effects on a few responses of structure (Table 2.3a), while having inconsistent or non-significant effects on most (Table 2.3b). For many aspects of both structure and function patterns were indicated, but the power of the test was too low to detect an effect. These are areas where further study is needed in order to clarify patterns (Table 2.3b). Where significant responses were reported in the present study, we can be reasonably confident that these patterns are consistent across regions, because many of the studies were from broadly different locations (Section 2.3.1.). Significant responses of structure were seen in different components of the community, suggesting that particular stressors

Table 2.3: Summary of the patterns of the responses of structure and function which I am either a) fairly sure of because there was enough statistical power in the test to be confident of a true effect; b) unsure of, because the power of the test was low. Asterisks indicate that the significance of the test was not $p < 0.05$, but that it was not far off (i.e. $p < 0.086$) (see Table 2.2).

Stressor	Structure	Function
a) Patterns of which I am sure:		
Heavy metal contamination elicits...	• A decrease in whole community number of taxa. • A decrease in whole community density of individuals (and probably biomass). • No effect on the number of EPT taxa.	
Acidification elicits...	• A decrease in whole community number of taxa. • A decrease in whole community density of individuals* and drift density*. • A decrease in the number of EPT taxa. • An increase in the % of shredder taxa.	• No effect on the rate of leaf processing measured as *L*. • A decrease in the rate of leaf processing measured as *k**.
Organic pollution elicits...	• A decrease in whole community number of taxa. • No effect on whole community density. • No effect on the Shannon index. • A decrease in the number of EPT taxa.	• No effect on the rate of leaf processing measured as *L*. • An increase in the rate of leaf processing measured as *k*.
b) Patterns of which I am unsure, but which are indicated:		
Heavy metal contamination elicits...	• A decrease in whole community biomass. • No effect on density of EPT taxa. • No effect on the density of the shredder community.	• A decrease in the rate of leaf processing, measured as *k**.
Acidification elicits...	• No effect on the density of EPT taxa. • No effect on the density or biomass of the leaf bag community.	
Organic pollution elicits...	• No effect on whole community biomass. • No effect on Simpson's index. • No effect on the % EPT no. taxa or density. • No effect on the shredder community. • An increase in the density of individuals found in leaf bags. • No effect on the biomass or number of taxa found in leaf bags.	

affect different members of the community, as predicted (Section 2.1.2.). Of the two measures of function, only one was significantly affected by organic pollution, and neither were affected by heavy metal contamination or acidification (at the level of $p < 0.05$). There were no associations between responses of structure and function.

2.4.1. The direction of effects.

Most responses of structure and function to stress were negative (i.e. there was a decrease at contaminated relative to reference site). Exceptions to this were some positive responses at organically polluted sites (i.e. there was an increase at organically polluted sites relative to reference site) and a single structural measure which increased significantly in acidified streams (the percentage of shredder taxa) (Figure 2.2; Table 2.3). These results indicate that heavy metal contamination and acidification are more likely to generate decreases in structure and function than organic pollution. However, responses of structure to organic pollution were not consistently positive; there were also significant decreases across organically polluted streams in terms of the number of taxa and the number of EPT taxa.

2.4.2. The consistency of the effects across streams and across stressors.

When considering the consistency of the effects, interpretation is complicated because the statistical tests used were not equivalent in terms of their power to reject the null hypothesis. This is caused by variability in n values, i.e. the number of data points or studies included in each analysis (Table 2.2). A failure to reject the null hypothesis when $n = 4$ is not of the same importance when $n = 23$. Because of the nature of the data (i.e. there were multiple t-tests being performed, each with different levels of replication) we need to be cautious when interpreting the results.

2.4.2.1. Effects on function.

In the present study, data from a total of 28 independent studies were incorporated

into the meta-analysis of the effects of stressors on ecosystem functioning. Patterns were not statistically significant for heavy metal contamination or for acidification (though in some cases the results were close enough to be suggestive) (Table 2.2). The results shown in Figure 2.3b indicate that similar patterns exist in the present study to those indicated by Gessner & Chauvet (2002) in that there is likely to be a decrease in leaf processing downstream of both heavy metal contamination and acidification, and an increase downstream of organic pollution (Section 2.1.1.; Table 2.1).

In the present study, the power of the tests used to detect the effect of stressors on ecosystem function was always very poor, except in the one instance where there was a significant effect (Table 2.2), i.e. an increase in the rate of leaf processing downstream of organic pollution ($p = 0.001$). This confirms that leaf processing is indeed sensitive to the effects of this stress. However, I cannot be confident that there truly was no effect of either heavy metal contamination or acidification on the rate of leaf processing, because of the low power and low sample sizes. More studies are needed to clarify patterns.

Other field studies, which were either unsuitable for inclusion, or have been published since completion of the meta-analysis, have also documented increases in the rate of leaf processing in streams subject to organic pollution. This phenomenon has mostly been attributed to nitrate addition stimulating decomposition rates (Elwood *et al.* 1981; Grattan & Suberkropp 2001; Nikolcheva & Bärlocher 2005; Ferreira *et al.* 2006; Gulis *et al.* 2006) and increases in the activity of the microbial community have been implicated (Pascoal *et al.* 2001; Pascoal *et al.* 2003; Pascoal & Cassio 2004; Pascoal *et al.* 2005a). In contrast, Lecerf *et al.* (2006) conducted a field survey of structure and function in 9 streams in France, subjected to low to high levels of eutrophication. They found that rates of leaf processing in coarse mesh leaf bags (i.e. including macroinvertebrates) were negatively related to ammonia concentrations, whereas rates of leaf processing in fine mesh leaf bags (i.e. excluding macroinvertebrates) remained constant across a gradient of ammonia concentrations. This suggests that the change in the rate of leaf processing was directly attributable to a reduction in the amount of leaf material processed by macroinvertebrate shredders,

and that the microbial community was unable to compensate for the loss of shredder leaf processing.

The results of the present study indicate that the leaf breakdown coefficient (k) (see Equation 2.1) is a better indicator of ecosystem stress than the alternative, the percentage leaf mass loss (L) (see Equation 3.2). This was because significant, or marginally significant, effects of heavy metal contamination or organic pollution were detected for k, where no effects were reported for L for these two stressors. No comparison can be made for acidification because there was no data reported for L. However, k was not significantly affected by acidification ($p = 0.086$).

2.4.2.2. The effect of stressors on macroinvertebrate community structure.

The results of the present study indicate that some, but not all, aspects of macroinvertebrate community structure respond predictably to anthropogenic stressors, which is something we already knew (Simon 2003) but was a reassuring find as it confirms the validity of the method. The most frequently reported response of macroinvertebrate community structure, the total number of taxa, was the most consistently sensitive to all three stressors (Table 2.2; Figure 2.2H). It was reported in a total of 71 out of 97 studies. The significance of these results, in comparison to other tests, may have either been due to a clear effect, or because the larger sample size gave better power to detect the effect. The results were highly significant for all three stressors, allowing confidence that these results reflect a real effect. This result indicates that empirical studies which have involved manipulation of species richness (e.g. the majority of studies in the biodiversity - ecosystem function literature, see Kinzig *et al.* 2001; Loreau *et al.* 2001a; Loreau *et al.* 2002) (Section 1.2) are, indeed, relevant to natural (i.e. stressed) ecosystems.

Another frequently reported response was whole community density, reported in 54 studies. This response was sensitive to heavy metal contamination, but was not sensitive to either acidification or organic pollution, although, once again, the relative

power to detect an effect was so low that we cannot be confident that there was truly no effect of acidification or organic pollution. Likewise, we may be confident that the number of EPT taxa was sensitive to acidification and organic pollution, because the results were significant, but we may have very little confidence that heavy metal contamination had no effect, because the power of the test was so low. We can also be fairly confident that heavy metal contamination affected whole community biomass, but there was no or little data for acidification or organic pollution.

Despite there being very few studies, and low power associated with the tests, we still see that the shredder community was sensitive to the effects of stressors: acidification increased the percentage of shredder taxa present at contaminated relative to reference sites (n = 5), leaf bag community density was significantly increased by organic pollution, and there was indication that heavy metal contamination might have reduced the density of shredder taxa at sites (Tables 2.2. & 2.3). If there is a simple linear relationship between structure and function we would predict that these changes would translate to changes in the rate of leaf processing. Indeed, an increase in the number of taxa inhabiting leaf bags was associated with an increase in the rate of leaf processing in organically polluted streams, and the reduction in shredder density at heavy metal contaminated sites was associated with a decrease in the rate of leaf processing. However, the increase in the number of shredder taxa at acidic sites was not associated with an increase in leaf processing (Figures 2.2 and 2.3). It is important to note that these patterns of structure and function are not necessarily from the same study and were not tested formally. A more rigorous test for an association between structure and function (Sections 2.2.6. and 2.3.7.) is discussed in the following section.

2.4.3. The relationship between structure and function.

In the present study I tried to understand whether a relationship exists between the structure of the macroinvertebrate community and ecosystem function, measured as the rate of leaf processing (Figure 2.4). Ideally, I would have examined the relationship between aspects of structure of the macroinvertebrate shredder functional

feeding group, and the rate of leaf processing. However, due to the limited number of studies (n = 10) reporting information at the shredder community level (Table 2.2) this was not possible. This represents a disparity in the kinds of structural and functional responses being reported in studies, which future studies might try to rectify. Despite these concerns, statistical tests were performed on the data and no relationships were detected between structure and function, where structure was characterised at the level of the whole community (density and number of taxa), the EPT community (number of taxa), the leaf bag community (density and biomass). The lack of any significant association between these aspects of structure and rates of leaf processing might either be because there was no relationship between any aspect of structure and function, or be because the components of structure and function considered were not directly related. In order to determine whether or not there is a relationship between community structure and function we need more studies reporting aspects of structure which directly relate to function. If then there is still no relationship between the macroinvertebrate community structure and ecosystem function, we may be more confident that there truly is no relationship.

2.4.4. Which taxonomic level is most relevant for monitoring the effects of stressors on stream structure and function?

Only at the level of the whole community were the effects of heavy metal contamination and organic pollution detected. The results suggest that responses at the level of the number of taxa in the whole community are both relevant and useful when monitoring the effects of stressors on freshwater ecosystems. The effects of acidification were detected at the level of the number of EPT taxa and the percentage of shredder taxa. The number of EPT taxa is often used to report responses of structure to pollution (e.g. Wallace *et al.* 1996) (Section 2.2.4), because many of the individual taxa are sensitive. The results of this study indicate that a more consistent response is seen at the level of the whole community than at the level of the EPT community (although there were far fewer studies reporting at the level of the EPT community).

The results show that the structural metric 'number of EPT taxa' was not very sensitive to the effects of stressors. Underpinning this are two possibilities: either that EPT taxa are simply more tolerant to pollution than many believe them to be, or that the loss of pollution-sensitive EPT taxa allowed other pollution-tolerant taxa to colonise the contaminated sites relative to reference. Winner *et al.* (1980) first suggested that as levels of heavy metal pollution increases, the more sensitive insects representing EPT orders appear in communities less frequently. However, *Baetis tricaudatus* Dodds (Ephemeroptera: Baetidae) has been shown to be one of the least tolerant insects to heavy metal pollution (Kiffney & Clements 1994a) and yet it was present at heavy metal impacted sites in the Coeur d'Alene River, Idaho, US (Hoiland *et al.* 1994) suggesting some taxa of these orders are able to develop tolerance to heavy metals. This brings into question the use of the EPT index (Section 2.2.4.) as a biomonitoring tool.

2.4.5. Caveats.

As with any analysis of this sort a number of possible biases and limitations should be borne in mind. Working with published results raises the issue of publication bias: the failure to publish non-significant results (Gurevitch & Hedges 1999). If this phenomenon was acting on my results, it would manifest as a positive bias in the mean effect size (Begg 1994; Jennions & Møller 2002). Since the mean effect size tested for each response was not usually statistically significant greater than zero, this suggests that publication bias was not acting strongly on these results or that the effects are even rarer than observed here.

Another issue of interpretation is the number of tests carried out. A total of twenty-seven *t*-tests were performed (Table 2.2). This raises a small, but important, possibility that some significant results could be spurious. However, with 27 tests we would only expect 1-2 results to appear significant by chance at the 0.05 level. In fact, most of the results that were significant were at much lower *p* values, providing

little support for the view that the effects seen here are a statistical artefact.

When interpreting the results from this study it is also important to note that several of the measures are not independent of each other. Measures of function (i.e. k and L) are not independent of each other. Measures of structure are also not independent of each other, for example, changes in the EPT community and the shredder community are not independent of each other, as some shredders belong to the Plecoptera and Trichoptera orders. The implications of this non-independence of results would be of greater importance if, for example, most of the significant patterns had been seen at the level of the EPT community, and these translated to weaker effects seen at the level of the whole community. Certainly for acidification and organic pollution, the number of EPT taxa was significantly affected by stress, and so were measures of the whole macroinvertebrate community. Ideally here we would also have a measure of how the non-EPT community was affected, but these data were never presented by the primary researcher, and would, if I had extracted the information from species lists, have had a low sample size, as full species lists were given in few studies.

2.4.6. Conclusions.

Stressors have strong and significant effects on the number of taxa of the whole macroinvertebrate community. This pattern can be generalised across stream ecosystems located in different regions. Stressors may have strong effects on other aspects of macroinvertebrate community structure. However, the results were inconsistent and the power of the analyses on these aspects were limited, due to low sample sizes.

Evidence from the present study suggests that there may be effects of some stressors on the rate of leaf processing (e.g. there was a significant increase in the rate of leaf processing downstream of organic pollution). There is a need for more studies to be undertaken in order to increase the power of analyses like these and to clarify the patterns.

In the present study structure was not indicative of function. This may have been because information was not available on the functional group most closely related to function (i.e. macroinvertebrate shredders). There is also a strong need for studies reporting information on functionally relevant species in communities, i.e. for more studies reporting information on both structure and function measured simultaneously.

3. The effect of heavy metal contamination on structure-function relationships in streams.

3.1. Introduction.

Determination of the nature of the relationship between ecological structure and function is fundamental to our understanding of ecosystems (Section 1.2.). Consideration of the effects of stressors on this relationship will aid in the protection and management of ecosystem services, in the context of current severe environmental changes resulting from human activities. In Chapter 2, I examined the effects of various stressors on the relationship between structure and function in stream ecosystems. A major gap was identified in the information available on the structure of the macroinvertebrate shredder functional feeding group and the rate of leaf processing from the same studies, preventing a comparison of the effects of stressors on both. The present study aimed to address this gap by using synoptic measurements of community structure and function to assess the effect of heavy metal contamination.

3.1.1. The effects of heavy metal contamination on macroinvertebrate community structure and function.

So far in this study I have reviewed the effects of heavy metal contamination on macroinvertebrate community structure, in terms of the kinds of responses we might expect to see and the mechanisms by which heavy metals affect macroinvertebrate community structure (Section 1.6.1.1.). I have also provided a quantitative review of these effects (Chapter 2) demonstrating that heavy metal contamination is likely to elicit effects on the structure of the whole community measured as the number of taxa, density of taxa and biomass (Table 2.2).

I have also reviewed the effects of heavy metal contamination on the rate of leaf processing (Chapter 2). Across the seven studies that documented this effect there was on average no significant effect of heavy metal contamination on the rate of leaf processing, although the power to detect an effect was low. The mechanisms by which stressors generate effects on the rate of leaf processing are poorly

understood. In Chapter 1 I hypothesized that effects may operate through direct or indirect pathways (Section 1.6.3.). Not much is known about how direct effects might operate (Section 1.6.3.1.), but to illustrate, if heavy metal contamination contains iron, leaves may become coated in a layer of ochre (Fe(III) oxide) rendering them unpalatable to macroinvertebrate shredders and preventing colonisation by microbes. If this happens leaf breakdown will occur at a much reduced rate, if at all. Indirect effects might result if metals affect rates of leaf processing by macroinvertebrate shredders (Section 1.6.3.2.). For example, Taylor *et al.* (1994) observed feeding avoidance behaviour in a key shredder species, *Gammarus pulex* L. (Amphipoda: Gammaridae), when exposed to natural sediments contaminated with copper. Recent evidence suggests that feeding inhibition by *G. pulex* results from aqueous, rather than dietary exposure, to heavy metals (zinc) (Wilding & Maltby 2006). Alternatively, effects may be mediated through the microbial community (Section 1.6.3.3.). For example, Bermingham *et al.* (1996a) found reduced rates of leaf processing downstream of metal mining were associated with reductions in fungal activity.

3.1.2. The relationship between structure and function in heavy metal contaminated streams.

In Chapter 2 I identified five studies which documented the effects of heavy metal contamination on measures of structure and function simultaneously in the same streams. All of these studies took place in the USA and they were all field surveys (Table 3.1). Most of the studies followed the same design, i.e. sample sites were located at multiple locations along a single stream (Schultheis *et al.* 1997; Nelson 2000; Chaffin *et al.* 2005; Woodcock & Huryn 2005). The design of one study differed and sample sites were located across independent streams (Carlisle & Clements 2005). Each study reported different aspects of macroinvertebrate community structure (Table 3.1), and there was some indication that aspects of structure and function were associated. No studies formally tested for a relationship between structure and function.

Table 3.1: Summary of the five previous studies to have documented responses of structure and function to heavy metal contamination in streams.

Reference	Location	Study Design	Metal stress	Pattern at contaminated site, relative to reference		Leaf type
				Macroinvertebrate community structure	Rate of leaf processing	
Schultheis *et al.* (1997)	Virginia, USA	Single stream: 4 upstream reference sites, 3 downstream contaminated sites	Copper	Decrease shredder abundance, and changes in shredder community composition.	Decrease	*Acer rubrum* (L.) (red maple)
Nelson (2000)	Arkansas River, Colorado, USA	Single stream: 2 upstream reference sites, 2 downstream contaminated sites	Manganese, zinc	Reduced no. taxa and shredder density.	No change	*Populus tremuloides* (Aspen)
Woodcock & Huryn (2005)	Maine, USA	Single stream: 4 upstream reference sites, 4 downstream contaminated sites	Iron, manganese and zinc	No change no. taxa. Change in no. EPT taxa and total community biomass. *Tipula* biomass compensated for loss of other shredder taxa.	No change	*Acer rubrum* (L.) (red maple)
Carlisle & Clements (2005)	Colorado, Rocky Mountain streams, USA	Multiple independent streams: 3 contaminated, 2 reference	Zinc	Decrease in total shredder production. No compensation for dominant shredder taxa.	Decrease	*Salix* spp.
Chaffin *et al.* (2005)	Appalachian mountains, USA	Single stream: 4 upstream reference sites, 4 downstream contaminated sites	Arsenic	Reduced densities of shredders.	Decrease	Red maple and white oak

3.1.3. Aims and objectives.

The overall aim of this chapter was to conduct field studies to document the effects of heavy metal contamination on the relationship between macroinvertebrate community structure and ecosystem function in streams. This was addressed by collecting field data on both aspects of the community at multiple contaminated and reference sites.

Shredders are the functional feeding group which feed directly on, and assimilate energy from, large fragments of leaf litter. It is expected that this group will have a distinct association with rates of leaf processing. To separate out this potentially important group, the structures of the shredder and non-shredder components of the community were considered separately. Despite the primary role of shredders, it is also important to consider the non-shredder community, as changes in other functional feeding groups may have indirect influences on the rate of leaf breakdown. For example, some species of mayfly larvae (Ephemeroptera), belonging to the collector functional feeding group, scrape fine particles from the surface of leaves, with potential effects on leaf breakdown. Some predator species may also play a role in influencing the rate of leaf processing through indirect effects on shredders (e.g. Oberndorfer *et al.* 1984; Malmqvist 1993).

3.2. Methods.

The study design consisted of comparisons between pairs of sites, one site with a history of heavy metal contamination, and the other a nearby uncontaminated ('reference') site. At all sites direct measurements were made of metals, a suite of abiotic factors (Section 3.2.2.) and macroinvertebrate community structure and function. Sites were visited on two occasions three weeks apart, during the months of July and August 2004.

3.2.1. Study site selection.

Two regions were selected for investigation from several regions in the UK known to be historically contaminated with heavy metals (Appendix B). The first eight sites were located around the town of Leadhills, Lanarkshire in south west Scotland (henceforth 'the Leadhills'), with the remaining twelve sites located in Cornwall, south west England. Four site pairs were selected in the Leadhills (Figures 3.1 & 3.2a) and six in Cornwall (Figures 3.1 & 3.2b). Site pair dossiers, which were compiled *a priori*, are detailed in Appendices C and D respectively. For basic site descriptions see Table 3.2 and environmental data see Table 3.3. Sites were selected using information detailed in previous studies and from routine monitoring programmes of the Environment Agency (EA) and the Scottish Environmental Protection Agency (SEPA).

Contaminated sites had to fulfil all of the following criteria:

1. There was information which indicated contamination, primarily by heavy metals, e.g. water chemistry data from the EA or from a previous study (Appendices C & D).
2. There was information which indicated no secondary contamination, e.g. no organic pollution, acidic deposition (pH of sites had to be > 6), acid mine drainage, coal mine effluent, or agricultural influence.
3. There was a nearby reference site.
4. The site was independent of the other contaminated sites.
5. That there were several contaminated sites (minimum of 4) all located in close proximity to each other (< 1 hour driving distance of each other).

It was preferable that some background information was available of the macroinvertebrate community at both contaminated and reference sites and that there was the potential for detritus processing (i.e. that macroinvertebrate shredders were present) (Appendices C & D).

Following identification of suitable contaminated sites, it was necessary to locate a nearby partner reference site. Reference sites were selected to minimize variation in environmental and abiotic factors, separate from the heavy metal

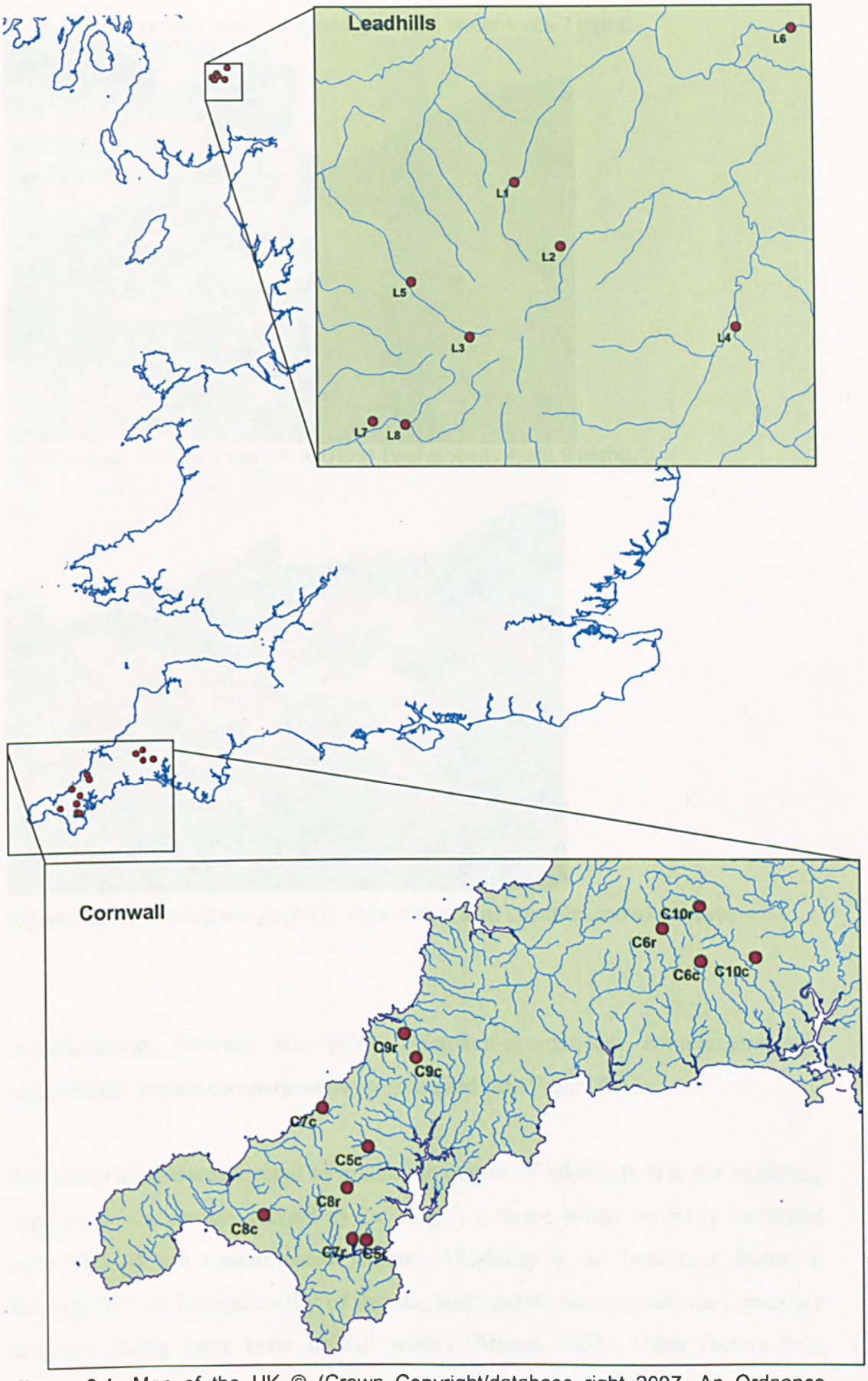

Figure 3.1: Map of the UK © (Crown Copyright/database right 2007. An Ordnance Survey/EDINA supplied service). The location of the 20 sample sites are indicated by red circles and labelled with either a capital L = Leadhills, or a capital C = Cornwall. In the Leadhills, sites were numbered 1-8; in Cornwall, sites were paired (site pairs C5-C10) and were either denoted small letter c = contaminated or small letter r = reference site.

a) Glengonnar Water = site 1 (left) and Mennock Water = site 7 (right).

b) Porthtowan Stream = site 13 (left) and Twelveheads = site 9 (right).

Figure 3.2: Example photographs of stream sites in a) Leadhills and b) Cornwall.

contamination, between site pairs. Upstream-downstream comparisons and independent stream comparisons were accepted (see Figure 2.1).

Reference sites were selected to match the degree of alkalinity (i.e. the buffering capacity of the stream, related to Ca^{2+}/Mg^{2+}, a factor which normally correlates with pH) of the contaminated stream. Alkalinity is an important factor in determining the bioavailability of metals, with metals like copper, lead, mercury and zinc being more toxic in soft waters (Mason 2002). Other factors (e.g. locality, stream order, and lack of any contamination) were also considered when choosing reference sites.

Table 3.2: Summary of site descriptive information for the twenty streams used in the field study. Sites 1-8 were in the Leadhills, and sites 9-20 were in Cornwall. Sites in Cornwall were paired (*contaminated vs. reference*): odd numbered sites were contaminated with heavy metals and even numbered sites were reference sites. Tr. = tributary. Data for site 1-8 was estimated from an OS Explorer 1: 25,000 map. Stream order was estimated using the Strahler method (Dobson & Frid 1998). All data for sites 9-20 came from the Environment Agency central database, using the procedure documented in Murray-Bligh *et al.* (1997), with the exception of stream order and land use type, which were extracted from a CEH online database (http://www.ceh.ac.uk/sections/seo/lcm2000_home.html). Mean annual discharge is estimated as a category 1-10.

Site no.	Site pair no.	Site name	NGR	Altitude (m above sea level)	Distance from source (km)	Mean annual discharge (m^3/s)	Slope (m/km)	Land use type	Stream order
1	-	Glengonnar Water	NS887177	330	8.0	-	-	-	2
2	-	Tr. Elvan Water	NS901157	380	0.25	-	-	-	1
3	-	Wanlock Water A	NS873129	390	0.75	-	-	-	1
4	-	Allershaw Burn	NS955132	290	1.0	-	-	-	1
5	-	Wanlock Water B	NS855146	310	3.0	-	-	-	1
6	-	Tr. Camps Water	NS973224	265	2.0	-	-	-	3
7	-	Mennock Water	NS843103	195	6.1	-	-	-	3
8	-	Tr. Mennock Water	NS853102	225	4.3	-	-	-	3
9	5	Twelveheads	SW76154206	20	4.5	2	12	Arable cereals	3
10		Trenarth Bridge	SW75772830	5	3.6	1	18.2	Coniferous woodland	2
11	6	Crow's Nest	SX26406938	195	1.8	1	5	Improved grassland	1
12		Harrowbridge	SX20667440	210	8.25	3	9.9	Broad-leaved/mixed woodland	3
13	7	Porthtowan Stream	SW69544740	5	3.2	1	19.5	Suburban/rural development	2
14		Polwheveral Bridge	SW73772900	12	6.6	1	50	Broad-leaved/mixed woodland	3
15	8	Godolphin Stream	SW60433208	39	1.95	1	4.6	Broad-leaved/mixed woodland	2
16		Tregolls Bridge	SW72953605	120	5.3	1	8.7	Acid grassland	3
17	9	East Wheal Rose Bridge	SW834552	49	1.4	1	14.3	Improved grassland	1
18		Rosecliston	SW81715877	17	2.55	1	18.2	Improved grassland	2
19	10	Haye Farm	SW 346 701	95	1.2	1	16.7	Broad-leaved/mixed woodland	2
20		Trebartha Road Bridge	SX 2629 7782	130	10.1	3	8.7	Improved grassland	3

Table 3.3: Summary of environmental data collected for the twenty streams used in the field study (mean and SE in parentheses). Sites 1-8 were in the Leadhills, and sites 9-20 were in Cornwall. Sites in Cornwall were paired (contaminated *vs.* reference): odd numbered sites were contaminated with heavy metals and even numbered sites were reference sites. Tr. = tributary. Flow rate was measured using a Valeport Electromagnetic Flow Meter. Canopy cover and substrate types were estimated using a visual judgement (see text).

Site no.	Site pair no.	Site name	NGR	Width (m)	Depth (cm)	Flow rate (m/s)	Canopy cover (%)	Substrate type (%)			
								Boulders/ Cobbles	Pebbles/ gravel	Sand	Silt
1	-	Glengonnar Water	NS887177	2.7 (0.19)	21.5 (2.89)	0.41 (0.08)	0 (0)	22.5 (10.5)	63.3 (11.5)	11.7 (4.6)	2.5 (1.7)
2	-	Tr. Elvan Water	NS901157	3.2 (0.67)	26.7 (5.94)	0.30 (0.04)	0 (0)	50 (15.3)	48.3 (15.5)	0 (0)	1.7 (1.7)
3	-	Wanlock Water A	NS873129	1.4 (0.21)	8.5 (1.68)	0.23 (0.05)	20 (17)	75 (9.6)	38.3 (13.8)	0 (0)	0 (0)
4	-	Allershaw Burn	NS955132	0.7 (0.14)	11.2 (1.42)	0.10 (0.05)	0 (0)	63.3 (12.0)	36.7 (12.0)	0 (0)	0 (0)
5	-	Wanlock Water B	NS855146	0.7 (0.08)	4.4 (0.72)	0.24 (0.07)	0 (0)	11.7 (5.4)	81.7 (4.0)	6.7 (2.1)	0 (0)
6	-	Tr. Camps Water	NS973224	1.4 (0.21)	13.8 (3.50)	0.22 (0.10)	0 (0)	94.2 (3.3)	4.2 (3.3)	1.7 (1.1)	0 (0)
7	-	Mennock Water	NS843103	5.9 (0.19)	13.7 (2.96)	0.42 (0.08)	0 (0)	65 (7.3)	29.2 (4.7)	5.8 (2.7)	0 (0)
8	-	Tr. Mennock Water	NS853102	2.1 (0.20)	14.7 (3.49)	0.49 (0.11)	0 (0)	60.8 (7.5)	28.3 (3.8)	7.5 (3.6)	0 (0)
9	5	Twelveheads	SW76154206	2.3 (0.43)	14.4 (1.91)	0.22 (0.05)	16.7 (8.82)	0 (0)	42.5 (14.8)	42.6 (12.0)	16.7 (7.6)
10		Trenarth Bridge	SW75772830	2.4 (0.12)	12.5 (1.87)	0.06 (0.01)	33.3 (33.3)	41.7 (14.0)	27.2 (9.5)	19.2 (7.2)	10.0 (3.9)
11	6	Crow's Nest	SX26406938	1.9 (0.16)	10.7 (0.72)	0.23 (0.06)	100.0 (0)	56.7 (12.8)	36.7 (11.4)	3.8 (1.5)	2.8 (1.8)
12		Harrowbridge	SX20667440	5.7 (0.25)	22.7 (2.33)	0.21 (0.02)	95.0 (5.0)	45.83 (9.87)	16.7 (7.6)	35.0 (3.2)	2.5 (1.1)
13	7	Porthtowan Stream	SW69544740	1.3 (0.09)	21.6 (2.40)	0.14 (0.06)	0 (0)	30.8 (17.6)	45.0 (12.5)	19.2 (6.8)	7.5 (4.0)
14		Polwheveral Bridge	SW73772900	2.7 (0.23)	9.9 (1.25)	0.14 (0.03)	80.0 (10.0)	33.3 (10.8)	62.5 (12.3)	0.8 (0.8)	0 (0)
15	8	Godolphin Stream	SW60433208	1.8 (0.05)	24.7 (2.39)	0.10 (0.01)	100.0 (0)	0 (0)	11.7 (5.9)	20.0 (9.0)	66.7 (15.0)
16		Tregolls Bridge	SW72953605	2.7 (0.06)	11.6 (1.22)	0.08 (0.02)	100.0 (0)	32.5 (10.5)	35.0 (12.8)	21.7 (6.0)	10.8 (7.9)
17	9	East Wheal Rose Bridge	SW834552	0.9 (0.20)	20.7 (2.31)	0.05 (0.02)	0 (0)	13.3 (8.43)	29.2 (6.4)	27.2 (2.3)	25.0 (6.8)
18		Rosecliston	SW81715877	2.6 (0.49)	14.5 (2.85)	0.11 (0.03)	100.0 (0)	0 (0)	12.0 (7.4)	40.0 (15.7)	48.0 (16.5)
19	10	Haye Farm	SW 346 701	2.9 (0.17)	9.4 (0.72)	0.13 (0.03)	100.0 (0)	57.5 (11.4)	26.7 (7.9)	10.8 (2.4)	5.0 (1.8)
20		Trebartha Road Bridge	SX 2629 7782	5.8 (0.45)	28.2 (1.41)	0.10 (0.02)	100.0 (0)	46.7 (6.15)	28.3 (4.0)	17.5 (1.7)	5.8 (2.7)

3.2.2. Quantifying abiotic variables.

Physicochemical factors:

Measurements of dissolved oxygen (DO) (% saturation), temperature (°C), pH, conductivity (mV/s) and flow rate (m/s) were made using hand held meters (DO with a Hanna H19142 meter, conductivity with a Jenway 4071 meter, pH and temperature with a Jenway 3310 meter, and flow rate with a Valeport 801 Electromagnetic Flow Meter, model no.: 801). Readings were taken three or more times, on both visits to sites.

Environmental factors:

A visual judgement of percentage canopy cover and estimation of stream substrate cover was made on both visits. Stream substrate cover was estimated using RIVPACs guidelines (Murray-Bligh *et al.* 1997) by assigning substrate to one of four categories: boulders (> 64 mm), pebbles (2 – 64 mm), sand (0.06 - 2 mm) and silt (< 0.06 mm), then by judging the percentage cover of each. These measurements, along with stream width and depth, were recorded three times on each visit.

Water chemistry:

Water chemistry was only sampled and tested on the second visit to each site. Three one-litre water samples from each site were analysed for nutrient concentrations (nitrate, nitrite, phosphate, ammonia) and alkalinity ($CaCO_3$), using a Palintest® Photometer 5000 kit, within six hours of sampling.

Heavy metal concentrations:

In addition, on both visits replicate 200 ml water samples were collected (on first visit 6 x 200 ml water samples were collected from Cornwall, and 3 x 200 ml samples from Leadhills; on second visit 6 x 200 ml samples were collected from both Cornwall and Leadhills). Samples were immediately acidified with 100 µl nitric acid (HNO_3) per sample, and later frozen and analysed in the lab for a series of metallic elements (Fe, Zn, Pb, Mn, Cu, Ni, Sn, Cr, Al, Cd) using a flame mass spectrometer. Minimum detection limits for each element were as follows: Mn < 0.007 mg/l, Fe < 0.01 mg/l, Pb < 0.02 mg/l, Zn < 0.004 mg/l, Cu < 0.014 mg/l, Sn

< 0.011 mg/l, Al < 0.08 mg/l, Ni < 0.02 mg/l, Cd < 0.5 µg/l, Cr < 0.011 mg/l. Three out of the six 200 ml water samples collected on each visit were filtered on site (using Whatman® No. 1 Filter papers and a funnel) and were tested for 'dissolved' metal concentrations. The three remaining 200 ml water samples were not filtered and were tested for 'total' metal concentrations. Total metals were not sampled on the first visit to the Leadhills. On analysis of heavy metal concentrations, site pairs were once again checked for suitability: i.e. that contaminated sites had elevated heavy metal concentrations and reference site did not (i.e. using criteria detailed in Section 3.3.1.).

3.2.3. Quantifying biotic variables.

Stream benthic macroinvertebrates were sampled on the first visit to sites using a Surber sampler. This method was chosen because it measures density (number of individuals per unit area), where other methods (e.g. kick sampling) do not. Ten 0.1 m^2 samples were taken at each site, moving diagonally across and upstream in order to sample as many stream benthic habitat types as possible. The contents of each sample were preserved with 70 % industrial methylated spirits (IMS) in sealed and labelled pots for storage.

In the laboratory, macroinvertebrates were identified to species level where possible and counted. Taxa were distinguished into shredder or non-shredder functional feeding groups using published information (Merritt & Cummins 1996; Bis & Usseglio-Polantera 2004). The biomass of individual shredder species per Surber sample was calculated after drying in pre-weighed foil cups in an oven at 60 °C for 8 days and weighing on a Cahn 25: Automatic Electrobalance (reading precision 0.1 µg). The biomass of non-shredders per Surber sample was calculated after oven-drying and weighing on a Mettler AT261 Delta range Electrobalance (reading precision 10 µg).

Macroinvertebrate community structure was characterised as number of taxa, density (ind/0.1m^2) and biomass (mg/0.1m^2) of the shredder, non-shredder community and whole macroinvertebrate community per site. Estimates of the

number of taxa may be conservative because not all taxa were identified to species level, e.g. large numbers of dipteran larvae.

3.2.4. Quantifying leaf processing.

The rate of leaf processing is usually measured at the time of peak leaf-fall (i.e. autumn in the UK) (Boulton & Boon 1991). In this study it was measured in the summer months, because this is the time when stressors were predicted to have the largest impact on the stream biota, due to low flow rates and nutrient limitations.

A single leaf type was used to quantify leaf processing rates. Alder (*Alnus glutinosa* (L.) Gaertner) is a common riparian tree species whose leaves are highly palatable to macroinvertebrate shredders (Leroy & Marks 2006) and known for their relatively fast breakdown rates (Chamier 1987). Leaves were collected from two locations close to Sheffield: Rivelin Valley (NGR: SK313878) and Harper Lees (NGR: SK234806). Leaves were collected just prior to abscission in autumn 2001 and air-dried at room temperature for one week prior to storage.

Mesh bags to hold the alder leaf material were constructed (roughly 20 x 10 cm). This relatively large size ensured that the leaf-material contained within the bags was exposed as much as possible to exogenous processing, such as abrasion and macroinvertebrate shredding (Benfield *et al.* 1977; Boulton & Boon 1991). There were two different mesh sizes: coarse and fine mesh. Coarse mesh was standard greenhouse shelter netting, whose aperture was 3.5 x 7 mm. This was large enough for most macroinvertebrates to pass through (i.e. allowing bacteria, fungi and macroinvertebrates access to the alder leaves). The fine mesh was a nylon mesh made by Plastok®, whose aperture was 400 x 600 μm. This aperture was large enough to allow the largest fungal spores to fit through sideways, but narrow enough to exclude macroinvertebrate shredders.

Each mesh bag was filled with 4 g (± 0.05 g) of alder material, along with some small pebbles to weigh it down. Twelve coarse and twelve fine mesh bags were

deployed at each site on the first visit. Litter bags were strung together in groups of four on fishing line (breaking strength 80 lb), leaving a gap of 0.5 m between each bag. Each string of bags was secured to a brick and placed on the river bed. Cobbles or boulders found *in situ* were used as additional anchors. At each site, the six strings of litter bags were positioned within the stream at intervals of approximately 5 m apart on alternating sides of the stream. I ensured that all litter bags were fully submerged.

Litter bags were deployed *in situ* for a period of 3 weeks. This provides enough time for micro-organisms to have colonised leaf bags (Bermingham *et al.* 1996a), but not so long that leaf processing would reach 100% mass loss. On the second visit to sites, each individual litter bag was detached from the fishing line, and placed into a labelled polythene bag. Litter bags were frozen and processed in the laboratory at a later date. After thawing, macroinvertebrates were removed from the leaves. Leaf material was sieved (through sieves constructed from the same mesh as used for the litter bags) to remove any small fragments of leaf material that may have been washed into the litter bag or retained after fragmentation by shredders, and to remove any silt. Leaves were then air dried at room temperature until a constant mass was achieved.

Leaf processing was expressed as both percentage leaf mass loss (L) and as the leaf breakdown coefficient (k). Both measures were calculated for both fine and coarse mesh leaf bags. Percentage leaf mass loss (L) was calculated as:

$$L = 100\left[1 - \left(\frac{W_z}{W_i}\right)\right] \qquad \text{Equation 3.1.}$$

where W_i was the initial mass of leaf material (mg, air dried) and W_z was the final mass of leaf material (mg, air dried).

I estimated the amount of leaf processing attributable to shredders as (NB: this is oversimplification as there will be other confounding differences between fine and

coarse mesh leaf bags and their rates of leaf processing, e.g. the effects of the exclusion of shredders on the microbial community):

$$L_{Shred} = L_{Coarse} - L_{Fine} \qquad \text{Equation 3.2.}$$

where L_{Coarse} is the percentage of leaf mass loss from coarse mesh leaf bags and L_{Fine} is the percentage of leaf mass loss from fine mesh leaf bags.

The leaf breakdown coefficient (k) represents the amount of leaf mass loss we would predict over time, where leaf breakdown is a non-linear (exponential) function of the number of days leaves are deployed in streams. It is calculated as (Wieder & Lang 1982; Gessner & Chauvet 1994):

$$k = -\frac{\ln\left(\frac{W_z}{W_i}\right)}{t} \qquad \text{Equation 3.3.}$$

where t is the number of days leaf bags were deployed in streams (i.e. 21 days).

3.2.5. Statistical analyses and data presentation.

A series of one-sample t-tests were used to test whether differences in measures of structure and function across site pairs were significantly different from zero. Here, the unit of replication was site pair.

A series of correlation analyses were used to examine relationships between structure and function. Here, the unit of replication was individual site. Six regression analyses of structure (shredder and non-shredder) *vs.* L_{Shred} (see above Equation 3.2) were performed on all three aggregate measures of structure: number of taxa, density of individuals and biomass. Within a panel of graphs, to reduce heteroscedasticity and to aid in comparability across measures and across graphs, where it was necessary for one graph to be transformed, then all graphs within the panel were log (base 10) transformed.

Principal Component Analysis (PCA) was used to assess the similarity in macroinvertebrate community composition among stream sites. Raw data were log transformed (*Ln(x+1)*) in order to overcome the sensitivity of PCA to abundance data that includes differences across orders of magnitude (Whittle 2000 unpublished). I removed any taxa which only occurred at a single site. Two separate PCAs were performed for: a) the whole community and b) the shredder community only, using covariance matrices (i.e. abundances were not standardised).

3.3. Results.

3.3.1. Assessment of heavy metal contamination at sites.

For a summary of heavy metal concentrations sampled in the water at the 20 stream sites see Table 3.4 for dissolved metal concentrations and Appendix E for total metal concentrations. Patterns of contamination were similar for both dissolved and total metals, so for brevity I focus on dissolved metal concentrations (Table 3.4). Ranges of metal concentrations in streams were as follows: Mn <0.007 to 0.93 mg/l; Fe 0.03 to 0.48 mg/l; Pb 0.02 to 0.37 mg/l; Zn 0.01 to 1.74 mg/l; Cu <0.014 to 0.47 mg/l; Sn <0.011 to 0.80 mg/l; Al <0.08 to 0.29 mg/l; Cd <0.50 to 20.38 µg/l; Ni <0.02 to 0.06 mg/l; Cr <0.011 to 0.012 mg/l. Levels of nickel and chromium were so low that I do not discuss them further in the results.

The pattern of heavy metal contamination in Cornwall showed reduced levels of heavy metals at reference sites relative to their paired contaminated sites (specifically zinc and cadmium concentrations) (Table 3.4) and as such the sites conformed to the study design (Section 3.3.1.). In the Leadhills, some of the 'contaminated' sites did not have significantly higher heavy metal concentrations than their respective 'reference' sites (specifically in cadmium concentrations) (Table 3.4). Sites with levels of cadmium above the 0.50 µg/l minimum detection

Table 3.4: Mean (SE) of dissolved heavy metal concentration collected across twenty stream sites. Data are mean (± SE) values from 6 replicate samples taken on two separate visits to site, three weeks apart. Minimum detectable concentrations were: Mn < 0.007 mg/l, Fe < 0.01 mg/l, Pb < 0.02 mg/l, Zn < 0.004 mg/l, Cu < 0.014 mg/l, Sn < 0.011mg/l, Al < 0.08 mg/l, Ni < 0.02 mg/l, Cd < 0.5 µg/l, Cr < 0.011 mg/l. - = no data. Site 1-8 were in the Leadhills, and sites 9-20 in Cornwall. Sites in Cornwall were paired (contaminated vs. reference): odd numbered sites were contaminated sites and even numbered sites were reference sites.

Site no.	Dissolved metals									
	Mn (mg/l)	Fe (mg/l)	Pb (mg/l)	Zn (mg/l)	Cu (mg/l)	Sn (mg/l)	Al (mg/l)	Cd (µg/l)	Ni (mg/l)	Cr (mg/l)
1	0.07 (0.02)	0.07 (0.02)	0.07 (<0.01)	0.09 (<0.01)	<0.014 (0.00)	<0.011 (0.00)	0.09 (<0.01)	3.23 (0.24)	0.02 (<0.01)	<0.011 (0.00)
2	<0.007 (0.00)	0.03 (<0.01)	0.03 (<0.01)	0.09 (0.05)	<0.014 (0.00)	0.14 (0.03)	<0.08 (0.00)	3.17 (0.33)	<0.02 (0.00)	<0.011 (0.00)
3	0.007 (<0.01)	0.03 (<0.01)	0.04 (<0.01)	0.02 (<0.01)	<0.014 (0.00)	0.14 (0.03)	<0.08 (0.00)	0.67 (0.17)	0.02 (<0.01)	<0.011 (0.00)
4	0.02 (<0.01)	0.05 (0.01)	0.03 (<0.01)	0.01 (<0.01)	<0.014 (0.00)	<0.011 (0.00)	0.09 (<0.01)	<0.50 (0.00)	0.02 (<0.01)	<0.011 (0.00)
5	0.04 (0.02)	0.05 (<0.01)	0.03 (<0.01)	0.01 (<0.01)	<0.014 (0.00)	<0.011 (0.00)	0.09 (<0.01)	<0.50 (0.00)	<0.02 (0.00)	<0.011 (0.00)
6	0.007 (<0.01)	0.09 (0.01)	0.03 (<0.01)	0.05 (0.02)	<0.014 (0.00)	0.80 (0.40)	0.09 (<0.01)	1.10 (0.46)	<0.02 (0.00)	<0.011 (0.00)
7	<0.007 (0.00)	0.03 (<0.01)	0.03 (<0.01)	0.01 (<0.01)	<0.014 (0.00)	<0.011 (0.00)	<0.08 (0.00)	<0.50 (0.00)	<0.02 (0.00)	<0.011 (0.00)
8	<0.007 (0.00)	0.05 (0.02)	0.03 (<0.01)	0.01 (<0.01)	<0.014 (0.00)	<0.011 (0.00)	<0.08 (0.00)	<0.50 (0.00)	<0.02 (0.00)	<0.011 (0.00)
9	0.01 (<0.01)	0.04 (<0.01)	0.03 (<0.01)	0.47 (0.05)	0.02 (<0.01)	<0.011 (0.00)	<0.08 (0.00)	3.95 (0.72)	<0.02 (0.00)	<0.011 (0.00)
10	0.02 (<0.01)	0.10 (<0.01)	<0.02 (0.00)	0.04 (0.04)	<0.014 (0.00)	-	<0.08 (0.00)	0.90 (0.31)	<0.02 (0.00)	<0.011 (0.00)
11	0.06 (<0.01)	0.08 (0.05)	0.03 (<0.01)	0.27 (0.05)	0.47 (0.09)	<0.011 (0.00)	0.29 (<0.01)	3.21 (0.58)	<0.02 (0.00)	<0.011 (0.00)
12	<0.007 (0.00)	0.09 (0.01)	0.03 (<0.01)	0.01 (<0.01)	<0.014 (0.00)	<0.011 (0.00)	0.09 (<0.01)	0.52 (0.02)	<0.02 (0.00)	<0.011 (0.00)
13	0.30 (<0.01)	0.05 (<0.01)	0.03 (<0.01)	1.74 (0.10)	0.30 (0.02)	<0.011 (0.00)	0.13 (0.02)	13.28 (2.13)	0.03 (<0.01)	<0.011 (0.00)
14	<0.007 (0.00)	0.03 (<0.01)	0.03 (<0.01)	0.02 (0.00)	0.03 (<0.01)	<0.011 (0.00)	<0.08 (0.00)	<0.5 (0.00)	<0.02 (0.00)	<0.011 (0.00)
15	0.01 (<0.01)	0.21 (0.07)	0.02 (<0.01)	0.44 (0.02)	0.09 (<0.01)	<0.011 (0.00)	0.23 (0.04)	8.43 (1.62)	<0.02 (0.00)	<0.011 (0.00)
16	0.01 (<0.01)	0.06 (<0.01)	0.03 (<0.01)	0.05 (0.02)	<0.014 (0.00)	<0.011 (0.00)	0.19 (0.10)	0.73 (0.16)	<0.02 (0.00)	0.012 (<0.01)
17	0.93 (0.05)	0.48 (0.05)	0.37 (0.03)	0.96 (0.05)	0.03 (<0.01)	<0.011 (0.00)	0.22 (0.02)	20.38 (4.01)	0.06 (<0.01)	<0.011 (0.00)
18	0.02 (<0.01)	0.21 (0.07)	0.03 (<0.01)	0.03 (0.01)	<0.014 (0.00)	<0.011 (0.00)	0.10 (<0.01)	<0.5 (0.00)	<0.02 (0.00)	<0.011 (0.00)
19	0.21 (0.01)	0.09 (0.01)	0.03 (<0.01)	0.67 (0.06)	0.03 (<0.01)	<0.011 (0.00)	0.09 (<0.01)	13.77 (1.34)	0.03 (<0.01)	<0.011 (0.00)
20	0.02 (<0.01)	0.11 (0.01)	0.03 (<0.01)	0.02 (<0.01)	<0.014 (0.00)	<0.011 (0.00)	0.07 (0.02)	2.03 (0.43)	<0.02 (0.00)	<0.011 (0.00)

limit were site 1 = 3.23µg/l, site 2 = 3.17 µg/l, site 3 = 0.67 µg/l and site 6 = 1.10 µg/l. Thus sites 2 and 6 were deemed unsuitable as reference sites. There was also some tin and iron detected at site 6. Sites in the Leadhills did not conform to the paired study design. For this reason subsequent analyses of data from the two regions were dealt with separately.

3.3.2. Patterns from Cornwall.

3.3.2.1. Differences in heavy metal contamination across site pairs.

A summary of the differences in dissolved heavy metal contamination measured across the six site pairs in Cornwall is given in Table 3.5. Tin was consistently below detection. Very negligible differences were found between site pairs for lead (except at site pair 9). Some differences were found between site pairs for manganese, iron, copper, zinc, aluminium and cadmium. On the whole, values were positive indicating that levels of metals were higher at the contaminated site, relative to reference. Where negative values did occur they were of a smaller magnitude than positive values.

Table 3.5: Difference in mean dissolved total heavy metal concentration between site pairs (contaminated – reference) in Cornwall. For mean data see Appendix E. - = no data at site number 10 (reference site for site pair 5). Positive values indicate that concentrations were higher at the contaminated site relative to the reference site and negative *vice versa*. BD indicates that values at both sites were below detection. Where a single site was below detection, I estimated the difference between sites using 50 % of the minimum detection value as the value for that site. Zero values indicate no difference between contaminated and reference.

Site Pair	Dissolved metals							
	Mn (mg/l)	Fe (mg/l)	Pb (mg/l)	Zn (mg/l)	Cu (mg/l)	Sn (mg/l)	Al (mg/l)	Cd (µg/l)
5	-0.01	-0.06	0.01	0.43	0.01	-	0.0	3.05
6	0.06	-0.01	0.0	0.26	0.46	BD	0.20	2.61
7	0.30	0.02	0.0	1.72	0.27	BD	0.09	13.45
8	0.0	0.15	-0.01	0.39	0.08	BD	0.04	7.25
9	0.91	0.27	0.34	0.93	0.02	BD	0.12	20.62
10	0.19	-0.02	0.0	0.65	0.02	BD	0.02	11.74

3.3.2.2. Differences in environmental and water chemistry factors across sites and between site pairs.

A summary of environmental factors across sites in Cornwall is given in Table 3.3 (sites 9-20). Across stream sites, mean width ranged from 0.9 to 5.7 m, depth ranged from 9.4 to 28.2 cm, flow ranged from 0.06 to 0.23 m/s, canopy cover ranged from 0 to 100 %, and the percentage of each substrate type ranged from 0 to 66.7 %. Table 3.6 shows mean differences in environmental factors between sites pairs in Cornwall. Differences in width were always negative, indicating that reference sites were always wider than contaminated sites, although the magnitude of these differences was quite variable. Differences in depth were both negative and positive, and were of similar magnitudes in either direction. Differences in flow rate tended to be positive, indicating slightly faster flow at contaminated sites relative to reference sites, but the magnitude of differences was not great. Differences in canopy cover were negative and of a relatively large magnitude for site pairs 5, 7 and 9, indicating that the percentage canopy cover at contaminated sites was much greater than at reference sites. Differences in percentage canopy cover at the other three site pairs were either zero or 5 %. Differences in substrate type were variable.

A summary of the mean water chemistry data collected across sites is given in Table 3.7 (sites 9-20). In Cornwall, mean temperatures ranged from 15.2 to 17.9 °C, mean alkalinity from <0.01 to 90.0 mg/l $CaCO_3$, dissolved oxygen from 79.0 to 97.3 % saturation, conductivity from 0.04 to 0.52 mV/s, pH from 4.08 to 7.63, nitrite from <0.001 to 0.021 mg/l, nitrate from 0.18 to 1.00 mg/l, phosphate from <0.01 to 2.43 mg/l, and ammonia from <0.01 to 0.37 mg/l.

Table 3.6: Difference in mean environmental variables between site pairs (contaminated – reference). For mean data per site see Table 3.3. Positive values indicate that values were higher at the contaminated site relative to the reference site and negative *vice versa*. Zero values indicate no difference between the site pair.

Site pair	Width (m)	Depth (cm)	Flow rate (m/s)	Canopy cover (%)	Substrate type (%)			
					Boulders/ Cobbles	Pebbles/ gravel	Sand	Silt
5	-0.16	1.88	0.16	-16.6	-41.7	13.3	23.3	6.7
6	-3.72	-12.02	0.02	5.0	10.9	20.0	-31.2	0.3
7	-1.41	11.67	<0.01	-80.0	-2.5	-17.5	18.3	7.5
8	-0.99	13.06	0.03	0.0	-32.5	-23.3	-1.7	55.9
9	-1.75	6.14	-0.06	-100.0	13.3	17.2	-12.8	-23.0
10	-2.95	-18.77	0.03	0.0	10.8	-1.7	-6.7	-0.8

Table 3.7: Water chemistry data collected across twenty stream sites. Data are mean values (± SE). Site 1-8 were in the Leadhills, and sites 9-20 in Cornwall. Sites in Cornwall were paired (contaminated *vs.* reference): odd numbered sites were contaminated sites and even numbered sites were reference sites. For site names see Table 3.3. Minimum detection limits of nutrient concentrations were as follows: Nitrite <0.001 mg/l; nitrate, phosphate and ammonia < 0.01 mg/l.

Site no.	Site pair no.	Temperature (°C)	Alkalinity (mg/l $CaCO_3$)	Dissolved oxygen (% saturation)	Conductivity (mV/s)	pH	Nitrite (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Ammonia (mg/l)
1	-	12.5 (0.54)	82.7 (11.10)	111.3 (7.97)	0.14 (0.52)	7.48 (0.04)	0.165 (0.138)	<0.01 (0.00)	0.29 (0.01)	0.12 (0.04)
2	-	16.5 (0.86)	24.3 (1.67)	104.7 (19.0)	0.10 (2.63)	7.58 (0.08)	0.002 (0.001)	<0.01 (<0.01)	0.04 (0.01)	0.09 (0.01)
3	-	13.5 (0.39)	22.3 (6.60)	101.0 (0.00)	0.10 (0.26)	7.52 (0.12)	0.018 (0.010)	0.12 (0.04)	0.05 (0.02)	0.02 (<0.01)
4	-	16.7 (0.12)	106.0 (3.00)	80.7 (6.33)	0.19 (0.10)	7.44 (0.04)	0.000 (0.000)	<0.01 (0.00)	0.20 (0.07)	0.05 (0.02)
5	-	12.7 (0.22)	44.5 (6.25)	101.0 (0.00)	0.09 (0.07)	7.39 (0.05)	0.025 (0.011)	<0.01 (0.00)	<0.01 (0.00)	0.08 (0.04)
6	-	16.0 (0.03)	42.3 (2.60)	83.0 (9.07)	0.13 (2.25)	7.53 (0.03)	0.012 (0.009)	<0.01 (0.00)	0.29 (0.02)	0.09 (0.07)
7	-	16.7 (1.11)	26.2 (4.61)	100.0 (0.00)	0.07 (0.20)	7.33 (0.22)	0.014 (0.012)	0.46 (0.19)	0.19 (0.10)	0.20 (0.10)
8	-	15.0 (0.51)	39.5 (7.23)	100.0 (0.00)	0.08 (0.40)	7.48 (0.14)	<0.001 (<0.001)	0.11 (0.11)	<0.01 (0.00)	0.38 (0.05)
9	5	16.3 (0.59)	2.7 (2.67)	93.0 (3.33)	0.34 (0.09)	6.83 (0.03)	<0.001 (<0.001)	0.83 (0.17)	0.13 (0.06)	0.14 (0.13)
10		16.1 (0.62)	<0.01 (0.00)	91.5 (4.95)	0.20 (0.01)	7.21 (0.02)	<0.001 (<0.001)	0.98 (0.02)	0.10 (0.05)	0.17 (0.04)
11	6	16.0 (0.02)	<0.01 (0.00)	97.8 (1.14)	0.05 (0.02)	6.29 (0.06)	0.008 (0.007)	0.60 (0.03)	0.01 (<0.01)	0.08 (0.04)
12		16.5 (0.34)	<0.01 (0.00)	91.5 (3.43)	0.04 (0.01)	6.33 (0.14)	<0.001 (0.000)	0.18 (0.01)	<0.01 (0.00)	<0.01 (<0.01)
13	7	16.2 (0.28)	2.7 (2.67)	88.0 (5.48)	0.20 (0.07)	6.06 (0.03)	0.001 (<0.001)	1.00 (0.00)	2.43 (0.13)	0.37 (0.32)
14		15.7 (0.67)	9.7 (6.12)	92.8 (4.38)	0.16 (0.01)	6.87 (0.05)	0.003 (0.001)	0.98 (0.02)	0.10 (0.04)	0.03 (0.01)
15	8	15.2 (0.17)	17.0 (8.89)	90.3 (4.69)	0.19 (0.02)	6.70 (0.04)	0.007 (0.004)	0.88 (0.04)	0.01 (0.00)	0.18 (0.04)
16		17.9 (0.66)	<0.01 (0.00)	89.3 (5.70)	0.13 (0.01)	6.98 (0.10)	0.004 (0.003)	0.58 (0.02)	0.03 (0.02)	0.06 (0.03)
17	9	17.5 (0.54)	<0.01 (0.00)	79.0 (3.68)	0.52 (0.16)	4.08 (0.10)	<0.001 (0.000)	0.30 (0.04)	0.01 (0.00)	<0.01 (0.00)
18		17.1 (0.52)	90.0 (11.5)	88.3 (5.23)	0.36 (0.02)	7.63 (0.01)	0.021 (0.001)	1.00 (0.00)	0.17 (0.03)	0.02 (0.01)
19	10	17.3 (0.03)	<0.01 (0.00)	97.3 (1.36)	0.17 (0.01)	6.82 (0.02)	<0.001 (0.000)	0.30 (0.02)	<0.01 (0.00)	0.09 (0.03)
20		17.0 (0.11)	7.0 (7.0)	91.8 (3.87)	0.09 (0.00)	6.78 (0.09)	<0.001 (0.000)	0.90 (0.00)	<0.01 (0.00)	0.07 (0.05)

A summary of the differences in water chemistry data collected across the six site pairs is given in Table 3.8. Differences in temperature were of low magnitude (in comparison with daily or seasonal fluctuations) with the exception of site pair 8, whose reference site was considerably (2.8 °C) lower than the contaminated site. Differences in alkalinity were variable across site pairs, ranging from -90 to 17 mg/l $CaCO_3$. Differences in dissolved oxygen were of no great magnitude. Differences in conductivity were always positive, suggesting that the contaminated sites were more conductive than reference sites. Differences in pH were mainly negative (but slightly positive at site pair 10). The contaminated site in site pair 9 was 3.55 pH units below that of its reference site (i.e. the contaminated site was quite acidic, mean = pH 4.08 at site 17). A pH value this low at the contaminated site is likely to affect organisms, their behaviour and physiology. Differences in nitrite levels were of no great magnitude and were either below detection for site pairs 5 and 10, or either positive or negative. Differences in nitrate were either positive or negative and were of a similar magnitude in either direction (ranging from -0.70 mg/l at site pair 9 to 0.42 mg/l at site pair 6). Differences in phosphate ranged from 0.16 mg/l to a large difference of 2.33 mg/l at site pair 7. Differences in ammonia were both positive and negative and of similar magnitudes in either direction (ranging from -0.33 to 0.34 mg/l).

3.3.2.3. Differences in mean biotic factors across sites and between site pairs.

The total number of macroinvertebrate taxa found across the six site pairs in Cornwall was 121 (45 shredder taxa, 76 non-shredder taxa) (see Appendix G for taxa density). The total number of taxa per site ranged from 11 taxa (site 17) to 47 taxa (site 18). The total number of individual animals collected was 12,348, ranging from 126 ind (site 17) to 2,454 ind (site 16). Total community biomass ranged from 27.31 mg (site 17) to 2250.87 mg (site 16) (data are not presented).

Table 3.8: Difference in mean water chemistry data between site pairs (contaminated – reference). For mean data per site see Table 3.6. Positive values indicate that values were higher at the contaminated site than at the reference site and negative *vice versa*. BD indicates that values at both sites were below detection. Where a single site was below detection I used 50 % of the minimum detection value (see text).

Site pair	Temperature (°C)	Alkalinity (mg/l $CaCO_3$)	Dissolved oxygen (% saturation)	Conductivity (mV/s)	pH	Nitrite (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Ammonia (mg/l)
5	0.2	2.7	1.5	0.14	-0.38	BD	-0.15	0.03	-0.33
6	-0.5	BD	6.3	0.01	-0.04	0.008	0.42	0.005	0.08
7	0.5	-7.0	-4.8	0.04	-0.81	-0.002	0.02	2.33	0.34
8	-2.8	17.0	1.0	0.06	-0.28	0.003	0.30	-0.02	0.12
9	0.4	-90.0	-9.3	0.16	-3.55	-0.021	-0.70	-0.16	-0.02
10	0.3	-7.0	5.5	0.08	0.05	BD	-0.60	BD	0.02

For summarised information of macroinvertebrate community structure per site see Table 3.9. The mean number of shredder taxa per site ranged from 0.2 taxa/0.1 m^2 (sites 11 and 17) to 5 taxa/0.1 m^2 site 18). Mean shredder density was greatest at site 10 (152.3 ind/0.1 m^2, of which the majority were *Gammarus pulex* L. (Amphipoda: Gammaridae) (Appendix G) and lowest at site 17 (0.2 ind/0.1 m^2) (Table 3.9). Mean shredder biomass ranged from 1.418 mg/0.1 m^2 (site 13) to 197.7 mg/0.1 m^2 (site 16). The mean number of non-shredder taxa per site ranged from 1.1 taxa/0.1 m^2 (site 15) to 12.6 taxa/0.1 m^2 (site 18). Mean non-shredder density ranged from 12.4 ind/0.1 m^2 (site 17) to 241.8 ind/0.1 m^2 (site 16), of which the majority were Diptera: Chironomidae (Appendix G). Mean non-shredder biomass ranged from 2.46 mg/0.1 m^2 (site 17) to 99.4 mg/0.1 m^2 (site 18) (Table 3.9).

Differences in the mean biotic data collected from the six site pairs are shown in Figure 3.3. Nearly all differences were negative, with a few exceptions, mostly at site pair 5. Statistical tests (one-sample *t*-tests: $p < 0.05$) revealed that those responses which were significantly less than zero (i.e. structure was consistently lower at contaminated site relative to the reference site) were: density of individuals of the non-shredder community (Fig. 3.3b) and the whole community (Fig. 3.3c); number of taxa of the shredder (Fig. 3.3d), non-shredder (Fig. 3.3e), and whole community (Fig. 3.3f); and biomass of the whole community (Fig. 3.3i). The test on non-shredder biomass was only just non-significant ($p = 0.060$).

Table 3.9: Summary of biotic data collected across twenty stream sites. Data are mean values (± SE) from 10 Surber samples, except at site 4, where only 8 Surbers were collected. Site 1-8 were in the Leadhills, and sites 9-20 in Cornwall. Sites in Cornwall were paired (contaminated *vs.* reference): odd numbered sites were contaminated sites and even numbered sites were reference sites.

Site no.	Site pair no.	Taxa richness (taxa/0.1 m^2)			Density (ind/0.1 m^2)			Biomass (mg/0.1 m^2)		
		Whole community	Shredder	Non-shredder	Whole community	Shredder	Non-shredder	Whole community	Shredder	Non-shredder
1	-	10.2 (0.63)	4.4 (0.27)	5.8 (0.57)	143.0 (45.6)	101.6 (32.3)	41.4 (14.3)	138.2	95.5 (21.7)	42.7 (15.3)
2	-	15.0 (1.26)	2.7 (0.62)	12.3 (0.94)	203.8 (74.6)	13.3 (3.19)	190.5 (71.9)	119.9	16.8 (8.04)	103.1 (18.0)
3	-	14.8 (1.00)	3.9 (0.41)	10.9 (0.80)	89.2 (18.5)	15.1 (3.23)	74.1 (19.8)	88.4	20.0 (6.29)	68.4 (10.8)
4	-	6.6 (1.61)	1.7 (0.45)	4.9 (1.33)	68.7 (21.1)	8.5 (2.56)	60.2 (20.4)	36.0	8.2 (2.93)	27.8 (6.82)
5	-	7.1 (0.55)	2.3 (0.37)	4.8 (0.5)	19.7 (3.04)	3.9 (0.67)	15.8 (2.78)	11.7	4.0 (1.66)	7.8 (1.84)
6	-	12.3 (1.07)	2.6 (0.52)	9.7 (0.7)	291.0 (102.0)	15.8 (4.43)	276.0 (102.0)	148.1	28.3 (7.5)	119.8 (19.9)
7	-	14.2 (1.37)	2.3 (0.52)	11.9 (1.02)	72.3 (10.3)	8.9 (3.12)	63.4 (8.15)	57.8	14.2 (4.31)	43.5 (9.84)
8	-	12.6 (0.78)	3.1 (0.31)	9.5 (0.73)	60.0 (9.61)	11.9 (1.88)	48.1 (8.25)	48.4	17.0 (4.97)	31.4 (9.44)
9	5	6.4 (0.76)	0.8 (0.25)	5.6 (0.56)	62.5 (10.9)	1.4 (0.56)	61.1 (10.6)	22.5	8.5 (3.63)	14.0 (5.36)
10		7.1 (1.21)	1.7 (0.37)	5.4 (1.03)	176.3 (15.2)	152.3 (16.8)	24.0 (10.6)	87.1	76.4 (16.3)	10.7 (6.32)
11	6	3.0 (0.76)	0.2 (0.2)	2.8 (0.63)	18.9 (7.36)	0.3 (0.3)	18.6 (7.13)	15.5	12.8 (0.0)	2.7 (1.37)
12		12.7 (1.94)	3.7 (0.62)	9.0 (1.37)	166.3 (39.0)	12.6 (3.71)	153.7 (36.3)	44.9	14.4 (3.6)	30.6 (7.69)
13	7	4.1 (0.84)	0.3 (0.15)	3.8 (0.81)	49 (15.7)	0.4 (0.22)	48.6 (15.7)	8.4	1.4 (0.47)	7.0 (3.17)
14		10.1 (0.74)	3.2 (0.47)	6.9 (0.53)	86.3 (13.8)	6.8 (0.84)	79.5 (13.9)	41.0	19.7 (9.57)	21.3 (5.63)
15	8	3.1 (0.66)	2.0 (0.0)	1.1 (0.66)	68.7 (40.4)	0.5 (0.34)	68.2 (40.1)	60.4	47.2 (46.1)	13.2 (5.32)
16		11.0 (1.01)	2.6 (0.45)	8.4 (0.76)	245.4 (46.4)	3.6 (1.24)	241.8 (45.7)	240.7	197.7 (90.9)	43.0 (11.4)
17	9	2.5 (0.58)	0.2 (0.13)	2.3 (0.58)	12.6 (4.17)	0.2 (0.13)	12.4 (4.13)	7.5	5.1 (4.98)	2.5 (1.05)
18		17.6 (1.28)	5.0 (0.44)	12.6 (0.93)	148.7 (14.8)	39.3 (7.93)	109.4 (12.4)	131.6	32.2 (11.0)	99.4 (35.0)
19	10	6.6 (1.01)	1.2 (0.29)	5.4 (0.75)	51.1 (15.7)	2.0 (0.63)	49.1 (15.5)	21.8	9.5 (6.52)	12.4 (3.15)
20		14.7 (1.64)	4.3 (0.80)	10.4 (1.42)	151.0 (55.8)	9.9 (2.88)	141.1 (56.0)	58.1	9.1 (3.41)	49.0 (13.3)

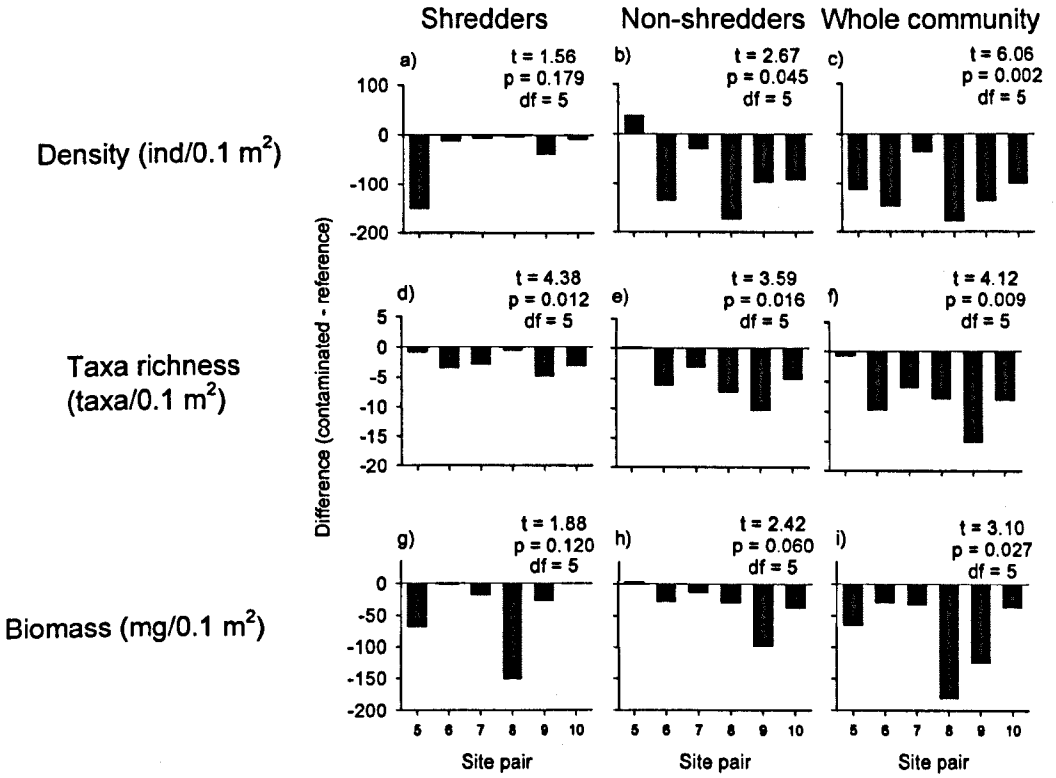

Figure 3.3: Differences in structural measures between site pairs in Cornwall: a) density of shredders; b) density of non-shredders; c) density of the whole community; d) number of shredder taxa, e) number of non-shredder taxa, f) number of taxa in the whole community taxa, g) biomass of shredders, h) biomass of non-shredders, i) biomass of the whole community. Positive values indicate an increase at contaminated site relative to reference and negative *vice versa*. *t* and *p* values indicate the results of one-sample *t*-tests (null hypotheses of $\mu = 0$).

3.3.2.4. Differences in mean rates of leaf processing across sites and between site pairs.

Patterns were similar for both measures of leaf breakdown at sites in Cornwall (i.e. both L and k) (Table 3.10). Mean L_{Fine} (i.e. percentage leaf mass loss from fine mesh leaf bags) ranged from 24 % (site 11) to 82 % mass loss (site 16). Mean L_{Coarse} (i.e. percentage leaf mass loss from coarse mesh leaf bags) ranged from 29 % (site 11) to nearly 100 % mass loss (site 16). Mean k_{Fine} ranged from 0.0133 (site 11) to 0.0996 (site 16). Mean k_{Coarse} ranged from 0.0165 (site 11) to 0.2948 (site 16). Mean L_{Shred} (i.e. the amount of leaf breakdown attributable to shredders: see Equation 3.2) ranged from -0.04 % (site 9) to 33.13 % (site 20).

At some sites rates of leaf processing in both types of leaf bag were practically identical. For example, at site 9 mean L_{Fine} was 74 % and L_{Coarse} was also 74 %. This suggests that shredders did not play a large role in leaf breakdown at this site.

The differences in the rate of leaf processing across site pairs are shown in Figure 3.4. All site pairs show a similar pattern of a decrease in the rate of leaf processing at contaminated sites relative to reference sites, for both L and k from both fine and coarse mesh leaf bags. The exception was site pair 5, where the rate of leaf processing was increased at the contaminated site relative to reference site. Statistical tests (one-sample t-tests: $p < 0.05$) reveal that the responses which were significantly different across site pairs were the percentage mass loss (L) from coarse mesh bags (Figure 3.4b) and the difference between coarse and fine mesh (Figure 3.4c). This indicates that shredder leaf processing was being affected by heavy metal contamination to a greater degree than the other processes associated with leaf breakdown. Other measures of function were not significantly different across site pairs (Figure 3.4a, c, d). L_{Shred} was the only measure which was consistently greater at reference sites, than at contaminated sites (Figure 3.4c).

Table 3.10: Mean (± SE) leaf breakdown rates from coarse and fine mesh leaf bags across twenty stream sites. L = the percentage leaf mass loss (Equation 3.1). L_{Fine} = percentage leaf mass loss from fine mesh leaf bags. L_{Coarse} = percentage leaf mass loss from coase mesh leaf bags. L_{Shred} = the percentage leaf mass loss from coarse - fine mesh leaf bags (Equation 3.2) and is the amount of leaf processing attributable to macroinvertebrate shredders. k is the leaf breakdown coefficient (Equation 3.3). k_{Fine} = the leaf breakdown coefficient calculated from leaf mass loss in fine mesh leaf bags. k_{Coarse} = the leaf breakdown coefficient calculated from leaf mass loss in coarse mesh leaf bags. Site 1-8 were in the Leadhills, and sites 9-20 in Cornwall. Sites in Cornwall were paired (contaminated vs. reference): odd numbered sites were contaminated sites and even numbered sites were reference sites.

Site no.	Site pair no.	Fine mesh		Coarse mesh		L_{Shred} (i.e. shredder processing only)
		L_{Fine}	k_{Fine}	L_{Coarse}	k_{Coarse}	
1	-	34.88 (1.32)	0.0205 (0.001)	54.42 (2.99)	0.0386 (0.003)	19.54
2	-	24.62 (0.47)	0.0135 (<0.001)	27.48 (1.07)	0.0154 (<0.001)	2.86
3	-	32.82 (0.83)	0.0190 (0.001)	35.34 (1.34)	0.0209 (0.001)	2.52
4	-	25.40 (0.64)	0.0140 (<0.001)	35.86 (1.54)	0.0213 (0.001)	10.46
5	-	25.21 (0.51)	0.0138 (<0.001)	30.78 (0.48)	0.0175 (<0.001)	5.57
6	-	42.20 (2.23)	0.0265 (0.002)	71.42 (2.10)	0.0612 (0.004)	29.22
7	-	23.47 (0.47)	0.0128 (<0.001)	29.86 (0.75)	0.0169 (<0.001)	6.39
8	-	33.88 (1.73)	0.0199 (0.0014)	44.31 (0.75)	0.0279 (<0.001)	10.43
9	5	74.05 (3.15)	0.0687 (0.006)	74.01 (1.86)	0.0652 (0.004)	-0.04
10		52.15 (2.94)	0.0364 (0.004)	67.29 (4.06)	0.0580 (0.007)	15.14
11	6	24.37 (1.06)	0.0133 (0.001)	29.17 (1.63)	0.0165 (0.001)	4.8
12		59.61 (2.43)	0.0442 (0.003)	72.65 (3.28)	0.0668 (0.007)	13.04
13	7	46.61 (1.28)	0.0300 (0.001)	51.95 (3.93)	0.0369 (0.006)	5.34
14		49.75 (2.44)	0.0334 (0.002)	59.10 (2.52)	0.0435 (0.003)	9.35
15	8	49.56 (0.95)	0.0327 (<0.001)	56.52 (3.22)	0.0406 (0.004)	6.96
16		81.96 (4.32)	0.0996 (0.013)	99.63 (0.12)	0.2948 (0.020)	17.67
17	9	30.68 (0.76)	0.0175 (<0.001)	32.17 (0.67)	0.0185 (<0.001)	1.49
18		59.42 (3.04)	0.0443 (0.003)	89.41 (1.78)	0.1182 (0.012)	29.99
19	10	38.92 (0.83)	0.0235 (<0.001)	46.61 (1.68)	0.0302 (0.002)	7.69
20		41.71 (1.09)	0.0258 (<0.001)	74.84 (4.69)	0.079 (0.012)	33.13

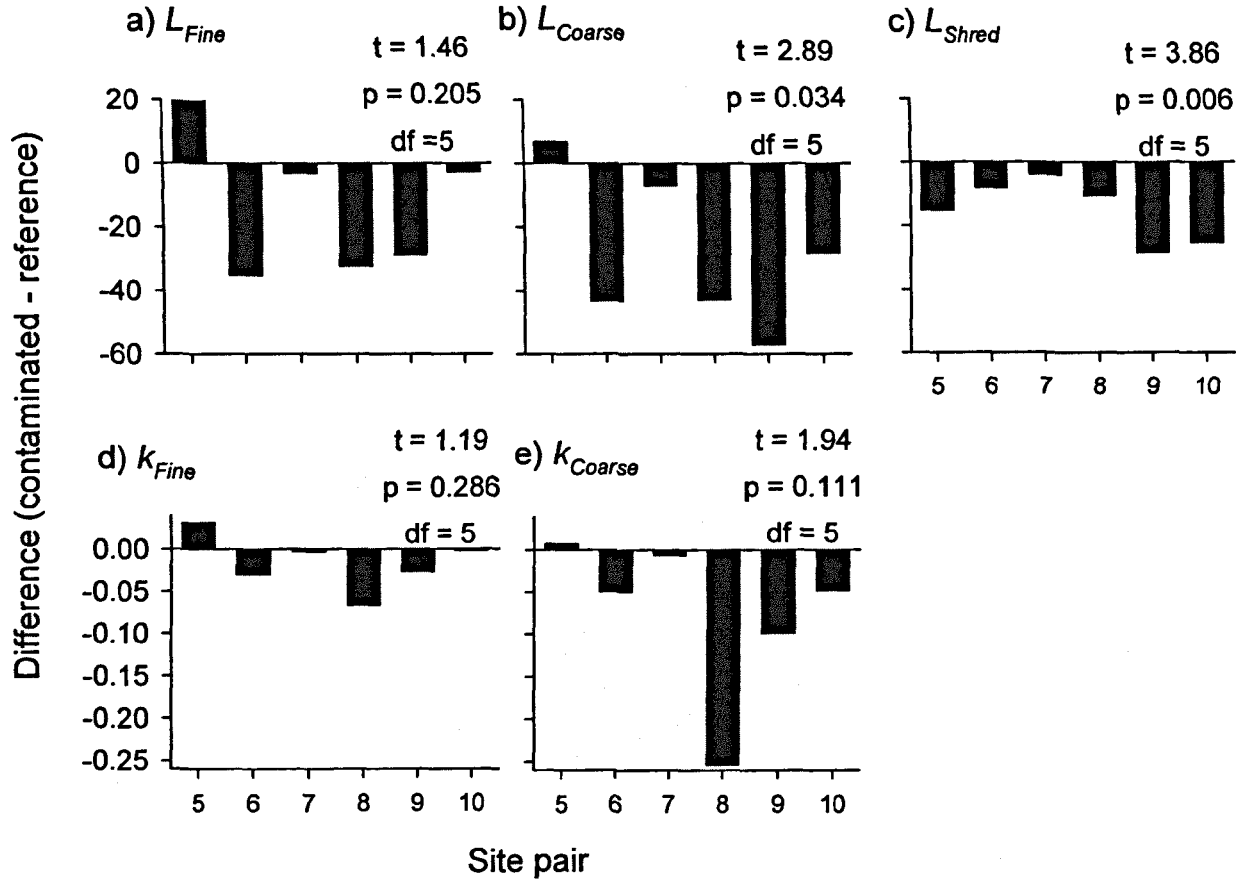

Figure 3.4: Differences in mean rate of leaf processing between site pairs in Cornwall (contaminated – reference) measured as: a) difference in mean *L* from fine mesh leaf bags (i.e. excluding shredders); b) mean *L* from coarse mesh bags (i.e. including shredders); c) mean *L* from coarse – fine mesh bags (i.e. breakdown attributable to shredders); d) mean *k* from fine mesh bags; e) mean *k* from coarse mesh bags (see text). Positive values indicate that the value of either the amount of or rate of leaf breakdown was higher at the contaminated site relative to reference and negative *vice versa*. *t* and *p* values indicate the results of one-sample *t*-tests (null hypotheses of $\mu \approx 0$).

3.3.2.5. Were structure and function associated across sites?

If heavy metal contamination affects function indirectly through structure, then structure and function should be strongly correlated, and this is what we observe in Cornwall. Correlation analyses reveal that two structural measures of the shredder community were correlated with L_{Shred} (i.e. the amount of leaf breakdown attributable to shredders: see Equation 3.2): 1) the mean number of shredder taxa, and 2) the density of shredders ($p < 0.05$) (Figures 3.5 a & b). Mean shredder biomass was not related to L_{Shred} ($p > 0.05$) (Figure 3.5c). All three structural measures of the non-shredder community were strongly associated with L_{Shred} ($p < 0.05$) (Figures 3.5 d-f).

However, to rule out the alternative explanation, that heavy metal contamination is driving the pattern of structure and function directly, structure and function must be shown to be related in the absence of heavy metal contamination. There were no significant correlations between any aspect of structure and function in the absence of heavy metal contamination (i.e. at reference sites only; Figure 3.5 white dots) (Pearson Correlation: $p > 0.05$). This suggests that heavy metal contamination affects structure and function directly and independently. The position of the black dots *vs.* white dots on Figure 3.5 shows the potential for the direct effects of heavy metal contamination to produce the strong correlation observed when data were considered together (see above).

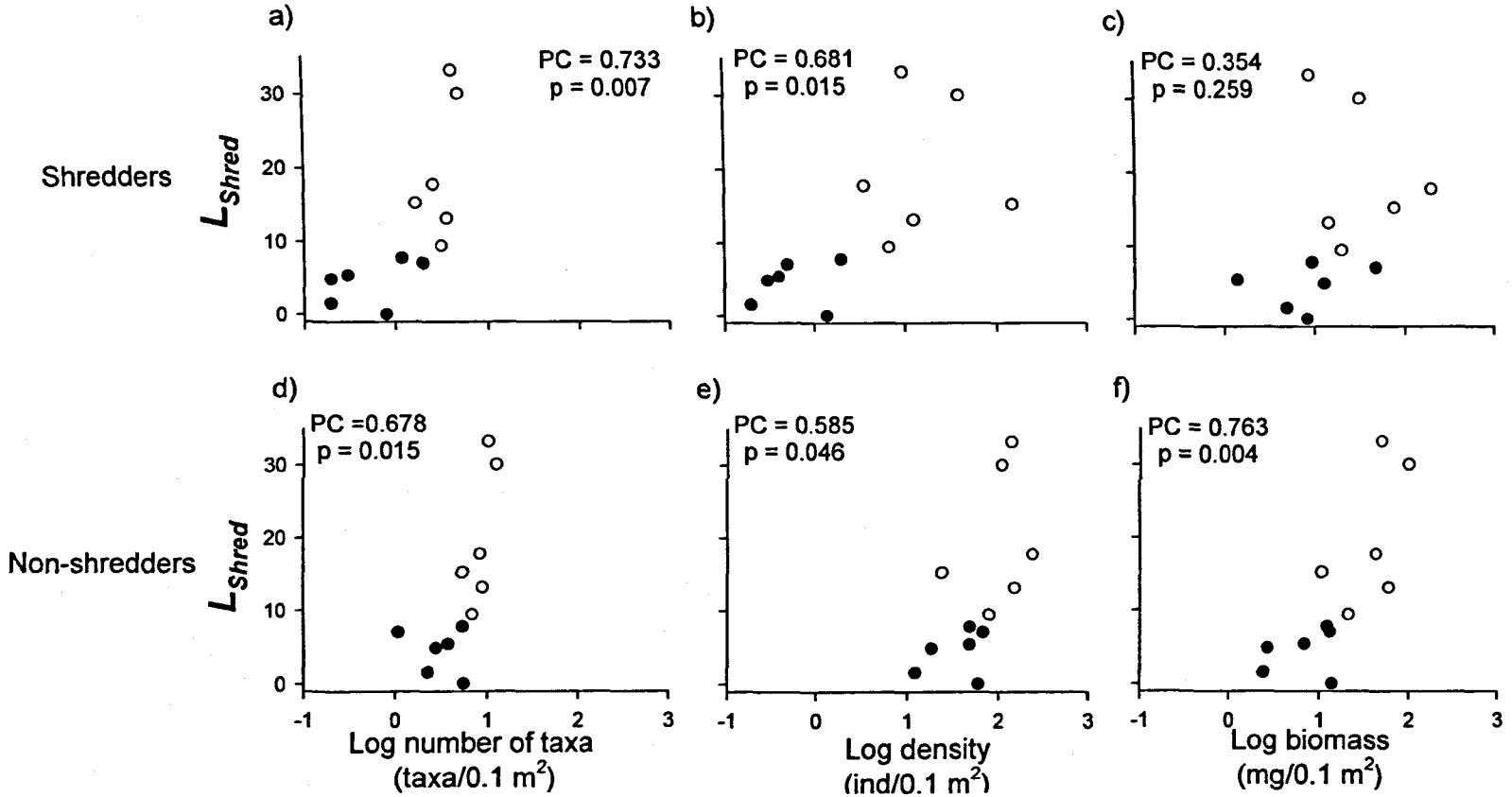

Figure 3.5: Structure *vs.* function relationships for stream sites across Cornwall. Open symbols = reference sites, closed symbols = contaminated sites. Function = L_{Shred} = leaf processing due to macroinvertebrate shredders (i.e. % leaf mass loss from coarse – fine mesh leaf bags). Structure = a) log number of shredder taxa, b) log density of shredder individuals, c) log shredder biomass, d) log number of non-shredder taxa, e) log density of non-shredder individuals, f) log non-shredder biomass. All relationships were significantly positively associated (see *p* values). PC is the Pearson Correlation coefficient, tested at $p < 0.05$.

3.3.3. Patterns from the Leadhills.

3.3.3.1. Heavy metal contamination and abiotic factors.

Sites in the Leadhills showed variability in the degree of their respective dissolved (Table 3.4) and total (Appendix E) heavy metal contamination. Once again, for brevity I focus on dissolved metals and continue by considering differences in the profile of heavy metal contamination between the two regions. Zinc, aluminium and cadmium were all much less prevalent in the Leadhills than in Cornwall (two-sample *t*-tests; Table 3.11). The range of each of these metals in the Leadhills was as follows: Zn 0.01 to 0.09 mg/l; Al <0.08 to 0.09 mg/l; Cd <0.50 to 3.23 µg/l (Table 3.4). These were all of low magnitude in comparison with the contaminated sites in Cornwall. There were no differences between the two regions in the amounts of dissolved manganese, iron or lead detected. There was some tin at sites in the Leadhills, whereas there was none in Cornwall.

Mean environmental data recorded for each site in the Leadhills region can be found in Table 3.3 (sites 1-8). Across streams, mean width ranged from 0.7 to 5.9 m, depth ranged from 4.4 to 26.7 cm, flow ranged from 0.10 to 0.49 m/s, canopy cover ranged from 0 to 20 %, and percentage of each substrate type ranged from 0 to 94.2 %.

Table 3.11: Differences in mean heavy metal concentrations between sites in the Leadhills and Cornwall (two-sample *t*-tests). Significant differences are highlighted in bold ($p < 0.05$). Where metals concentrations were below detection I entered a value based on 50 % of the minimum detection value (see Table 3.4 for minimum detection limits). BD = little or none was detected at any of the sites. – = no data.

Metal	Statistics			Leadhills		Cornwall	
	T	p	d.f.	Mean	SE	Mean	SE
Mn (mg/l)	1.46	0.172	11	0.0193	0.0085	0.133	0.077
Fe (mg/l)	2.15	0.055	11	0.05	0.0076	0.129	0.036
Pb (mg/l)	0.67	0.514	11	0.0363	0.005	0.0558	0.029
Zn (mg/l)	2.35	**0.039**	11	0.033	0.013	0.393	0.15
Cu (mg/l)	-	-	-	BD	-	1.6	0.112
Sn (mg/l)	-	-	-	1.384	0.097	BD	-
Al (mg/l)	2.38	**0.032**	14	0.036	0.0094	0.1275	0.024
Cd (µg/l)	2.28	**0.042**	12	1.15	1.46	5.68	1.9

Mean water chemistry data of the eight sites in the Leadhills region can be found in Table 3.7 (sites 1-8). Mean temperatures ranged from 12.5 to 16.7 °C, mean alkalinity from 22.3 to 106.0 mg/l $CaCO_3$, dissolved oxygen from 83.0 to 111.3 % saturation, conductivity from 0.07 to 0.19 mV/s, pH from 7.33 to 7.58, nitrite from <0.001 to 0.165 mg/l, nitrate from <0.01 to 0.46 mg/l, phosphate from <0.01 to 0.29 mg/l, and ammonia from 0.02 to 0.38 mg/l.

3.3.3.2. Macroinvertebrate community structure.

The total number of macroinvertebrate taxa found across the eight sites in the Leadhills was 63 (21 shredder taxa, 42 non-shredder taxa) (see Appendix F for taxa density). The total number of taxa collected from each site ranged from 18 taxa/0.1 m^2 (site five) to 41 taxa/0.1 m^2 (site two). The total number of individual animals collected across all sites was 7901, with a range of 197 ind/0.1 m^2 (site five) to 2914 ind/0.1 m^2 (site six). Total community biomass ranged from 117.20 mg/0.1 m^2 (site five) to 1424.01 mg/0.1 m^2 (site six) (data are not presented).

The mean number of shredder taxa per site ranged from 1.7 taxa/0.1 m^2 (site four) to 4.4 taxa/0.1 m^2 (site one) (Table 3.9). Shredder density was greatest at site one (101.6 ind/0.1 m^2, of which roughly 40 % were *Gammarus pulex* and 32 % were *Leuctra inermis* Kempny (Plectoptera: Leuctridae). Shredder density was lowest at site five (3.9 ind/0.1 m^2) (Appendix G). Shredder biomass ranged from 3.97 mg/0.1 m^2 (site five) to 95.5 mg/0.1 m^2 (site one). The number of non-shredder taxa per site ranged from 4.8 taxa/0.1 m^2 (site five) to 12.3 taxa/0.1 m^2 (site two). Non-shredder density was greatest at site six: 276.0 ind/0.1 m^2, of which roughly 70 % were Diptera: Chironomidae, and 15 % were *Serratella ignita* (formerly *Ephemerella ignita*) Poda (Ephemeroptera: Ephemerellidae) and lowest at site five: 15.8 ind/0.1 m^2. Non-shredder biomass ranged from 7.8 mg/0.1 m^2 (site five) to 119.8 mg/0.1 m^2 (site six).

3.3.3.3. Leaf breakdown.

Mean L_{Fine} (i.e. percentage leaf mass loss from fine mesh leaf bags) ranged from 23 % (site seven) to 42 % mass loss (site six) (Table 3.10). Mean L_{Coarse} ranged

from 27 % (site two) to 71 % mass loss (site six). Mean k_{Fine} ranged from 0.0128 (site seven) to 0.0265 (site six) and mean k_{Coarse} from 0.0169 (site seven) to 0.0612 (site six). Mean L_{Shred} (i.e. the amount of leaf breakdown attributable to shredders: see Equation 3.2) ranged from 2.52 % (site 3) to 29.22 % (site six).

3.3.3.4. Were structure and function associated across sites?

Correlation analyses reveal that no structural measures of either the shredder or non-shredder community were correlated with L_{Shred} ($p > 0.05$) (Figure 3.6 a-f). Sites which received higher amounts of cadmium (Figure 3.6: white dots) were not associated with lower values of either structure or function than sites with lower levels of cadmium (black dots).

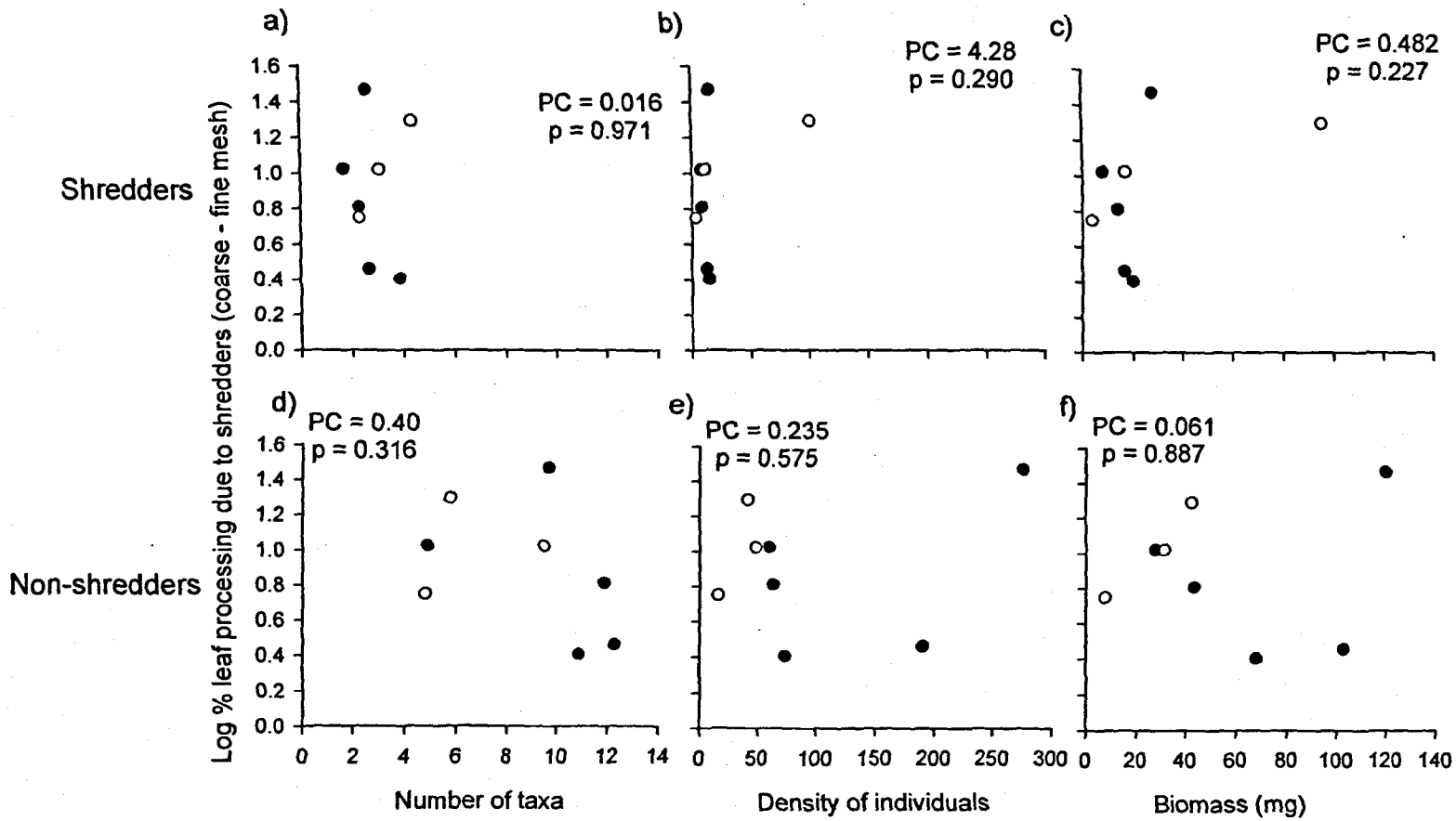

Figure 3.6: Structure *vs.* function relationships for stream sites across the Leadhills. Function = L_{Shred} = leaf processing due to macroinvertebrate shredders. Structure = a) number of shredder taxa, b) density of shredder individuals, c) shredder biomass, d) number of non-shredder taxa, e) density of non-shredder individuals, f) non-shredder biomass. No relationships were significantly positively associated (see *p* values). PC is the Pearson Correlation coefficient, tested at $p < 0.05$. Black dots indicate that levels of dissolved cadmium were lower than levels seen at contaminated sites in Cornwall (see Appendix E). White dots indicate that levels of dissolved cadmium exceeded the minimum level of contamination seen in Cornwall.

3.3.4. Differences between the two regions.

Given that there is a relationship between structure and function in Cornwall, but not in the Leadhills, it makes sense to try to characterise the differences in biotic and abiotic factors between the regions which might explain the results. I did not have any information on broad scale geographic drivers (e.g. climate, underlying geology, etc.) which might differ between the two regions, but we would expect them to be very different. Differences in environmental and water chemistry data between the sites in the Leadhills region and Cornwall are shown in Table 3.12 (two sample *t*-tests). There were no differences in mean width or depth between the two regions. The rate of flow was significantly faster on average in the Leadhills than in Cornwall. There was significantly less percentage canopy cover at sites in the Leadhills than at sites in Cornwall. There were also some differences in substrate composition between the two regions: a) there was a greater percentage of boulders/cobbles in the Leadhills than in Cornwall; b) there were no significant differences in the percentage of pebbles/gravel in the Leadhills and Cornwall; c) there was a lesser percentage of sand and silt in the Leadhills than in Cornwall. Mean temperature was lower and more variable in the Leadhills than in Cornwall. On average Cornish sites had lower alkalinity, were more acidic, and had higher levels of nitrate than sites in the Leadhills. There were no significant differences between the regions in the amount of dissolved oxygen, conductivity, nitrite, phosphate or ammonia present in streams.

I tested for differences in mean biotic data between the two regions (Table 3.13) (two sample *t*-tests). There were no significant differences in the mean species richness, density of individuals or biomass of the whole community, shredder community or the non-shredder community between sites in the Leadhills region and Cornwall. A PCA for the whole community (Figure 3.7a) represented 39.7 % of the variation among stream sites in species composition on the first two principal components. Sites in the two regions occupied different areas across the plot. Cornish sites (white dots) and Leadhills sites (black dots) were both spread along the length of the 'Principle Component 1' axis (PC1), but were split along the 'Principle Component 2' axis (PC2). Taxa that characterised sites towards the negative end of PC2 (i.e. Leadhills sites) were *Caenis rivulorum* Eaton (Ephemeroptera: Caenidae), *Rhithrogena semicolorata* Curtis (Ephemeroptera:

Table 3.12: Differences in environmental and water chemistry data between sites in the two regions (two-sample *t*-tests). Significant differences are highlighted in bold ($p < 0.05$). Where nutrient concentrations were below detection I entered a value based on 50 % of the minimum detection value (see Table 3.6 for minimum detection limits).

Factor		Statistics			Leadhills		Cornwall	
		T	p	d.f.	Mean	SE	Mean	SE
Environmental data	Width (m)	0.65	0.527	13	2.26	0.61	2.75	0.44
	Depth (cm)	0.78	0.448	14	14.31	2.5	16.74	1.9
	Flow (m/s)	3.47	**0.007**	9	0.301	0.046	0.1308	0.018
	Canopy cover (%)	5.57	**<0.001**	11	0.025	0.025	68.8	12
	Boulders/cobbles (%)	2.23	**0.045**	12	55.3	9.5	29.9	6.2
	Pebbles/gravel (%)	1.08	0.304	10	41.3	8.3	31.1	4.2
	Sand (%)	4.23	**0.001**	14	4.18	1.6	21.5	3.8
	Silt (%)	2.73	**0.020**	11	0.525	0.35	16.7	5.9
Water chemistry data	Temperature (°C)	2.38	**0.044**	8	14.95	0.64	16.567	0.23
	Alkalinity (mg/l $CaCO_3$)	2.91	**0.012**	13	48.5	11	10.8	7.4
	Dissolved oxygen (% saturation)	1.72	0.119	9	97.7	3.7	90.89	1.4
	Conductivity (mV/s)	2.15	0.051	13	0.1125	0.014	0.204	0.04
	pH	3.59	**0.004**	11	7.47	0.028	6.55	0.25
	Nitrite (mg/l)	1.31	0.233	7	0.0296	0.02	0.0039	0.0017
	Nitrate (mg/l)	5.93	**<0.001**	17	0.089	0.056	0.711	0.089
	Phosphate (mg/l)	0.57	0.0577	12	0.134	0.043	0.250	0.20
	Ammonia (mg/l)	0.54	0.599	14	0.129	0.04	0.102	0.03

Table 3.13: Biotic differences between sites in the Leadhills and Cornwall (two-sample *t*-tests). Significant differences are highlighted in bold ($p < 0.05$).

Factor		Statistics			Leadhills		Cornwall	
		T	p	d.f.	Mean	SE	Mean	SE
Taxa richness (taxa/0.1 m^2)	Whole community	1.81	0.088	17	11.6	1.2	8.24	1.4
	Shredder community	1.35	0.195	17	2.875	0.32	2.10	0.48
	Non-shredder community	1.74	0.101	16	8.73	1.1	6.14	1.0
Density (ind/0.1 m^2)	Whole community	0.41	0.693	12	118.5	32	103.1	21
	Shredder community	0.19	0.849	17	22.4	11	19.1	13
	Non-shredder community	0.33	0.747	12	96.2	32	84.0	19
Biomass (mg/0.1 m^2)	Whole community	0.74	0.470	17	81.0	18	61.6	19
	Shredder community	0.56	0.581	17	25.5	10	36.2	16
	Non-shredder community	1.90	0.084	11	55.6	14	25.5	8.0
Leaf breakdown	L_{Fine}	3.83	**0.002**	15	30.31	2.4	50.7	4.8
	k_{Fine}	3.03	**0.011**	12	-0.01750	0.0017	-0.0391	0.0069
	L_{Coarse}	2.66	**0.016**	17	41.2	5.3	62.8	6.1
	K_{Coarse}	2.00	0.069	12	-0.0275	0.0055	-0.0724	0.022
	L_{Shred}	0.26	0.0796	16	10.87	3.3	12.0	3.0

Heptageniidae). Toward the opposite end of PC2 (i.e. Cornish sites) were *Limnius volckmari* Panzer and *Elmis aenea* Mull (both Coleoptera: Elmidae), and *Lepidostoma hirtum* Fabricius (Trichoptera: Lepidostomatidae) and *Sericostoma personatum* Spence (Trichoptera: Sericostomatidae).

A second PCA for just the shredder community was performed (Figure 3.7b) and captured 55.2 % of the variation in shredder community composition among sites on the first two principal components. A strong influence on both axes was *Gammarus pulex* (Amphipoda: Gammaridae). Sites in the two regions were quite evenly spread across the plot, although no sites in the Leadhills fell below -4 on PC2, which was influenced by two stonefly species (*Leuctra inermis* Kempny and *Leuctra geniculata* Stephens, both Plecoptera: Leuctridae). Sites within regions did not separate out into two separate areas in the same way as seen for the whole community (Figure 3.7a), indicating that perhaps the composition of shredders between the two regions was not as distinct as some other members of the community.

On both Figure 3.7a and b, contaminated sites in Cornwall (white dots, codes: C5c, C6c, C7c, C8c, C9c, C10c) were more similar in community composition than reference sites (codes C5r, C6r, C7r, C8r, C9r, C10r), all clustering in the top right corner of the graph in Figure 3.7b. In Figure 3.7a reference sites were quite similar, clustering in the top half of the graph, while reference sites were below and to the left.

The rate of leaf processing was significantly faster in Cornwall than in the Leadhills from fine mesh bags (L_{Fine} and k_{Fine}: Table 3.13). L_{Coarse} was faster in Cornwall than in the Leadhills, but k_{Coarse} was not significantly different. There was no difference in L_{Shred} between the two regions.

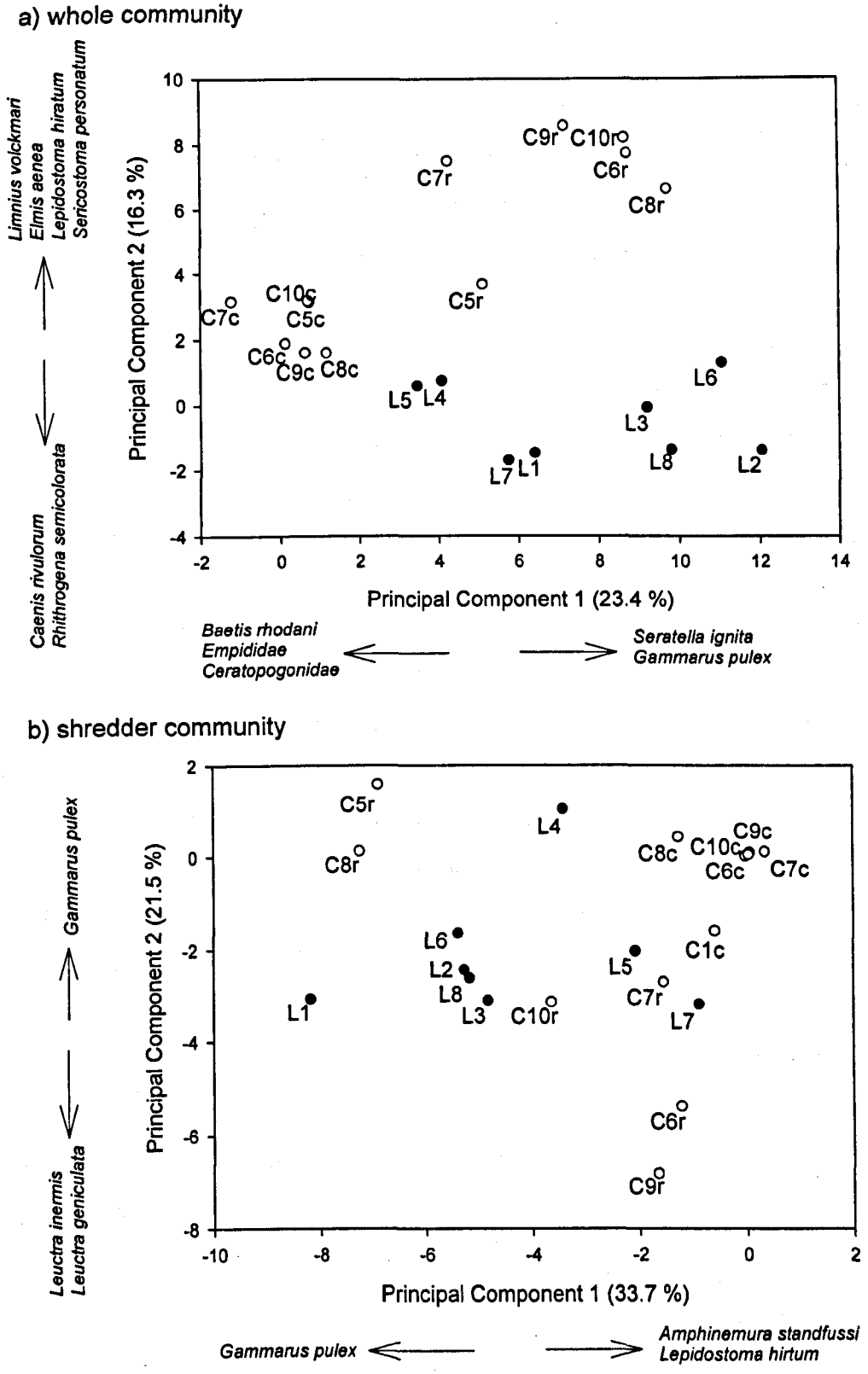

Figure 3.7: Principal Components Analyses for the a) whole community for b) shredder community. Black dots are sites in the Leadhills and white dots are sites in Cornwall. Sites are labelled with either a capital L = Leadhills, or a capital C = Cornwall. In the Leadhills, sites were numbered 1-8; in Cornwall, sites were paired (site pairs C5-C10) and were either denoted small letter c = contaminated or small letter r = reference site.

3.4. Discussion.

The overall aim of this chapter was to conduct field studies to document the effect of heavy metal contamination on the relationship between macroinvertebrate community structure and function in streams. This was addressed by collecting field data on both aspects of the community at multiple contaminated and reference sites in both Cornwall and the Leadhills, Scotland. Sites in the two regions were analysed separately because of differences in the respective profiles of heavy metal contamination between the two regions (see Section 3.3.1.). Sites in Cornwall conformed to the study design (i.e. there were pairs of sites: contaminated *vs.* reference). This permitted differences within pairs of sites to be assessed (e.g. Figure 3.4). As such, the patterns of the effects of heavy metal contamination on community structure and function were easier to interpret in the Cornish data set (see following Sections 3.4.1. and 3.4.2.). Historic data indicated levels of heavy contamination at four sites in the Leadhills region, and no/low levels of contamination at four other sites (Appendices B and C). However, analysis of water chemistry in the present study indicated no/low levels of heavy metal contamination at sites which historic data had indicated were contaminated and *vice versa* (Table 3.4). The dataset therefore prevent definitive statements being made about the influence of heavy metal pollution on structure and function in the Leadhills region. For this reason the majority of this discussion will focus on the Cornish data set.

3.4.1. The effect of heavy metal contamination on macroinvertebrate community structure.

In Cornwall, nearly all responses of structure to heavy metal contamination were negative, with few exceptions (Figure 3.3). That the number of taxa, density of individuals and biomass of the whole community were significantly reduced by heavy metal contamination supports evidence of the same patterns from the meta-analysis in Chapter 2.

Unsurprisingly, patterns of response were similar for those structural measures which co-varied or where co-linearity occurred. For example: 1) at the level of the

whole community, where there was a significant difference in density, there was also a significant difference in biomass (Figures 3.3c and i); 2) at the level of the shredder community, the lack of significant difference in density was matched by an absence of a difference in biomass (Figures 3.3a and g).

Some previous studies of the effects of heavy metal contamination have shown that macroinvertebrate communities under heavy metal stress have been dominated by chironomids (Diptera: Chironomidae) (Winner *et al.* 1980; Hoiland *et al.* 1994; Hickey & Clements 1998; Maret *et al.* 2003). In the present study the number of chironomid individuals was greater than all the other taxa combined at five out of six contaminated sites in Cornwall (sites 9, 11, 15, 17, 19) (Appendix G). This suggests that the pattern of chironomid domination at contaminated sites is operating in the streams.

3.4.2. The effect of heavy metal contamination on the rate of leaf processing.

In Cornwall, the responses of function to stress were predominantly negative, with the exception of site pair five. Rates of leaf processing from coarse mesh were significantly different from zero across site pairs, suggesting that the effect of heavy metal contamination was on average to reduce the rate of leaf processing. This pattern was observed in the meta-analysis in Chapter 2, although the result was not significant. The results of the present study clarify these patterns and support the contention that heavy metal contamination is likely to have a profound effect on the overall rate of leaf breakdown.

The present study incorporated the novel examination of the relationship between structure and the amount of leaf processing attributable to macroinvertebrate shredders (i.e. L_{Shred}; see Equation 3.2) (Figure 3.4c). By doing so I isolated the processing by shredders from that of physical and microbial processes. This aspect of leaf processing was most consistently affected by heavy metal contamination across site pairs in Cornwall ($p = 0.006$). This indicates that at contaminated sites most leaf processing was due to microbial and/or physical processes, rather than due to shredders. At reference sites up to 30 % of the

overall leaf processing was attributable to shredders (Table 3.10). This, and the lack of any significant difference across site pairs in physical and microbial processing (i.e. fine mesh bags) (Figure 3.4a and d: $p > 0.05$), suggests that shredder processing is more sensitive to heavy metal contamination than any other contributing process. This is a similar finding to that of Niyogi *et al.* (2001), who concluded that at sites downstream of acid mine drainage, contamination induced changes in the microbial community were less important in determining overall reductions in the rate of leaf processing than associated changes in leaf consumption by macroinvertebrate shredders.

Results suggest that microbial processing is not able to compensate for the loss of shredder processing in heavy metal contaminated streams. This is in contrast to Nelson (2000), who suggested that aquatic fungi were responsible for maintaining rates of leaf processing in heavy metal (zinc and manganese) contaminated streams. In support of this argument, evidence exists to suggest that aquatic hyphomycetes are able to withstand the effects of zinc (Miersch *et al.* 1997). However, evidence also exists to the contrary (Duarte *et al.* 2004; Duarte *et al.* 2007). The findings of this study, along with the lack of general consensus, suggest that the capacity for microbial compensation may vary on a case by case basis.

3.4.3. Was there an association between structure and function?

There were significant positive relationships between five different responses of macroinvertebrate community structure and function to heavy metal contamination across the 12 sites in Cornwall (Figure 3.5) but not across the eight sites in the Leadhills (Figure 3.7). No previous studies have formally tested for a relationship between structure and function in heavy metal contaminated streams (Section 1.1.4.). However, in some previous studies, positive associations have been indicated: between shredder abundance and rates of leaf processing (Schultheis *et al.* 1997); between secondary shredder production and rates of leaf processing (Carlisle & Clements 2005); between densities of macroinvertebrates and rates of leaf processing (Chaffin *et al.* 2005).

In Cornwall, positive associations existed between structure and function (Figures 3.5a, b, d, e, f) where structure was measured as number of taxa and density of individuals of the shredder and non-shredder community, and non-shredder biomass. This result indicates that the rate of leaf processing is indicative of the status of both aspects of the shredder and non-shredder community. There was no relationship for shredder biomass and rates of shredder leaf processing (Figure 3.5c). This is in contrast with Niyogi *et al.* (2001) who found that shredder biomass was associated with reduced leaf processing downstream of acid mine drainage, primarily attributable to heavy metal toxicity.

In the present study the positive associations between structure and function (Figures 3.5a, b, d, e, f) could have been driven by the direct effects of contamination on both structure and function, rather than through an indirect effect of stress on function, via changes in the macroinvertebrate community. Analyses presented in Section 3.3.2.5. indicate that in the absence of contamination, there was no relationship between structure and function. This suggests that stressors are affecting structure and function independently. However, the power of the test is limited (n = 6). Therefore, whilst there was no evidence for an indirect effect of heavy metals on ecosystem functioning via community structure, future studies at greater levels of replication are required to confirm this result. The lack of significant associations between structure and function in streams in the Leadhills (Figure 3.7; correlations $p > 0.05$) would similarly benefit from confirmation at greater levels of replication.

3.4.4. Why might these differences between regions exist?

In the present study I observed a positive association between aspects of macroinvertebrate community structure and function in Cornish streams, but no association in the Leadhills (Section 3.4.1.2.). There are several differences between the two regions which might explain these different patterns:

1) There were significant differences in scale between the two regions; some of the site pairs in Cornwall were quite a distance apart, whereas sites in the Leadhills were within a much smaller area.

2) Levels of contamination were significantly greater in Cornwall than they were in the Leadhills (specifically zinc, cadmium and aluminium) (Table 3.11).
3) The relative power of the analyses to detect a relationship in the Leadhills was weaker (8 data points) (Figure 3.7) as opposed to Cornwall (12 data points) (Figure 3.5).
4) There were differences in macroinvertebrate community composition between the two regions (Figure 3.6).

3.4.5. Caveats and considerations.

Given that very few studies have looked at relationships between structure and function in freshwater ecosystems, there are no established 'rules' about which measures are most appropriate to use. In this study I used to range of different measures to characterise the community, in order to provide the maximum possible information about the appropriate measures. However, there is a degree of non-independence in the reported measures of structure and function. Several measures of structure were non-independent: e.g. measures of the whole community are not independent of the shredder/non-shredder community, rather they are an aggregate measure of all of them. Similarly k is not independent of L (see also discussion in Section 2.4.5.).

3.4.6. Conclusions.

- The effect of heavy metal contamination is to reduce aspects of community structure and function.
- There were positive associations between several aspects of structure and shredder leaf processing in Cornwall, but not in the Leadhills.

4. Are rates of leaf processing by mixed-species assemblages predictable from the sum of their constituent parts?

4.1. Introduction.

The previous two chapters have focussed on identifying whether a positive association exists between ecological structure, specifically macroinvertebrate community structure and ecosystem function measured as the rate of leaf processing (Chapters 2 and 3). In this chapter I focus on evaluating the importance of species identity and compositional effects in determining rates of ecosystem processes. Justification for this focus comes from studies which have considered how species losses might affect ecosystem functioning (Section 1.2.), and recent recognition by ecologists that species identity and compositional effects are important functional components of biodiversity (Hooper *et al.* 2005) (Section 1.3.). An understanding of how interspecific differences in ecological attributes affect ecosystem functioning will enable ecologists to better predict the likely effects of biodiversity loss on ecosystem function. If ecosystem processes are simply the sum of their constituent (structural) parts, then structure reveals function, and from an ecosystem management perspective there is less of a need to develop tools to assess ecosystem function.

4.1.1. Species identity effects in freshwater ecosystems.

Species identity effects can be expected when species differ in their relative contribution to a particular ecosystem function. Covich *et al.* (1999) highlighted the importance of individual species functional traits in their contribution to freshwater ecosystem functions, such as sediment mixing, nutrient cycling and energy flow through food webs. Covich *et al.* reviewed the literature of the interactions between benthic species and ecosystem processes and indicated that some species have disproportionately large effects on certain ecosystem functions. Despite this emphasis, relatively few empirical studies have considered the role of species identity in driving structure-function relationships (Section 1.3.1.).

Species addition or removal experiments (Section 1.3.1.) have provided evidence to suggest that species are not equal in their relative contribution to determining rates of ecosystem processes in freshwater ecosystems (Ruesink & Srivastava 2001; Jonsson & Malmqvist 2003a). In a laboratory study by Jonsson & Malmqvist (2003a) the addition of one stonefly species had large effects on the rate of leaf processing, while the addition of a second stonefly species did not alter rates of leaf processing. Ruesink & Srivastava (2001) reported a similar pattern when two dominant leaf-eating species (this time, one stonefly and one caddisfly) were removed separately from field enclosures; the resulting change in the rate of leaf processing depended upon the identity of the species lost.

Evidence also comes from surveys of natural streams to suggest that shredder species are not equal in their relative contribution to rates of leaf processing (Carlisle & Clements 2005). Carlisle and Clements (2005), measured rates of leaf processing in heavy metal contaminated and nearby reference streams. Rates across contaminated sites were found to be similar, but lower than at nearby reference steams. In the study, three species of macroinvertebrate shredders were associated with leaf processing in streams: *Paraleuctra* spp. (Plecoptera: Leuctridae), *Taenionema pallidum* (Plecoptera: Taeniopterygidae), and *Zapada* spp. (Plecoptera: Nemouridae). The collective biomasses of these three species decreased with increasing heavy metal contamination. A significant reduction in the rate of leaf processing was associated with a significant decrease or even loss of *T. pallidum* individuals. In contrast, despite large differences in *Paraleuctra* spp. biomass and production between reference streams, there was no associated change in leaf breakdown rates, suggesting that this taxon had little influence on the overall rate of leaf processing.

Dangles & Malmqvist (2004) examined temporal data over a period of one year in three headwater streams in north-eastern France and determined the identity of the three dominant shredder species, which were: *Gammarus fossarum* (Amphipoda: Gammaridae), *Sericostoma personatum* (Trichoptera: Sericostomatidae) and *Chaetopteryx villosa* (Trichoptera: Limnephilidae). Rates of leaf processing were measured in the streams between December 1998 and August 1999. Rates varied with time and across streams. In the stream dominated by *G. fossarum* rates of leaf processing were maintained year round, despite low shredder diversity. In the

other two streams there was seasonal variation in breakdown rates, corresponding to peaks in the population densities of the dominant shredding trichopterans. This illustrates that rates of leaf processing may closely reflect the patterns of dominance of shredder species in natural streams.

4.1.2. Compositional effects in freshwater ecosystems.

Compositional effects are where certain combinations of species have disproportionately large effects on rates of ecosystem processes. A few studies in freshwater ecosystems have manipulated community composition and measured changes in ecosystem process rates, and, like most biodiversity – ecosystem function studies, have manipulated it within levels of species richness (Section 1.3.2.), including various studies by Jonsson *et al.* (Jonsson & Malmqvist 2000; Jonsson *et al.* 2002; Jonsson & Malmqvist 2003b; 2005). Two of these experimental studies have found community composition to be an important determinant of function, where function was measured as either secondary production (Jonsson & Malmqvist 2005), or rates of three distinct freshwater ecosystem processes: a) filtration rates, b) predation and c) grazing (Jonsson & Malmqvist 2003b). In the first study, secondary production was measured as growth of suspension feeding black-fly larvae when in the company of all possible combinations of 1-3 shredder species. Shredders were responsible for increasing the number of particles of leaf material (>0.1 mm) available to the black-fly larvae (Jonsson & Malmqvist 2005). In the second study, rates of the three distinct ecosystem processes were measured in assemblages of all possible combinations of 1-3 species of each of three related invertebrate functional feeding groups (Jonsson & Malmqvist 2003b).

Two separate studies measured the rate of leaf processing at the level of either 1, 2 or 3 shredder species (Jonsson & Malmqvist 2000; Jonsson *et al.* 2002). The results of these two studies appear to contradict each other. Jonsson *et al.* (2002) found that the effects of community composition were above and beyond those of species richness, whereas Jonsson & Malmqvist (2000) found that the effects of species richness were more important than those of community composition. These differences may be attributable to the identities of the shredder species

used. In Jonsson & Malmqvist (2000) three closely related species of stonefly (Plecoptera) species were used: *Taeniopteryx nebulosa* (Taeniopterygidae), *Nemoura avicularis* and *Protonemura meyeri* (Nemouridae). These species are closely related and so are unlikely to be immensely 'complementary' (Section 1.2.2.2.), whether in their resource use or in facilitative interactions. In contrast, in Jonsson *et al.* (2002) the shredder species were less closely related taxonomically: *Gammarus fossarum* (Amphipoda: Gammaridae), *Sericostoma personatum* (Trichoptera: Sericostomatidae) and *Nemurella picteti* (Plecoptera: Nemouridae). Therefore there is greater possibility that species were complementary. The authors proposed facilitative behavioural interactions between shredder species as the mechanism underpinning the compositional effects (Jonsson *et al.* 2002). This was because they observed *N. picteti* feeding on the surface of the leaves, *G. fossarum* feeding on the edges of the leaves, and *S. personatum* cutting leaf material into smaller pieces. Hence the most obvious case of facilitation would have been between *S. personatum* and *G. fossarum*, since *S. personatum* greatly increased the availability of leaf edges, which *G. fossarum* seem to prefer.

Other studies also document differences in the ways which shredder species differ in the way they feed on leaves. For example, for: *Gammarus pulex* (Graça 1993a), which probably feeds in the same way as *G. fossarum* (see above); *S. personatum* (see above) (Friberg & Jacobsen 1994); *A. aquaticus* which seems to graze the leaf surface (Graça 1993a). Other shredder species prefer to feed on small pieces of leaves e.g. *L. hippopus* (personal observation). However, the extent to which these differences between shredder species manifest into either positive or negative interactions between species is unknown. Observations of the feeding behaviour of other macroinvertebrate feeding groups indicates the presence of facilitative interactions between species. For example, Cardinale *et al.* (2002) observed facilitation between three species of suspension feeding caddisfly larvae in stream mesocosms. They proposed that differences in the morphology of the nets used for filter feeding allowed different species to facilitate each other's resource capture through biophysical interactions. In summary, differences in species traits may lead to complementarity between certain species, which might mean that rates of ecosystem processes by assemblages are greater than we would predict.

4.1.3. Philosophical/ Theoretical approach.

A relatively large body of literature indicates that rates of ecosystem processes increase with increases in species richness (reviews by Balvanera *et al.* 2006; Cardinale *et al.* 2006) (Section 1.2.1.3.1.). However there has been debate as to whether changes in function reflect changes in species richness *per se* or the loss of particularly dominant species with strong effects (Section 1.2.2.). Theory states that two effects can cause a diverse mixture of species to perform functionally differently than would be expected based on the functional performance of species growing in monoculture (Section 1.2.2.). Firstly, the sampling effect reflects the increasing possibility of selecting important or dominant species which might drive rates of ecosystem processes (Huston 1997) (Section 1.2.2.1.). Secondly, complementarity effects reflect niche differences and/or facilitative interactions between species (Section 1.2.2.2.).

Whereas most studies have considered the effects of species identity and composition within levels of species richness, in the present study I consider the relative importance of species identity and composition, in the situation where there is no manipulated diversity gradient. In this situation, sampling effects are analogous to species identity effects, because they reflect differences in species individual contributions to process rates, and compositional effects are analogous to complementarity effects, because they reflect interactions between particular species. If species identity *per se* is important in determining rates of ecosystem processes then we will be able to predict the aggregate functioning of a diverse ecosystem from individual processing rates, and points will lie along to 1:1 line between predicted and observed (Figure 4.1.). Interspecific differences will manifest themselves as variation in data points falling along the 1:1 line of predicted *vs.* observed, whereas compositional/complementarity effects will manifest themselves as data points falling either above (positive species interactions) or below (negative species interactions) the 1:1 line (Figure 4.1.). To illustrate, imagine that there is a pool of six species: species A, species B, species C, species D, species E and species F. In isolation, the rates of processing by individual species are greater for some species than for others, such that the amount processed by species A is less than species B, which is less than species C... to species F (i.e. A < B < C < D < E < F). If we were then to make predictions of the amount of processing by combinations of 3-species

assemblages, we would predict that an assemblage comprising species A, B and C would process a lesser amount than an assemblage comprising species D, E and F. So a prediction of the rate of processing of assemblage ABC would be toward the bottom left of the 1:1 line on Figure 4.1, whereas prediction of assemblage DEF would be toward the top right of the 1:1 line. The variation between points along the 1:1 line thus being driven by interspecific differences.

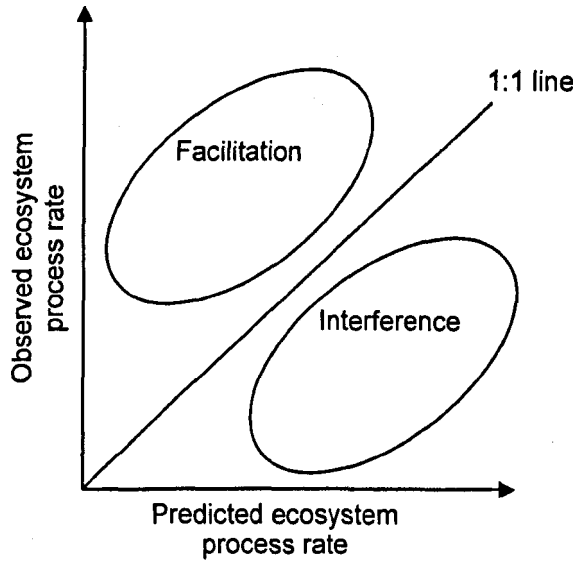

Figure 4.1: The hypothetical relationships between predicted and observed ecosystem process rates (see text). If an accurate prediction can be made, then points will lie on the 1:1 line. If data points fall above the 1:1 line, then facilitative interactions are occurring, and below the 1:1 line, interference.

4.1.4. Study system and mechanistic basis for identity effects.

The following experiment was designed to examine whether rates of leaf processing are predictable from individual species rates of leaf processing in isolation. Interspecific variation in the traits of macroinvertebrate shredders includes biomass (usually measured as dry weight) and the amount of leaf material they process over a given time period, usually expressed as a mass specific consumption rate and expressed as the letters *Cm*. Previous studies have indicated that there is variation in species-specific feeding rates (Jonsson & Malmqvist 2000; Inglis 2003 unpublished) suggesting that species identity effects are a possibility. One source of variation in species consumption rates could be differences in body size (Peters 1983). Cammen (1980) collected data on a range

of detritus-feeding invertebrates, and showed that consumption rate was related to body size:

$$Y = aM^b \qquad \text{Equation 4.1.}$$

where Y is the rate of consumption to be predicted, M is the animal body mass, and a and b are empirically derived constants, with $a = 0.381$ and $b = 0.742$. For example, rates of leaf processing by individuals of each of 3 species of shredding stoneflies (*Protonemura meyeri, Nemoura avicularis, Taeniopteryx nebulosa*) were found to be significantly different (mg/ind), but this difference was removed when biomass was accounted for (i.e. mass-specific leaf processing rate *Cm*) (mg/mg/ind) (Jonsson & Malmqvist 2000).

4.1.5. Aims.

The overall aim was to test whether a prediction could be made of the rate of leaf processing of assemblages of shredder species, based on knowledge of individual shredder species leaf processing rates. This was addressed by asking the following questions:

1) What are the mass-specific leaf consumption rates of individual shredder species?
2) What are the net leaf consumption rates of various assemblages of the same shredder species?
3) Can leaf consumption rates in assemblages be predicted from information on species consumption rates in isolation?

These were addressed by: 1) quantifying leaf consumption rates of seven shredder species on a single leaf resource; 2) quantifying the leaf consumption rates of three-species shredder assemblages in thirty stream mesocosms; 3) using data from the feeding trials to generate a prediction of the rate of leaf processing in assemblages.

4.2. Methods.

4.2.1. Choice, collection and acclimation of shredder test species.

Shredder species were selected using two criteria: representation of a range of taxonomic groups, and availability. The shredder species used, their taxonomic groups and collection locations are summarised in Table 4.1. Some of the test species have been found to exist together in local streams and have been successfully used in previous indoor stream mesocosm and feeding trial experiments (Jonsson *et al.* 2002; Inglis 2003 unpublished).

Shredders were collected by taking kick samples from the streambed with a standard kick-net (Murray-Bligh *et al.* 1997). The kick-net contents were then sorted in trays; species were identified and collected using a pipette or forceps where appropriate. This process was repeated until the target number of individuals had been collected (which was roughly 10 % greater than the required number, to allow for species emergence and mortality) (for relative abundances of animals stocked into stream mesocosms see Section 4.2.3.3.). Collection techniques were modified slightly for *A. aquaticus*, which were collected by dredging leaf litter from the bottom of the pool with a kick net and then separating the animals from the leaf litter for collection. Shredders were transported back to the laboratory in stream water aerated with small air pumps.

Shredders were acclimatised to laboratory conditions, which differed between the two experiments (see Sections 4.2.3.1. and 4.2.4.2.), for a minimum period of three days. Animals were held in species-specific tanks and provided with alder leaves (*Alnus glutinosa* (L.) Gaertner) as a food source. Tanks were aerated and kept in the same room as experimental system, and were therefore exposed to a similar light and temperature regime as throughout the duration of the experiment. After 24 hours in the tanks, the stream water was gradually replaced with Artificial Pond Water (APW) (H.S.E. 1982).

Table 4.1: Shredder species used in mesocosm experiments, taxonomic order and collection locations. NGR = National Grid Reference.

		Collection location	
Species Name	**Order**	**Watercourse name**	**NGR**
Asellus aquaticus Linnaeus	Isopoda	Rivelin Pond	SE324889
Gammarus pulex Linnaeus	Amphipoda	Crags stream	SK497744
Leuctra hippopus Kempny	Plecoptera	Berrymoor (River Dove)	SE292029
Nemurella picteti Klapalek	Plecoptera	Pigeon Bridge Brook	SK480852
Potomophylax latipennis Curtis	Trichoptera	Crags Stream	SK497744
Protonemura praecox Morton	Plecoptera	Strines Dike	SE220908
Sericostoma personatum Spence	Trichoptera	River Lathkill	SK223647

4.2.2. Conditioning leaf material.

Shredders were provided with a single leaf resource: alder (*Alnus glutinosa*). Leaves were collected just prior to abscission during October/November 2001 at the same locations as used previously (Section 3.2.4.). Leaf material was air-dried for one week prior to storage.

Thirty-five coarse mesh leaf bags were constructed from standard greenhouse shelter netting (mesh size: 3.5 x 7 mm) and filled with approximately 8 g of air-dried leaf material. Seven weeks prior to the start of the experiments leaf bags were conditioned by deployment into a local stream, the Porter Brook (NGR: SK318855) in order that they could be colonised by a variety of micro-organisms (i.e. to make leaves more suitable for detritivores in general). Mesh bags were attached to fishing wire (80 lb breaking strength) and deployed into the stream for a period of 3 weeks. On collection, leaves were rinsed in distilled water and air-dried prior to use in the experiments.

4.2.3. Species-specific feeding rates.

4.2.3.1. Experimental system.

The leaf consumption rates of the seven shredder species were quantified in a series of feeding trials. Consumption rates were quantified for individual animals exposed to five leaf disks in small glass jars (Section 4.2.2.2.). Feeding trials were performed in February 2006. Animals were collected in January/ February 2006,

and held in holding tanks maintained in a constant temperature room prior to the start of the experiments.

Individual animals of each of the seven shredder species (Table 4.1) were exposed to leaf disks in small (60 ml) glass jars (Figure 4.2). Each glass jar contained 40 ml APW, three small pieces of pea gravel, five leaf disks and a single animal. Jars were aerated through a syringe needle. There were 25 replicates of each shredder species treatment, plus 25 replicates with no animals present (this treatment provided a control for mass loss due to bacterial/fungal breakdown and physical abrasion). Treatments were assigned to jars in a stratified order. Jars were contained in trays of 35 replicates (Figure 4.2) in a constant temperature room at 15 °C with lights operating on a 12-hr light: 12-hr dark photoperiod. Water levels were maintained by refilling with distilled water. Animals were monitored daily for mortality or emergence; replicates where this happened were subsequently removed from the analysis. Moults were removed from jars. Feeding trials were terminated when leaf resources in any of the replicates had almost completely been consumed (i.e. 6 d).

Leaf disks were cut from alder leaves (see Section 4.2.1.2.), using a cork borer (10 mm diameter) and were oven-dried at 60 °C for 7 days before being weighed. Leaf disks were rehydrated in APW for 4 days prior to the addition of shredders to the jars. After 6 days of feeding animals and leaf disks were removed from jars, oven-dried at 60 °C and weighed.

4.2.3.2. Statistical analyses.

Mean rates of shredder feeding (C, mg/ind/d) were calculated as the amount of leaf material consumed per animal per day:

$$C = \frac{(W_i \times F) - (W_z)}{t} \qquad \text{Equation 4.2.}$$

where W_i is the start weight of leaf material (mg, oven-dried), W_z is the end weight of leaf material (g, oven-dried), and t is the number of days (d). F is a correction

Figure 4.2: Photograph of the feeding trials (i.e. the experimental system used for quantifying leaf consumption rate by individuals of different shredder species) (see Section 4.2.2.2.).

factor representing the mean proportional change in leaf mass for control leaf material in the non-shredder treatment (W_z/W_i).

I compared mean rates of shredder feeding (C) and body size (M) estimates from the feeding trails with the equation by (Cammen 1980) to verify that animals conform to standard allometric scaling relationships (Section 4.1.4.):

$$C = 0.381 \times M^{0.742} \qquad \text{Equation 4.3.}$$

where C is mg of organic matter eaten per animal per day and M is the mg dry weight of each animal.

4.2.4. Leaf consumption rates of shredder assemblages.

4.2.4.1. Experimental design.

In January and February 2005 thirty replicate artificial stream mesocosms were used to investigate whether macroinvertebrate shredder community composition was important for determining rates of leaf processing. The use of indoor mesocosms permits control of many biotic and abiotic variables, which could not be controlled outdoors. Each stream mesocosm was allocated a shredder assemblage of three species, each being taxonomically distinct, from a species pool of seven. There are 35 possible three-species combinations, 30 of which were used in this experiment (Table 4.2). Shredder assemblages were selected to represent a range of processing rates and shredder species, with a bias toward those with either particularly low- or high- predicted processing rates.

4.2.4.2. Experimental system.

Artificial stream mesocosms were constructed from white plastic electrical ducting material and small pumps (Aquaclear Powerhead 201, Hagen®) were used to recirculate APW through plastic tubing (Figure 4.3). Each stream was compartmentalised with fine nylon mesh (120 μm x 200 μm mesh size from Plaskok®) and coarse woven wire mesh (0.15 x 0.17 cm mesh size) in order to create compartments that were assigned to particular functions (Figure 4.3). Both kinds of mesh acted as barriers to enclose shredders within particular compartments, while coarse mesh was placed immediately upstream of each fine mesh barrier in order to prevent fine mesh becoming clogged with coarse particulate organic matter (CPOM), thereby restricting flow. Each stream channel was 150 cm long, 9.5 cm wide and 9 cm deep and was filled to a depth of 3 cm with pea sized gravel in certain compartments (see Figure 4.3). Mesocosms were filled to a depth of 6 cm with APW, the water level was marked and maintained throughout the experiment through the addition of distilled water. Throughout the course of the experiment the fine meshes were periodically scraped with a spatula in order to remove any fine particulate organic matter and to maintain flow.

Table 4.2: Possible assemblage composition of the 7 shredder species, and their allocation to each stream mesocosm. - = Assemblage was not included. * = this stream mesocosm was eliminated from analyses (see text).

Species present			Assemblage/ Stream no.
G. pulex	*S. personatum*	*P. latipennis*	1
G. pulex	*P. praecox*	*P. latipennis*	2
S. personatum	*N. picteti*	*P. praecox*	3
A. aquaticus	*S. personatum*	*N. picteti*	4
A. aquaticus	*G. pulex*	*P. latipennis*	5*
L. hippopus	*N. picteti*	*P. latipennis*	6
L. hippopus	*P. praecox*	*P. latipennis*	7
A. aquaticus	*N. picteti*	*P. latipennis*	8
G. pulex	*L. hippopus*	*N. picteti*	9
A. aquaticus	*L. hippopus*	*P. praecox*	10
A. aquaticus	*S. personatum*	*L. hippopus*	11
A. aquaticus	*L. hippopus*	*P. latipennis*	12
A. aquaticus	*S. personatum*	*P. praecox*	13
A. aquaticus	*G. pulex*	*L. hippopus*	14
S. personatum	*N. picteti*	*P. latipennis*	15
G. pulex	*L. hippopus*	*P. praecox*	16
G. pulex	*N. picteti*	*P. praecox*	17
A. aquaticus	*G. pulex*	*N. picteti*	18
S. personatum	*P. praecox*	*P. latipennis*	19
L. hippopus	*N. picteti*	*P. praecox*	20
A. aquaticus	*S. personatum*	*P. latipennis*	21
G. pulex	*N. picteti*	*P. latipennis*	22
G. pulex	*L. hippopus*	*S. personatum*	23
A. aquaticus	*P. praecox*	*P. latipennis*	24
A. aquaticus	*G. pulex*	*S. personatum*	25
G. pulex	*L. hippopus*	*P. latipennis*	26
S. personatum	*L. hippopus*	*N. picteti*	27
S. personatum	*L. hippopus*	*P. praecox*	28
G. pulex	*S. personatum*	*P. praecox*	29
A. aquaticus	*L. hippopus*	*N. picteti*	30
A. aquaticus	*G. pulex*	*P. praecox*	-
A. aquaticus	*N. picteti*	*P. praecox*	-
G. pulex	*S. personatum*	*N. picteti*	-
N. picteti	*P. praecox*	*P. latipennis*	-
S. personatum	*L. hippopus*	*P. latipennis*	-

Each mesocosm consisted of seven compartments in total (Figure 4.3). The 'community compartment' contained the experimental shredder assemblages, measuring 98 cm long x 9.5 cm wide. Three of the smaller compartments were assigned as 'monitoring compartments'. In each of these monitoring compartments, five individuals (of each of the three species in the corresponding community compartment) were kept with a small amount of alder leaf material and a few pieces of pea gravel in order that any emergence could be monitored. The fourth small compartment was assigned as a control, for leaf mass loss due to microbial and physical abrasion, i.e. it contained leaves, but no shredders.

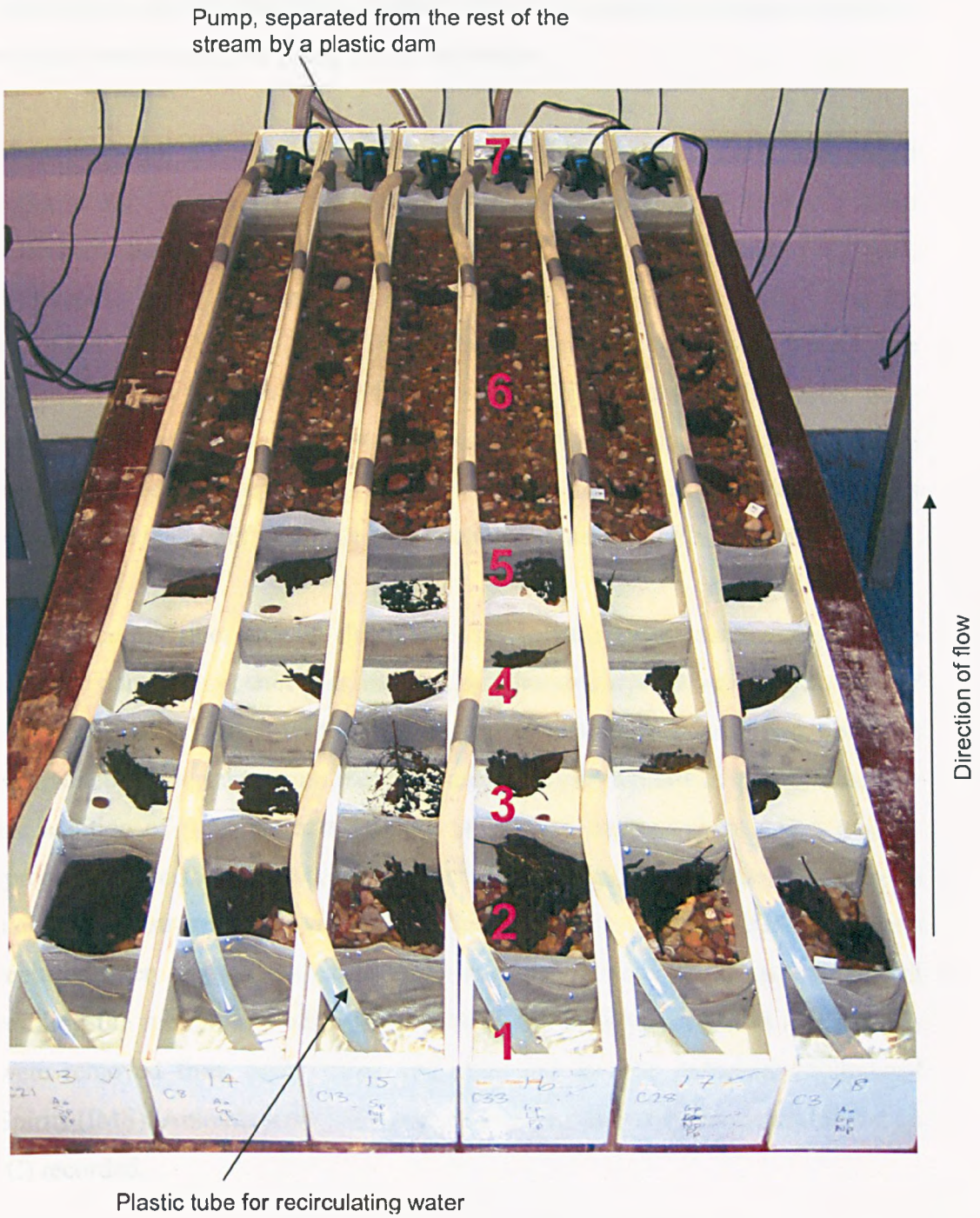

Figure 4.3: Six of the artificial stream mesocosms. Individual compartments were as follows: 1 = water inflow; 2 = gravel and control leaf material; 3, 4 and 5 = single species monitoring compartment with 3 pieces of gravel, 5 animals and some alder leaves as a food resource; 6 = community compartment with shredder assemblage, experimental leaf material and gravel; 7 = pump area.

Each stream was randomly assigned one of thirty treatments and labelled accordingly (Table 4.2). The mesocosms were placed side-by-side on laboratory benches (6 to a bench). After stocking with leaves and shredders (see Sections 4.2.3.4 and 4.2.3.4.) the mesocosms were left to run for 21 days in a room with natural light. Stream mesocosm number 5 had to be eliminated from the analyses as there was a leak at the pump end of the stream.

Maximum and minimum room temperatures were recorded daily and varied between 9.5 °C and 15.5 °C (mean minimum temperature = 11.0 °C, mean maximum temperature = 13.1 °C). Stream water temperature (°C), pH, conductivity (mV) and dissolved oxygen (mg/L) were recorded daily (with the exception of days 6, 14 and 21) for each stream with hand-held meters (see Section 3.2.3.). pH was also not recorded on days 1 and 2. Flow rates were estimated every second day by draining water from the plastic tubing in which the water was recirculating, into a measuring cylinder for 10 seconds. This was repeated and the pump speed adjusted to approximately 83 ml/s for each stream.

Emergence and mortality in the single-species monitoring compartments were recorded daily. Emergence was recorded by removing and counting the number of final instar empty cases found in or on the sides of each stream. Any observed emergence or mortality from the monitoring compartments was compensated by the addition of fresh animals into the monitoring compartments and a number (proportional to the number of individuals of the corresponding species originally stocked into streams) was added into the community compartments. APW was replaced after 14 days, by draining the mesocosms from the plastic tubing and refilling the mesocosms with fresh APW. After 21 days all gravel and animals were removed from each stream and preserved in 70% Industrial Methylated Spirits (IMS). Animals were later counted and their dry weights (oven-dried at 60 °C) recorded.

4.2.4.3. Mesocosm stocking.

Animals were collected over a period of one week (in early February 2005). Therefore, although animals were acclimatised to mesocosm conditions in tanks for a standard period of time, not all the animals were acclimatised to laboratory

conditions in tanks for a standard period of time. Shredders were transferred into mesocosms 24 hours prior to the addition of leaf material.

Mesocosms were stocked with approximately equal target shredder biomasses for each species of shredder. A range of shredder species of different size categories were used, and therefore equalising biomass across the treatments minimising weight-specific feeding rate differences. This design assumes that each of the seven shredder species used would be able to achieve similar biomasses in nature. The mean dry weight of 20 individuals of each shredder species, collected between November 2004 - January 2005, was used to calculate the target biomass for stocking stream mesocosms (mg/dry weight) (Table 4.3). The target biomass for each species was estimated as the lowest number of individuals of the heaviest species that were deemed sensible for inclusion, i.e. 3 individuals of the heaviest species, in this case *P. latipennis* (= 26.4 mg) (see Table 4.3). Target stocking densities were then estimated for each species as the target biomass/ mean biomass per species and rounded to the nearest whole number (see Table 4.3: "Actual relative abundance of individuals used to stock mesocosms") (*StartA*).

Although I attempted to control and standardise animal biomass across streams, this proved very difficult. For this reason, despite efforts to avoid it, there were changes in animal biomass between the start and the end of the experiment. These are documented in Appendix H (Section H.1), as is the replacement of animals into the stream mesocosms (community compartments) over the 21-day period (Section H.2).

Table 4.3: Target and actual stocking levels for each shredder species in the artificial stream mesocosms. Mean target biomasses were calculated as the mean of 20 individuals (dry weight) collected between November 2004 and January 2005 from the locations described above (Table 4.1). * = target biomass was calculated as three times the weight of the heaviest species, which was *P. latipennis*. Actual relative abundance of individuals used to stock mesocosms was calculated as the target biomass/biomass per species, and rounded to the nearest whole number (*StartA*). Actual biomasses stocked per species were calculated from the biomass of animals collected from stream mesocosms at the end of the experiment ($M_{Individual}$).

Species	Target stocking levels		Actual stocking levels		
	Mean biomass per species from animals collected Nov 2004 – Jan 2005 (mg/individual dry weight)	Target biomass (mg/species)*	Actual relative abundance of individuals used to stock mesocosms (*StartA*)	Mean biomass per individual animal collected in Feb 2005 ($M_{Individual}$) (mg/individual dry weight)	Actual biomass of each species stocked ($M_{Individual}$ x *StartA*) (mg/species)
Asellus aquaticus	6.0	26.4	4	4.4	17.8
Gammarus pulex	7.6	26.4	3	2.9	8.7
Leuctra hippopus	0.8	26.4	32	0.4	11.9
Nemurella picteti	0.8	26.4	31	0.4	13.8
Potomophylax latipennis	8.8	26.4	3	7.8	23.3
Protonemura praecox	2.2	26.4	12	1.0	11.9
Sericostoma personatum	3.6	26.4	7	2.6	18.3

4.2.4.4. Quantifying leaf processing.

Each stream community compartment was initially stocked with 0.7 g ± 0.05 g (air-dried) of alder leaves at the start of the experiment. A further 0.25 g of leaf material was added on Day 13 of the experiment. Each stream was also stocked with 0.5 g ± 0.05 g of control alder leaves, to provide a measure of leaf processing in the absence of shredders (see Figure 4.3: compartment 2). The exact air dry weight of each set of leaves was recorded, and each set was identified using a code on a plastic plant label. Leaves were rehydrated with APW 24 hours prior to the start of the experiment. After 21 days all leaf material was removed from the mesocosms, rinsed with distilled water, and placed in a labelled dish for air-drying. Leaf mass was air-dried until a constant mass was achieved.

4.2.4.5. Statistical analyses.

The observed leaf mass loss (L_{OBS}) per stream mesocosm was calculated using the following equation:

$$L_{OBS} = (W_i \times F) - W_z \qquad \text{Equation 4.4.}$$

where W_i is the start weight of leaf material (mg, air-dried), W_z is the end weight of leaf material (g, air-dried). F is a correction factor representing the mean proportional change in leaf mass for control leaf material (W_z/W_i) across streams.

An estimate of shredder assemblage biomass (mg/dry) ($M_{Assemblage}$) for each stream mesocosm was calculated as:

$$M_{Assemblage} = \sum_{i=1}^{n} \left(M_{Individual\ i} \frac{StartA_i + EndA_i}{2} \right) \qquad \text{Equation 4.5.}$$

where $M_{Individual}$ is the average mass of each shredder species collected at the end of the experiment across streams, *StartA* is the relative abundance of individuals of each shredder species stocked into each stream at the start of the experiment, *EndA* is the relative abundance of individuals recovered from each stream at the

end of the experiment and n is the number of shredder species in each mesocosm ($n = 3$).

Regression analysis was used to ascertain whether there was a significant linear relationship between shredder assemblage biomass ($M_{Assemblage}$) and the observed rate of leaf processing (L_{OBS}) in streams over the 21 day experimental period.

4.2.5. Predicting the rate of leaf processing in assemblages.

Leaf consumption rates (C) of shredder species in isolation as measured in the species-specific feeding trials (Section 4.2.2.) were used to make predictions of leaf mass loss from the shredder assemblages in stream mesocosms (Section 4.2.3.). It was appropriate to use allometric scaling relationships (see Section 4.1.4.) for generating this prediction because there were differences in the average body sizes of species between the feeding trials and the mesocosm study (data not presented). Predicted leaf mass loss (L_{EXP}) in each of the stream mesocosms was calculated as:

$$L_{EXP} = \left(\sum_{i=1}^{n} C_i \frac{StartA_i + EndA_i}{2} \right) \times t \qquad \text{Equation 4.6.}$$

where n is the number of shredder species in each stream ($n = 3$), t is the time in days mesocosms ran for ($t = 21$ d), C is the rate of leaf consumption per animal per day (mg/d), *StartA* is the relative abundance of individuals of each shredder species stocked into streams at the start of the experiment, *EndA* is the relative abundance of individuals recovered from streams at the end of the experiment. The results of the feeding trials reveal that making a prediction of the rate of leaf processing from body size, for most shredder species is a valid assumption, with the exception of *A. aquaticus* (see Section 4.3.1.). Therefore, C was calculated using Equation 4.3 for all shredder species except *A. aquaticus*. To do this I estimated the mass (mg/ind) of each shredder species as the average mass per individual collected at the end of the experiment across streams ($M_{Individual}$). The results of the feeding trials revealed that *A. aquaticus* did something else to the other shredder species (Section 4.3.1.). Therefore I fitted a new relationship to the

small amount of information we have for this species, by changing the *y* intercept. Thus, for *A. aquaticus*, *C* was calculated using Equation 4.7 (see Section 4.3.1).

In order to test whether predictions equal observed rates of leaf processing, residual values (i.e. observed minus predicted) of leaf processing rates were calculated and tested (one-sample *t*-test) for residuals being significantly different from zero.

4.3. Results.

4.3.1. Species-specific feeding rates.

Species-specific feeding rates ranged from 0.18 mg/ind/d to 2.11 mg/ind/d with larger shredder species (e.g. *Potamophylax latipennis*) having proportionally lower rates of leaf consumption (*C*) than smaller species (e.g. *Leuctra hippopus*) (Table 4.4). There were significant differences in rates of leaf consumption (mg/ind/d) between shredder species (One-way ANOVA: $F = 59.45$, $p < 0.001$, d.f. = 6, 139). There were no significant differences within the two insect orders, but they were significantly different from the non-insect orders (Table 4.4: letter 'b' = stoneflies, and letter 'd' = caddisflies) (Tukey Multiple Comparison test, $p < 0.05$), with the exception of *A. aquaticus* which was not significantly different from two of the stonefly species (Table 4.4: letter 'a'). *G. pulex* was significantly different from all other shredder species (Table 4.4: letter 'c').

Table 4.4: Mean (N and standard error (SE)) rates of leaf consumption (*C*) and body size (*M*) of shredder species in the single-species feeding trials. Letters a – d indicate significant differences in leaf consumption rates between species (Tukey Multiple Comparison test, $p < 0.05$).

Species	N	Leaf consumption rate (*C*) (mg/ind/d)			Body size (*M*) (mg dry mass)	
		Mean	SE		Mean	SE
Asellus aquaticus	22	0.18	0.03	a	10.0	0.4
Nemurella picteti	21	0.28	0.04	ab	1.2	0.1
Leuctra hippopus	17	0.30	0.05	ab	0.7	<0.1
Protonemura praecox	18	0.75	0.09	b	1.7	0.2
Gammarus pulex	24	1.24	0.13	c	6.3	0.2
Sericostoma personatum	23	2.01	0.11	d	7.1	0.6
Potomophylax latipennis	21	2.11	0.18	d	22.6	1.8

Six out of seven of the shredder species conformed to a standard allometric scaling relationship between body size and feeding rates (Cammen 1980) (Figure 4.4). Data from previous feeding trials of the same shredder species as used in the present study (Inglis 2003 unpublished) (Figure 4.4: open symbols) indicate that these patterns are generally applicable. Species included in both sets of feeding trials fit well within the spread of species used to generate the allometric equation (Figure 4.4: black circles), with the exception of *Asellus aquaticus*, which did not conform to the allometric scaling relationship in either feeding study (Figure 4.4: brown circles). The rate of leaf consumption of this species was disproportionately lower than expected based on its body size. Given this, and for the purpose of generating a prediction of the amount of leaf processing in streams (Section 4.2.5.), I generated a new allometric relationship for *A. aquaticus*, by keeping the slope of the line the same (i.e. $b = 0.742$) and using the data from both sets of feeding trials to calculate a (i.e. the y intercept; see Equation 4.1):

$$C_{Aa} = 0.0297 \times M^{0.742} \qquad \text{Equation 4.7.}$$

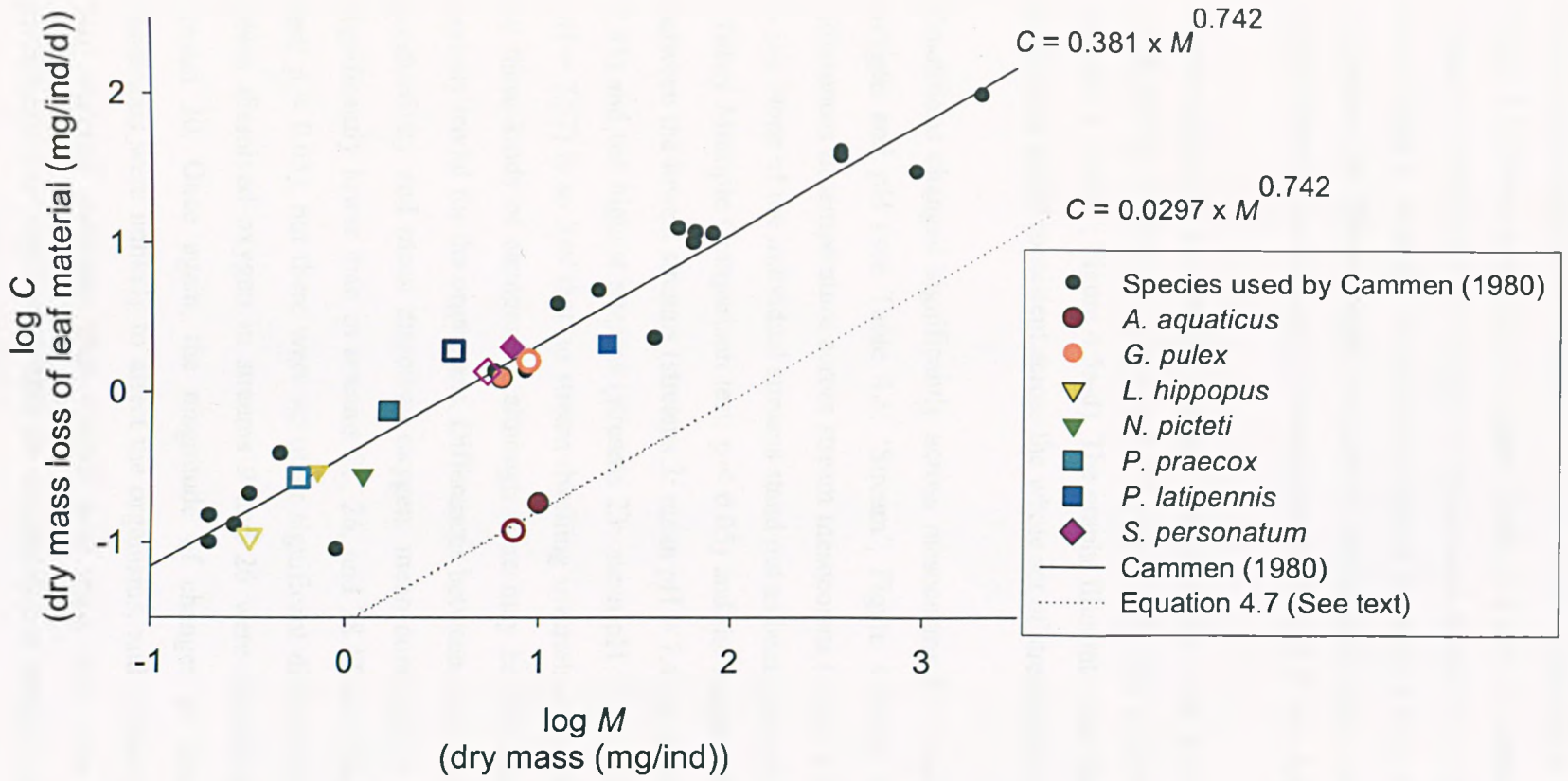

Figure 4.4: The relationship between body size (*M*) (mg/ind) and leaf consumption rate (*C*) (mg/ind/d) for different invertebrate species. Closed coloured symbols represent means from species as measured by the feeding trials in the present study. Open coloured symbols represent the same species in previous feeding trials (Inglis 2003). Black dots represent species of benthic invertebrate deposit feeders and detritivores used to generate the equation of Cammen (1980) (see Equation 4.3) (black line). The dotted line is Equation 4.7 (see text Section 4.3.1.) (i.e. the allometric scaling equation for *A. aquaticus*). All feeding was measured at 15 ˚C and individual species were maintained in isolation.

4.3.2. Physicochemical conditions in stream mesocosms.

Mean physicochemical conditions across stream mesocosms are summarised in Table 4.5. Mean conductivity ranged from 515 mV in stream 2 to 584 mV in stream 1. Mean dissolved oxygen in mesocosms ranged from 10.1 mg/L in stream 9 to 10.5mg/L in stream 30. Mean pH ranged from pH 7.43 in stream 3 to pH 7.57 in stream 26. These ranges are low in comparison with natural streams (e.g. physiochemical conditions in streams seen in Chapter 3: see Appendices C & G).

Physicochemical conditions changed significantly with time for temperature, conductivity, dissolved oxygen and pH over the 21-day experimental period (see Table 4.5: 'Time'; Figure 4.5a-d). The graphs illustrate that changes through time seem to be fairly consistent across the whole set of streams (Figure 4.5).

Conditions changed significantly across mesocosms for conductivity, dissolved oxygen and pH (see Table 4.5: 'Stream'; Figure 4.5b-d), but there were no differences in temperature across stream mesocosms (Table 4.5: 'Stream'; Figure 4.5a). None of the individual streams stand out as being very different in mean pH (Tukey Multiple Comparison test: $p < 0.05$) and the magnitude of the difference between the lowest streams (streams 3: mean pH = 7.43 and stream 6: mean pH = 7.43) and the highest streams (streams 23: mean pH =7.56 and stream 26: mean pH = 7.57) is so low that no stream dwelling invertebrate is likely to be affected by these kinds of changes, so although there may be differences, these may be entirely trivial for the organisms. Differences between streams were few for mean conductivity and mean dissolved oxygen: mean conductivity in stream 11 was significantly lower than in streams 21, 26, and 28 (Tukey Multiple Comparison test: $p < 0.05$), but there were no other significant differences between streams. Mean dissolved oxygen in streams 9 and 26 were significantly lower than in stream 30. Once again, the magnitude of changes in these physiochemical conditions were unlikely to affect the organisms, and evidence from the control leaf material indicates that overall leaf mass loss was not affected by physiochemical conditions within the streams (Mean control leaf mass loss = 0.05 g / 21 days, mean SE = 0.006).

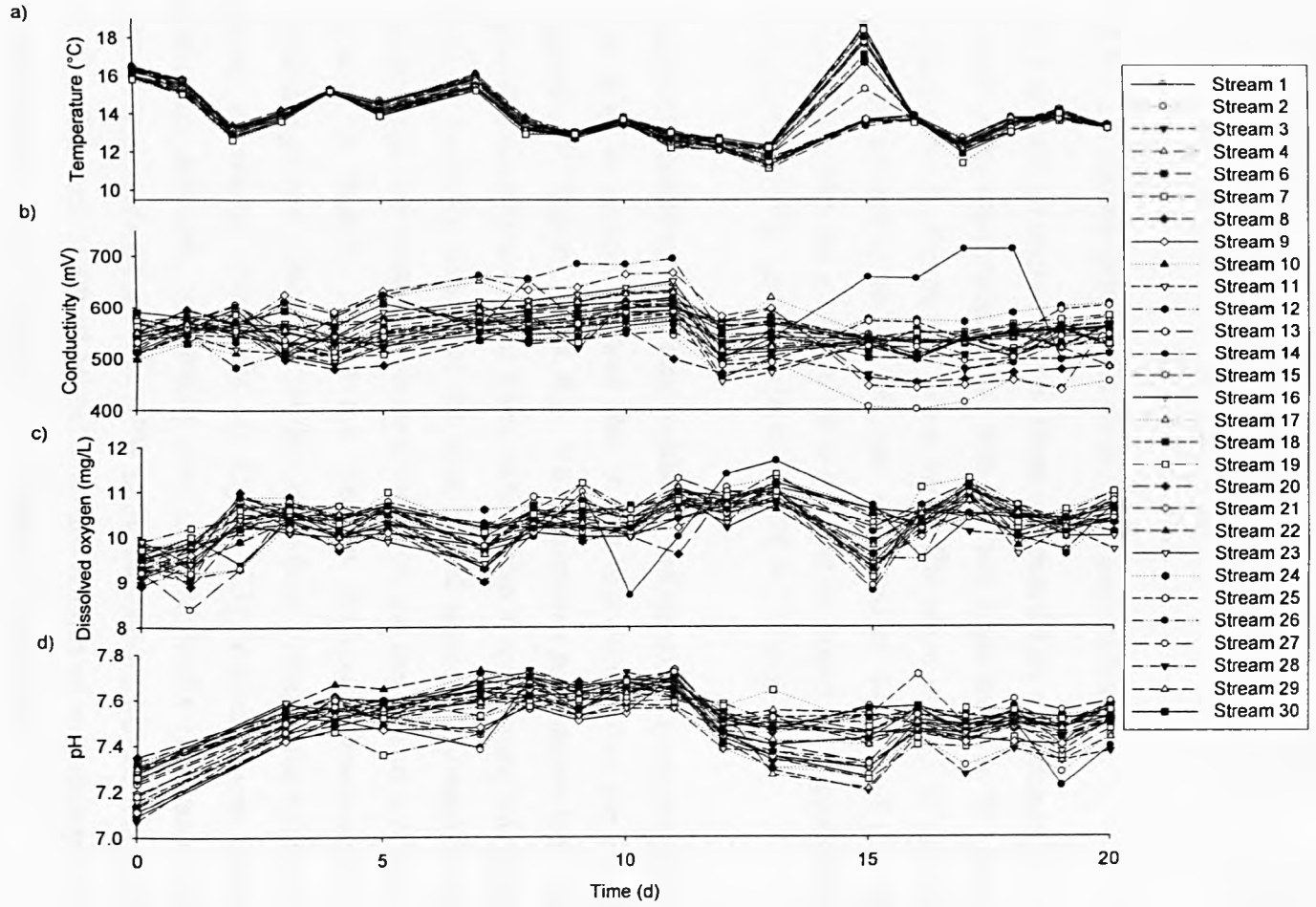

Figure 4.5: Mean physicochemical factors a) temperature, b) conductivity (mV), c) dissolved oxygen (mg/L) and d) pH over the 21 day experimental period in stream mesocosms.

Table 4.5: Mean, N, and Standard Error (SE) of physicochemical conditions in stream mesocosms over the 21 day experimental period, and results of statistical tests for differences in mean physicochemical conditions across mesocosms over the 21 day experimental period (repeated measures ANOVA between streams). *** = $p < 0.001$, NS = $p > 0.05$.

				Stream (d.f. = 28, 504)		Time (d.f. = 18, 504)	
Physicochemical factor	**N**	**Mean**	**SE**	**F**	**p**	**F**	**p**
Temperature (°C)	551	13.84	0.06	0.51	0.983	173.89	<0.001
Conductivity (mV)	551	553.33	1.88	4.62	<0.001	16.98	<0.001
Dissolved oxygen (mg/L)	551	10.31	0.02	2.40	<0.001	46.89	<0.001
pH	493	7.51	0.01	15.75	<0.001	146.22	<0.001

4.3.3. Leaf consumption rates of shredder assemblages.

The estimated amount of animal biomass stocked into each stream mesocosm at the start of the experiment ranged from 32.5 mg in stream 16 to 59.3 mg in stream 21 (Appendix H: Figure L.1: black bars). The mean was 45.32 mg (SE = 1.23 mg). The amount of animal biomass recovered at the end of the experiment ranged from 16.1 mg in stream 28 to 61.1 mg in stream 21 (Appendix H: Figure H.1: grey bars). The mean was 35.80 mg (SE = 2.15 mg).

There was variability in the total number of individuals and amount of dry mass of each shredder species replaced into stream mesocosms over the 21 day period (Appendix H: Figures H.2 & H.3). No *Sericostoma personatum* individuals were replaced into any mesocosms (their survivorship in mesocosms was exceptionally high). A moderate number of the large bodied species *Potamophylax latipennis* was replaced into streams (their survivorship in the mesocosms was low). Greater numbers of stonefly species (i.e. *Leuctra hippopus, Protonemura praecox, Nemurella picteti*) were replaced than other taxa, owing to the tendency of these species to emerge (Appendix H: Figure H.3). Animals were replaced into mesocosms relatively consistently over time (Appendix H: Figure H.4). Fewer animals were replaced after the APW in the mesocosms had been changed on day 14. The distribution of both animal mass and number of animals replaced was not even across mesocosms (Appendix H: Figures H.5 and H.6).

Rates of leaf processing by shredder assemblages ranged from 152 mg/21 d for assemblage 2 (*Gammarus pulex, Protonemura praecox, Potamophylax latipennis*) to 804 mg/ 21 d for assemblage 19 (*Sericostoma personatum, P. praecox, P. latipennis*) (Figure 4.6: *y* axis).

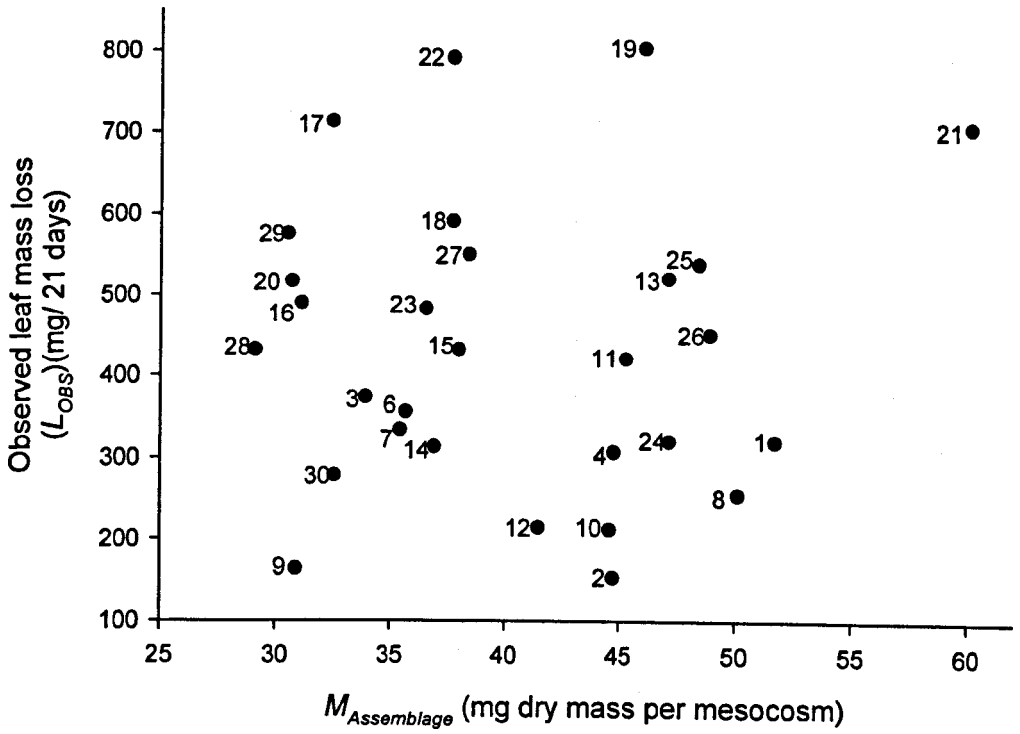

Figure 4.6: Observed rate of leaf processing (L_{OBS}) and the amount of shredder biomass ($M_{Assemblage}$) in each stream mesocosm over the 21 day experimental period. Numbers to the left of data points are the stream mesocosm/ assemblage number (see Table 4.2).

There was no significant relationship between shredder assemblage biomass ($M_{Assemblage}$) and the observed rate of leaf processing (L_{OBS}) in streams over the 21 day experimental period (Figure 4.6) (Regression: $F_{1,27} = 0.02$, $p = 0.9$, R^2 (adj.) = 0 %). This relationship does not improve if assemblage biomass is estimated using the relative abundance of individuals at either the start (*StartA*) or end (*EndA*) of the experiment, rather than the midpoint between the two ($M_{Assemblage}$).

4.3.4. Predicting the rate of leaf processing in assemblages.

Predictions of leaf processing rates of assemblages in stream mesocosms were generated using feeding rates for individual species (*C*, mg/ind/d) calculated using allometric relationships relating feeding rate to body mass (Equations 4.4 and 4.8). Predicted assemblage leaf processing rates (L_{EXP}) were then compared to those observed in stream mesocosms (L_{OBS}).

In 27 out of 29 assemblages, the observed rate of leaf processing was greater than predicted (Figure 4.7: points lying above the 1:1 line) (i.e. $L_{OBS} > L_{EXP}$), with the

exception of assemblages 2 (*Gammarus pulex, Protonemura praecox, Potamophylax latipennis*), and 9 (*Gammarus pulex, Leuctra hippopus, Nemurella picteti*) whose $L_{OBS} < L_{EXP}$ (points lie below the 1:1 line). Residuals (i.e. observed – expected leaf mass loss) were significantly greater than zero across streams (one-sample *t*-test: $n = 29$, $t = 6.04$, $p < 0.001$).

Examination of species presence/absence in the different stream assemblages for L_{EXP} *vs.* L_{OBS} reveals no strong patterns (Figure 4.8). Species seem to be well distributed amongst the range of predicted and observed rates of leaf processing. In streams where *A. aquaticus* was present there was a tendency for L_{EXP} to be low (i.e. clustered to the left hand side of the graph (Figure 4.8a), yet L_{OBS} varied from the 3rd lowest to the 4th highest rate of leaf processing in stream assemblages containing this species.

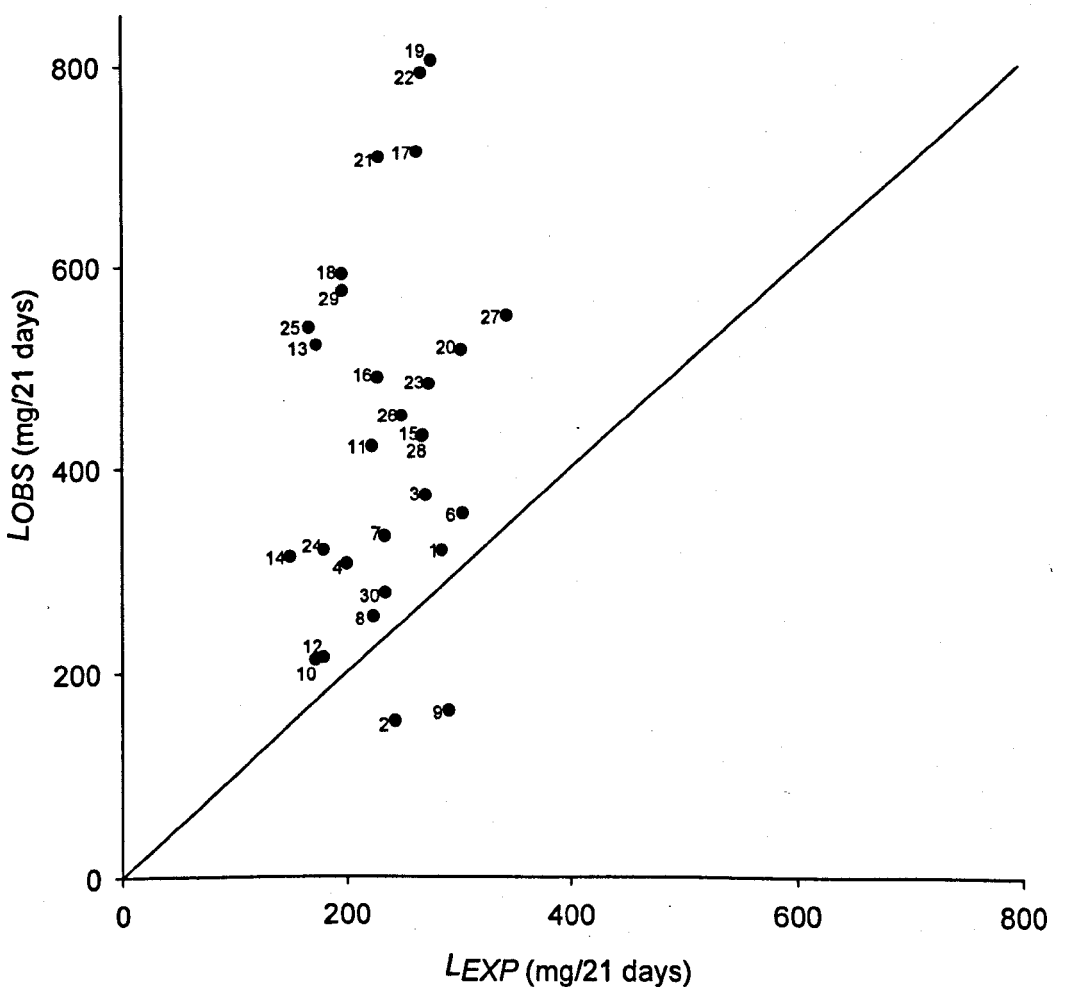

Figure 4.7: Predicted (L_{EXP}) *vs.* observed (L_{OBS}) rates of leaf processing and the observed rate of leaf processing. The black line shows the 1:1 relationship. Numbers to the left of data points are the stream/ assemblage numbers (see Table 4.2).

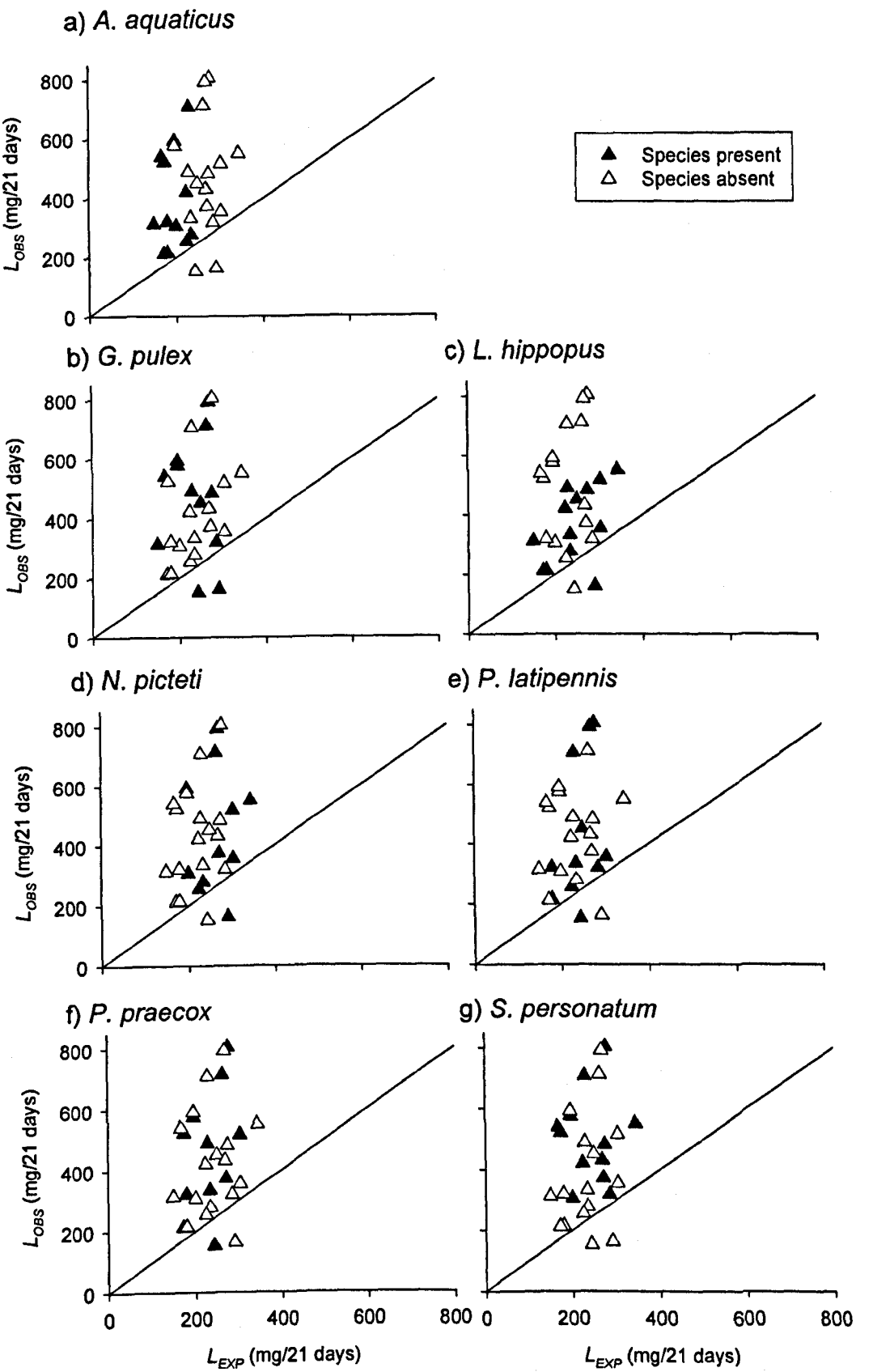

Figure 4.8: The predicted rate of leaf processing (L_{EXP}) *vs.* the observed rate of leaf processing (L_{OBS}) for each of 29 stream mesocosms. Points on the graphs are coded for species presence/absence for each of the seven shredder species: a) *Asellus aquaticus*; b) *Gammarus pulex*; c) *Leuctra hippopus*; d) *Potomophylax latipennis;* e) *Protonemura praecox;* f) *Nemurella picteti*; g) *Sericostoma personatum.* Black lines show the 1:1 relationship.

4.4. Discussion.

The overall aim of this chapter was to test whether a prediction could be made of the rate of leaf processing of assemblages of shredder species, based on knowledge of individual shredder species leaf processing rates. While this study did provide evidence for species-specific feeding rates of shredders conforming to standard allometric scaling relationships (of consumption rates to body size), there was no evidence to suggest that rates of leaf processing in assemblages of shredder species could be predicted from species-specific information. Rates of leaf processing by assemblages of shredder species were greater than predicted, which if they are not due to any other sources of erroneous variation, are indicative of positive species interactions or facilitation (Figure 4.1).

4.4.1. Species-specific feeding rates.

One of the assumptions underlying strong species identity effects is that species are inherently different. Differences between the shredder species-specific leaf consumption rates (mg/ind/d) (Table 4.4.: letters a-d) indicate that there is variation between species, enough to suggest that species identity effects might exist in the study system. Other studies to have examined interspecific variation in rates of ecosystem processes concur that species are not equal in their contribution to rates of ecosystem processes (Section 4.1.1.) (Ruesink & Srivastava 2001; Jonsson & Malmqvist 2003a).

Single-species feeding trials also confirmed that leaf consumption rates of species in isolation were allometrically related to body size for three species of stonefly, two species of caddisfly and the amphipod *Gammarus pulex* (Figure 4.4). This means that, in essence, differences between species' feeding rates in isolation are accountable for by variation in body size rather than anything unique to that species. This is a similar finding to that of Vanni *et al.* (2002) who found that interspecific differences in rates of nutrient cycling by various fish and amphibian species could partially be explained by allometric scaling theory. The importance of allometric scaling relations in understanding structure-function relationships is being increasingly recognised (Hildrew *et al.* 2007). Both natural and

anthropogenic influences can affect body size in streams (Townsend & Thompson 2007). Predators of shredding macroinvertebrates (e.g. fish, macroinvertebrates) are size selective and can greatly affect size structure and species composition of assemblages (Woodward & Warren 2007). Thus, the results of the present study indicate that disruptions of size-structure, as well as taxonomic composition, could have important indirect effects on rates of leaf processing.

The isopod *A. aquaticus* consumed less leaf material in isolation than was predicted by the allometric scaling equation of other detritivorous species. Why this species performed differently to the other species might be due to differences in its feeding action strategy. Food choice experiments have shown that *A. aquaticus* was observed to have a 'scraping' feeding action with selective feeding on micro-organisms colonizing leaf surfaces (Graça 1993a), whereas other species of shredders (e.g. *G. pulex*) show no discrimination between leaf substrates and the attached micro-organisms. Because this species has such a strong preference for feeding on micro-organisms, it may have been the case that the quality of micro-organisms on the leaf-disks were not sufficient for *A. aquaticus* in the feeding trials. The leaf disks were exposed to oven-drying at 60 °C. The effects of this treatment are unknown. However, there was no indication of elevated levels of mortality in these species, despite low rates of feeding.

4.4.2. Leaf consumption rates of shredder assemblages.

Before I discuss the various biological mechanisms that might be responsible for the rate of leaf processing in assemblages of shredders being greater than the sum of their parts (Sections 4.4.2.3 and 4.4.2.4.), I will first discuss some of the other factors which I cannot rule out for their potential to contribute to the effect (Sections 4.4.2.1. and 4.4.2.2.).

4.4.2.1. Differences between the experiments that might have influenced the results.

The species-specific feeding trials were highly replicated, and subject to minimum environmental fluctuation (Section 4.2.2.1.). I am therefore confident that the results of the feeding trial experiment were robust, reproducible and reliable. The

stream mesocosms, however, had a minimum level replication across assemblage treatments, were subject to relatively more variable environment conditions and were less easy to control and standardise. There were significant differences in physiochemical variables between stream mesocosms (Section 4.3.2.: Table 4.4.). However, given the range and magnitude of the differences (Figure 4.5), it seems unlikely that these differences would cause adverse effects on stream biota and will not have serious implications on animal feeding rates in the streams.

The most obvious way that the predictions I made could be inaccurate is because the conditions used to estimate rates of feeding in the single-species feeding trials were different in some way from the conditions in the stream mesocosms, which indirectly affected the way in which the animals feed between the experiments. Any differences in conditions which directly affected the rate of leaf breakdown (by microbes or physical abrasion, but not through shredder feeding) should have been controlled for by the control leaf material in both experiments. Without information on the physicochemical conditions in the feeding trials, it is impossible to judge whether there were differences in physiochemical conditions between the experiments. Temperature is unlikely to have generated faster rates of leaf processing in stream mesocosms than in the feeding trails, because mean air and water temperature was lower in the stream mesocosms than in the feeding trials. However, I can postulate that differences between the conditions in the two experimental systems which might have indirectly affected the physiology and behaviour of the animals in some way are: a) flow (circulating water in the stream system *vs.* agitated, but relatively lentic, water in feeding trials), b) size (large stream *vs.* small jar), c) levels of dissolved oxygen (gently flowing water in streams *vs.* aeration by needle in feeding trials). This list is not exhaustive, but covers the most likely differences.

The most obvious methodological difference between the two experiments is in the drying of leaf material post-conditioning and prior to being fed to shredders. Leaf material used for the stream mesocosms might have been far more palatable to the shredders, because the leaves and micro-organisms had not been subjected to oven-drying. For species such as *A. aquaticus* (as discussed above) for whom micro-organisms constitute an important part of their diet, this may cause large

differences in the amounts of leaf material eaten in the stream mesocosms relative to the feeding trials.

4.4.2.2. Assumptions which were made when generating predictions.

Another key reason why observed did not equal predicted might have been caused by inaccuracies in the assumptions I made to generate the predictions. To begin with, when generating the equation for *A. aquaticus* I decided to maintain the slope at a gradient of $b = 0.742$ and adjust the y intercept to $a = 0.0297$. There is a possibility that this regression line may generate a spurious prediction of the rate of processing of this species. Predictions of leaf processing in assemblages with this species present were mostly low (Figure 4.8a, black triangles). However, this species was also present in assemblages ranging from the lowest to the fourth highest rate of observed leaf processing overall (Figure 4.8a black triangles), suggesting that the observed rates of leaf processing are hugely variable and probably quite unpredictable.

The amount of biomass present in the streams was uncontrollable due to mortality and emergence. Every effort was made to maintain a standard level of biomass (Section 4.2.3.3.) but in the end there were differences between streams in the amount of animal mass replaced into streams and collected at the end of the experiment (Appendix H). When I made a prediction of the leaf processing over the course of the experiment I assumed that the average mass of the individuals of each species collected at the end of the experiment was a good indicator of the average mass of individuals in the streams over the course of the experiment. In reality it might have been that the animals left in the streams were smaller than most of the animals which had been in the streams over the course of the experiment. In addition the animals most likely to emerge are probably the larger ones, and so my predictions may have been low because the body size estimates were below average.

Given these considerations, I now go on to discuss the biotic mechanisms which might explain the patterns seen in the results and why the observed rate of leaf processing in stream mesocosms was greater than predicted.

4.4.2.3. Species identity effects.

Interspecific differences, as were shown to exist between species (Table 4.4., Section 4.4.1.), should have manifested as variation in rates of leaf processing by assemblages in the stream mesocosms (Section 4.1.3.). Indeed there was considerable variation in the observed rates between assemblages, by a factor of five across streams (Figure 4.7). This variation was not explained by differences in physicochemical conditions (Figure 4.5; Section 4.3.2.) or by differences in total assemblage biomass (Figure 4.6; Section 4.3.3.). Therefore, I suggest that this indicates the presence of strong species identity effects.

If identity effects *per se* were important in determining the rate of leaf processing then I should have been able to predict the aggregate processing of an assemblage from individual species' processing rates, and data points (predicted *vs.* observed) should have been on the 1:1 line (Section 4.1.3.). However, data points for 27 out of 29 assemblages were above the 1:1 line (Figure 4.7), suggesting that effects over and above those of species identity were operating, and these are discussed in the following section.

4.4.2.4. Compositional/complementarity effects.

Compositional effects are seen when certain combinations of species have disproportionately large effects on rates of ecosystem processes. If compositional effects were operating in assemblages, then rates of leaf processing were predicted to have been either above (positive species' interactions: facilitation) or below (negative species' interactions: interference) the 1:1 line of predicted *vs.* observed (Section 4.1.3., Figure 4.1). In this study I found evidence of observed rates of leaf processing exceeding predicted. When predicted *vs.* observed are plotted (Figure 4.7), most of the data points lie above the 1:1 line. This is indicative of facilitative (positive) interactions between shredder species (see Figure 4.1).

Using observations from previous studies, we can see that facilitation may operate between shredders. For example, leaf fragmentation of large particles of leaf material by the feeding action of larger shredder species, with chewing action, e.g. *Gammarus pulex* (Graça *et al.* 1993), or cutting action, e.g. *Sericostoma personatum* (Friberg & Jacobsen 1994), feeding strategies, into smaller particle

sizes that may be more readily consumed by smaller shredder species (e.g. stonefly larvae). If we assume that more closely related species (i.e. within an order) exhibit similar feeding strategies, and more distantly related species (i.e. across orders) exhibit different feeding strategies, then assemblages which were composed of three species with different feeding strategies (e.g. *G. pulex* or *A. aquaticus* or a stonefly species or a trichopteran species) might have greater possibility for facilitative interactions (see 4.1.2.). If this is the case then we might expect these taxonomically diverse assemblages to have had the highest rates of observed leaf processing (L_{OBS}), and assemblages composed of three closely related species to have had the lowest rates of leaf processing, or at least lying nearest to the 1:1 line. Results indicate that this was not the case. For example, some of the most taxonomically diverse assemblages and respective rates of processing were: *G. pulex* – Caddis spp. – stonefly spp.: assemblages 2, 22, 23, 26, 29 (see Table 4.2). The observed rates of processing for these assemblages ranged from the lowest to the second highest (Figure 4.7). In contrast the least taxonomically diverse assemblage was: stonefly spp. - stonefly spp. - stonefly spp.: assemblage 20 (Table 4.2) observed rate of process was well above the 1:1 line and was the 10th highest processing assemblage. Two quite similar assemblages: stonefly spp. - stonefly spp. – *A. aquaticus*: assemblages 10 and 30 were quite close to the 1:1 line, and were both among the lowest processing communities.

The level of species richness used in the present study makes the results directly comparable with the results of several other studies that have manipulated community composition at the level of three species (Jonsson & Malmqvist 2000; Jonsson *et al.* 2002; Jonsson & Malmqvist 2003b; 2005). The present study extends the scope of any study to have manipulated community composition in freshwater ecosystems in that manipulated the community composition of a total of seven different species. Of those other studies to have manipulated macroinvertebrate shredder community composition and measured the rate of leaf processing the results of the present study are in agreement with those of Jonsson *et al.* (2002), in that community composition is important. In the study by Jonsson *et al.* (2002), as well as in the present study, the kinds of species used were relatively taxonomically diverse (i.e. a range of amphipod, stonefly and trichopteran species).

It is becoming increasingly important to study community composition and species identity effects because anthropogenic disturbances are affecting species distributions and dominances of natural assemblages. The results of the present study indicate that even detailed knowledge of the kinds of species present in a community (i.e. community composition) cannot reveal rates of leaf processing. To illustrate this in the present study: community composition of the highest and lowest processing community were very similar in that they both contained *P. praecox*, and *P. latipennis*, plus one other species; both communities had very similar community biomasses ($M_{Assemblage}$ of the lowest processing assemblage (assemblage 2) = 44.8 mg) and highest (assemblage 19) = 46.1 mg). Yet, despite these similarities, the observed rate of leaf processing was over five times greater for one assemblage than for the other (assemblage 19 > assemblage 2).

4.4.3. Conclusions.

1) There are interspecific differences between shredder species leaf consumption rates, which can for most species be accounted for by differences in body size.

2) Net leaf consumption rates vary considerably between assemblages of the same shredder species, indicative of species identity effects operating in assemblages.

3) Leaf consumption rates of individual species are not good predictors of the net consumption rate of a set of species together in an assemblage.

4) Rates of leaf processing in assemblages were greater than the sum of their constituent parts.

5. The effect of fungal species richness on the rate of leaf processing mediated through macroinvertebrate shredders.

5.1. Introduction.

The rate of leaf breakdown (reviewed in 1.5.2.2.) has been proposed as a potential indicator of stream ecosystem function, because it integrates biological, chemical and physical conditions (Hagen *et al.* 2006). If leaf breakdown is to be successfully used as an indicator of stream ecosystem function, a greater understanding of the role of aquatic fungi in determining rates of leaf breakdown is required.

The importance of considering the effects of changes in fungal species richness are clear when we consider that pollutant stressors affect the composition of the fungal community, by inhibiting or excluding particular fungal species (Section 1.6.3.). Species losses may affect the rate of microbial leaf decomposition and/or the rate at which material is utilised by shredders, with implications for the transfer of energy through the freshwater food web (Section 1.5.2.).

5.1.1. The relationship between microbial conditioning and macroinvertebrate leaf processing.

Aquatic hyphomycetes are an important group of microbes in freshwater leaf decomposer systems (Bärlocher & Kendrick 1973b; Bärlocher 1992; Gessner & Chauvet 1994). These fungi mediate leaf breakdown, both directly and indirectly, by improving the leaf quality for macroinvertebrate shredders through 'conditioning' (reviewed by Suberkropp 2003). Conditioning occurs in part because of the tissue softening action of the fungal enzymes (Fisher & Likens 1973; Petersen & Cummins 1974; Graça *et al.* 1993) and in part through the provision of mycelia as food (Bärlocher & Kendrick 1974; Rossi & Fano 1979; Arsuffi & Suberkropp 1988).

Studies of the relationship between microbial leaf conditioning and rates of leaf consumption by macroinvertebrate shredders highlight that there are differences in

the ability of individual fungal species to condition leaves (Bärlocher & Kendrick 1973a; Arsuffi & Suberkropp 1984; 1986; Graça *et al.* 1994). Other studies report the ability of shredders to differentiate between leaves conditioned by different fungal species (Bärlocher & Kendrick 1973a; 1973b; 1974; Arsuffi & Suberkropp 1984; 1988; Graça *et al.* 1994). In addition, trichopteran and amphipod shredders can locate and selectively feed upon fungal patches on a single leaf (Arsuffi & Suberkropp 1985; Graça *et al.* 1993), and feed preferentially on conditioned leaves as opposed to unconditioned leaves (Fisher & Likens 1973; Petersen & Cummins 1974).

Fungi affect the energy budget of shredders and have been shown to have affects on shredder growth and survivorship (Kostalos & Seymour 1976; Willoughby & Sutcliffe 1976; Sutcliffe & Willoughby 1981; Naylor *et al.* 1989). This is likely to be because fungal species differ in their nutritional quality to shredders (Bärlocher & Kendrick 1973b).

5.1.2. The relationship between fungal species richness and macroinvertebrate leaf processing.

Theory predicts that a positive relationship between the number of fungal species and the rate of macroinvertebrate leaf processing may occur through the 'sampling effect' (*sensu* Aarssen 1997; Huston 1997; Tilman *et al.* 1997a) (Section 1.2.2.1.), whereby increasing the number of fungal species on the leaf surface increases the probability that a highly palatable and nutritious species will be present which will drive higher rates of leaf processing. Alternatively, fungal species may be 'complementary' in their abilities to condition leaf material, so that the overall rate of leaf processing will increase as the number of fungal species increases. This could happen through two mechanisms: 1) through 'facilitation' (see Section 1.2.2.2.1) between fungal species, such that the presence of certain species modifies the leaf surface in a way which is favourable for other fungal species, so that the overall rate of leaf processing by the shredder is greater when certain combinations of species are together; 2) through 'niche differentiation' (see Section 1.2.2.2.2.) whereby because fungal species differ in their ability to colonize and utilize the entire leaf surface, greater species richness promotes greater resource utilization and leaf palatability to shredders.

5.1.3. Previous studies of structure-function relationships in stream decomposer systems.

Most studies of the relationship between species richness and ecosystem functioning have concentrated on a single trophic level (Petchey *et al.* 2004), and there is a pressing need for further research into multiple trophic levels (Hooper *et al.* 2005). Previous experimental studies have examined the role of species richness within a single trophic level, either among shredders (Jonsson & Malmqvist 2000; Jonsson *et al.* 2001; Cardinale & Palmer 2002; Jonsson *et al.* 2002; Jonsson & Malmqvist 2003a; Dangles & Malmqvist 2004) or among fungi (Bärlocher & Graça 2002; Bärlocher & Corkum 2003; Treton *et al.* 2004; Dang *et al.* 2005; Duarte *et al.* 2006) in determining rates of leaf processing. Only one study to date has examined the relationship between the diversity of primary consumer microbes (i.e. fungi) and a single secondary consumer (i.e. macroinvertebrate shredder species). Lecerf *et al.* (2005) examined the relationship between rates of leaf consumption by *Gammarus fossarum* Koch (Amphipoda: Gammaridae) feeding on leaves conditioned with nine multi-species community combinations of fungi at four richness levels (2, 4, 6 and 8 species). Communities were drawn at random from a species pool of twelve species. In addition, all species were grown in monoculture. In the absence of the species with the highest processing performance in monoculture (*Goniopila monticola* Dyko) a positive relationship existed between species richness and leaf consumption rate. However, leaf consumption rates on *G. monticola* grown in isolation outperformed every other monoculture and multi-species polyculture treatment, by a factor of least two.

The design of Lecerf *et al.*'s (2005) experiment prevents determination of the relative importance of species composition and overall richness *per se* on rates of shredder leaf consumption (Allison 1999; Naeem 2002). Due to the limited number of random draws of species from a species pool it is inevitable that particular species compositions were repeated at more than one level of species richness. In order to separate the effects of composition from those of species richness, it is necessary to have every possible combination of species, at every level of species richness. It is rare that such high levels of replication will be possible in an experiment. In order to

exclusively detect the effect of species richness, composition within each level of species richness must be non-overlapping. Furthermore, Lecerf *et al.* (2005) did not attempt to determine the mechanisms through which the positive association between species richness and leaf mass loss arises (see Sections 1.2.2.3. and 5.1.4.).

5.1.4. Techniques for detecting complementarity effects.

The most unambiguous evidence for the existence of complementarity effects (Section 5.1.2.) is the detection of 'transgressive overyielding' (Trenbath 1974; Loreau 1998a; Hector *et al.* 2002) (Section 1.2.2.3.). Transgressive over- (or under-) yielding occurs if the observed response for a polyculture is greater (or less) than the yield of its highest (or lowest) component species in monoculture. Overyielding can only arise from complementarity between species (Loreau 1998a; Hector *et al.* 2002).

Other empirical studies in freshwater leaf decomposer systems have examined evidence for non-transgressive overyielding, e.g. Relative Yield Total (RYT) (Hector 1998). This is where the yield of a polyculture is greater than expected based on a weighted average of the monoculture yields of the constituent species (see Fridley 2001; Hector *et al.* 2002). Authors have used the RYT method to detect complementarity effects in freshwater fungi-decomposer systems. Studies have compared rates of leaf processing by multi-species assemblages of fungi to monocultures, and rates of microbial leaf processing have been measured in the absence of shredders (Bärlocher & Corkum 2003; Dang *et al.* 2005; Duarte *et al.* 2006). However, problems have arisen because the RYT method "*can only be fully applied in experimental systems where the response of individual species can be quantified in a mixture*" (Hector 1998) and this is not possible in these fungal systems. Therefore, in order to calculate the weighting of species' contributions in mixtures, researchers have either assumed that the proportion of fungal inocula present on leaves was equal to the mass of inocula added to flasks, with fungal biomass being directly proportional to leaf mass loss of each species (Bärlocher & Corkum 2003; Duarte *et al.* 2006), or that spore biomass output of any species in monoculture is directly proportional to the leaf mass loss caused by this species (Dang *et al.* 2005). In the end, Duarte *et al.* (2006) could not exclude the sampling

effect, since the mixtures did not outperform the highest processing monoculture species (*Articulospora tetracladia* Ingold), and Dang *et al.* (2005) found no consistent evidence for positive or negative species interactions, with RYT values being approximately equal to one, rather than consistently greater or less than one.

Loreau (1998a) introduced an index to assess the degree to which transgressive overyielding occurs, called D_{Max}. D_{Max} was used in the present study primarily because it is the most stringent test for complementarity effects (Hooper & Dukes 2004). I preferred to use this method, rather than test for the less stringent non-transgressive overyielding (RYT), because I was unwilling to make assumptions with regard to the relative proportional weighing of each fungal species in a mixture (see above). Evidence suggests that fungal species actually differ in their abilities to colonise, grow and produce biomass on leaves (Bärlocher & Schweizer 1983; Butler & Suberkropp 1986; Gessner *et al.* 1993; Gessner 1997; Fabre 1998a; 1998b). The detection of transgressive overyielding does not require us to make assumptions with regard to the proportion of each individual species' contribution to a mixture.

5.1.5. Aims and objectives.

My aim was to examine the relationship between fungal species richness and rates of leaf processing by two shredder species. I wanted to isolate the effects of species richness from those of community composition, and thus be able to understand whether or not species richness *per se* is an important determinant of leaf processing rates. Therefore, I created a gradient of fungal richness, where richness was manifested as sets of species with non-overlapping composition, thereby maximising the chance that any effect observed was due to richness on average, and not particular species compositions. In addition, the design of one of two of the experiments undertaken was such that it enabled me to test for the existence of complementarity effects (see Section 5.2: 'Experiment 2').

The objectives were as follows:

1) To determine whether there was a relationship between fungal species richness and the mean rate of leaf processing by macroinvertebrate shredders.
2) If there was a relationship between species richness and magnitude of rates of leaf processing, to determine what might be driving this (i.e. was there any evidence for species' complementarity or sampling effects?)
3) To establish whether the relationship differed between two different shredder species: *Gammarus pulex* and *Sericostoma personatum*.

In order to address these objectives I performed two mesocosm experiments. 'Experiment 1' addressed Objectives 1 and 3. 'Experiment 2' addressed Objectives 1 and 2. In Experiment 1, I investigated the relationship between fungal richness and rates of shredder leaf processing for communities of 3 or 12 species of fungi and two shredder species: *Gammarus pulex* Linnaeus (Amphipoda: Gammaridae) and *Sericostoma personatum* Spence (Trichoptera: Sericostomatidae). Whereas in Experiment 2, the relationship was investigated in more detail for communities with either 0, 1, 3 or 9 fungal species, but with only a single shredder species *Gammarus pulex*. In order to be able to differentiate between the different mechanisms which might be operating in the system, it is essential to compare the rate of leaf processing of monocultures *vs.* polycultures (see Section 1.2.2.3. and 5.1.4.).

5.2. Methods.

5.2.1. Experimental design.

In both experiments, shredder species were provided with a single-species leaf resource inoculated with different community treatment combinations of aquatic fungi (belonging to the aquatic hyphomycete group). The levels of fungal species richness differed between experiments. Experiment 1 had two species richness treatments: 12 different three-species polycultures and three different 12-species polycultures drawn from a pool of 36 aquatic hyphomycete species. Experiment 2 had three species richness treatments and included all possible monocultures, nine different three-species polycultures and three different nine-species polycultures

drawn from a pool of 27 aquatic hyphomycete species. A zero fungal species treatment was also included.

The whole species pool was exhausted within each species richness treatment, permitting 16 unique communities in Experiment 1, and 39 unique communities in Experiment 2. Species were selected so that no two species occurred together repeatedly, but were otherwise randomly allocated to communities (Table 5.1). This design ensures that the effects of species richness were not confounded with those of community composition (Tilman & Lehman 2001). The identity of some three-species polycultures were shared between the two experiments. Three-species communities included in Experiment 2 were selected primarily based on availability and also to ensure the representation of a range of processing rates.

Where possible, the same or similar methodologies were used for both experiments, although the experimental systems were different (Section 5.2.4.). Experiment 1 took place in January/February 2006, and Experiment 2 in October 2006.

5.2.2. Preparing leaf material and establishing communities of aquatic fungi.

Alder (*Alnus glutinosa* (L.) Gaertner) is a common UK riparian tree species, whose leaves are highly palatable to shredders (Leroy & Marks 2006). Leaves were collected just prior to abscission in autumn 2005 and air-dried at room temperature for one week prior to storage. Nine weeks prior to the start of the experiment, leaves were leached in distilled water for 2 days and disks of 1.6-cm diameter were cut using a no. 11 cork borer, avoiding the main veins. Leaf disks were dried in an oven at 60 °C for 4 days, and then weighed in 5 g (Experiment 1) or 2 g (± 0.05 g) (Experiment 2) batches. Sixteen 5-g batches were placed into 500-ml conical flasks with 350 ml of distilled water and forty 2-g batches were placed into 250-ml conical flasks with approximately 175 ml of distilled water. Flasks were then sealed and autoclaved at 120 °C for 20 minutes. Upon cooling, aquatic fungi were added into the flasks.

Table 5.1: Allocation of aquatic fungal species to communities. Numbers identify each community treatment. For three-species communities, differences between the two experiments are indicated by punctuation marks after the community treatment number: Experiment 1 = single apostrophe (e.g. 3.1'), and Experiment 2 = speech marks (e.g. 3.1"). Spp. is an abbreviation for species.

Species name	Community treatment				
	Experiment 1		Experiment 2		
	Polyculture		Monoculture	Polyculture	
	3 spp.	12 spp.		3 spp.	9 spp.
Taeniospora gracilis var. enecta (Marvanová)	3.1'	12.1	1.01	3.1"	9.1
Geniculospora inflate (Trigold)	3.2'	12.1	-	-	-
Dendrospora erecta (Ingold)	3.3'	12.1	1.02	3.2"	9.1
Cylindrocarpon ianthothele (Wollenw.)	3.4'	12.1	-	-	-
Cylindrocarpon aquaticum (Marvanová & Descals)	-	-	1.03	3.3"	9.1
Anguillospora rosea (Descals & Marvanová)	3.5'	12.1	1.04	3.4"	9.1
Tetracladium setigerum (Grove)	3.6'	12.1	-	-	-
Anguillospora filiformis (Greathead)	3.7'	12.1	1.05	3.5"	9.1
Tricellula aquatica (Webster)	3.8'	12.1	1.06	3.6"	9.1
Anguillospora crassa (Ingold)	3.9'	12.1	-	-	
Tricladium splendens (Ingold)	3.9'	12.3	1.07	3.7"	9.1
Culicidospora aquatica (Petersen)	3.10'	12.1	-	-	-
Tricladium chaetocladium (Ingold)	3.11'	12.1	1.08	3.8"	9.1
Anguillospora longissima (Ingold)	3.12'	12.1	1.09	3.9"	9.1
Tetracladium furcatum (Descals & Webster)	3.1'	12.2	1.10	3.1"	9.2
Articulospora tetracladia (Ingold)	3.3'	12.2	1.11	3.2"	9.2
Heliscella stellata (Ingold & Cox)	3.4'	12.2	1.12	3.3"	9.2
Actinospora megalospora (Ingold)	3.5'	12.2	1.13	3.4"	9.2
Clavariopsis aquatica (de Wild)	3.6'	12.2	-	-	-
Varicosporium delicatum (Iqbal)	3.7'	12.2	1.14	3.5"	9.2
Anguillospora furtiva (Descals & Marvanová)	3.8'	12.2	1.15	3.6"	9.2
Tricladium attenuatum (Iqbal)	3.9'	12.2	-	-	-
Lunulospora cuvula (Ingold)	3.10'	12.2	1.16	3.7"	9.2
Heliscus lugdunedensis (Sacc. & Therry)	3.11'	12.2	1.17	3.8"	9.2
Varicosporium elodeae (Kegel)	3.12'	12.2	1.18	3.9"	9.2
Lemonniera aquatica (de Wild)	3.1'	12.3	1.19	3.1"	9.3
Triscelophorus monosporus	3.2'	12.3	-	-	-
Dactylella aquatica (Ingold)	3.3'	12.3	1.20	3.2"	9.3
Lemonniera terrestris (Tubaki)	3.4'	12.3	1.21	3.3"	9.3
Flagellospora curvula (Ingold)	3.5'	12.3	-	-	-
Tetrachaetum elegans (Ingold)	3.2'	12.2	1.22	3.4"	9.3
Casaresia sphagnorum (Fragoso)	3.6'	12.3	-	-	-
Clavatospora longibrachiata (Ingold)	3.7'	12.3	1.23	3.5"	9.3
Tetracladium marchalianum (de Wild)	3.8'	12.3	1.24	3.6"	9.3
Varicosporium gigantum (Crane)	3.10'	12.3	1.25	3.7"	9.3
Tetracladium maxilliforme (Rostrup)	3.11'	12.3	1.26	3.8"	9.3
Alatospora acuminata (Ingold)	3.12'	12.3	1.27	3.9"	9.3

The aquatic hyphomycete species used in these experiments are all present in UK streams and were grown as isolated cultures on malt extract agar (by K. Botham, The University of Sheffield). Using aseptic technique, mycelial plugs were cut, using a no. 4 cork borer, from the edge of each of the colonies, and transferred into the conical flasks containing the sterile leaf disks and water. Each flask was inoculated with a different treatment (see Table 5.1). For Experiment 1, a total biomass of 3.6 g (wet weight) fungi was added to each flask (i.e. 1.2 g per fungal species for a 3-species assemblage and 0.3 g per fungal species for a 12-species assemblage) by K. Botham (The University of Sheffield). For Experiment 2, each monoculture treatment was inoculated with a single plug per flask. For each polyculture treatment a plug of each species was added, making 3 plugs for each 3-species treatment and 9 plugs for each 9-species treatment.

Flasks were placed on an orbital shaker at approximately 80-105 rpm at 15°C for a period of 6 weeks. Leaf disks were then removed from flasks, rinsed with sterile distilled water and separated.

5.2.3. Collection and acclimation of animals.

The two shredder species, *Gammarus pulex* and *Sericostoma personatum*, were selected because of their relatively large size and relatively fast rate of leaf processing (see Chapter 4: Table 4.4). It was expected that the effect of changes in fungal community structure on rates of shredder feeding would be subtle, and therefore species with large consumption potential were chosen for this experiment.

Gammarus pulex is common in British streams (Gledhill *et al.* 1993), plays an important role in the breakdown of leaf litter (Kaushik & Hynes 1971; Sutcliffe & Willoughby 1981) and is an important prey item for fish (Andersen *et al.* 1993). *Sericostoma personatum* is also a common British species (Elliott 1969; Wallace *et al.* 2003), and is widely distributed across Europe (Iversen 1980). It feeds on fallen leaves. It preferentially feeds on, and grows best on, a diet of alder leaves, because of their high nitrogen content (Iversen 1974). The two shredder species have different feeding strategies. *G. pulex* uses a chewing action to break off whole pieces of leaf

material, leaving only the central veins intact (Graça *et al.* 1993), whereas *S. personatum* has large mandibles (Friberg & Jacobsen 1994) which it uses to cut whole pieces from the leaf and consumes all parts of the leaf, including the veins (Jonsson *et al.* 2002; Inglis 2003 unpublished).

Animals (375 of each species for Experiment 1, and 800 *G. pulex* for Experiment 2) were collected from the same locations as described previously (Chapter 4: Table 4.2). To minimize variation in animal physiology, only adult *G. pulex* males were used in the feeding trials. It was not possible to determine the sex of *S. personatum*, so animals of this species were standardised using case length (between 12 – 16 mm). After collection, animals were maintained in holding tanks in the laboratory at a constant 15 °C for approximately one week. During the first 24 hours animals were acclimated to Artificial Pond Water (APW) (H.S.E. 1982), which was used thereafter both to maintain them and for carrying out the feeding trials. Animals were fed alder leaves inoculated with *Cladosporium* spp. (Naylor *et al.* 1989). Animals were transferred to a separate holding tank 24 hours prior to the start of the experiment, containing APW and pebble gravel but no food.

5.2.4. Experimental system.

The experiments were both conducted at a constant 15 °C, under a 12-h dark: 12-h light photoperiod. Test vessels were placed so that they were not adjacent and treatments were evenly distributed across the laboratory bench.

Experiment 1:

Two-hundred and seventy-five 1000-ml plastic containers were filled with 800 ml APW. A two millimetre mesh screen separated the containers horizontally into two chambers. Test vessels were aerated though a 10-ml short-form pipette connected to an air supply. There were 15 fungal community treatments, two shredder treatments, plus one non-shredder control treatment and five test vessels of each. The experiment was performed in four time blocks, starting sequentially every day for four days. The first three time blocks consisted of 25 test vessels of each shredder treatment (i.e. 25 non-shredder test vessels, 25 *G. pulex* test vessels and 25 *S. personatum* test vessels

per day), making a total of 225 test vessels. Due to experimental complications, the *G. pulex* test vessels from the time block 1 were removed from the analysis, and a fourth time block of 25 test vessels of each of the *G. pulex* and non-shredder treatment was run. This resulted in a nested design (*S. personatum* only occurred in the first time block and *G. pulex* only occurred in the fourth time block) (see Section 5.2.6.1.). In addition, some other test vessels were removed during the experiment due to the occurrence of uncontrollable events, e.g. animal mortality. A total of 242 test vessels were used in the analysis, of which 142 were shredder treatments.

Experiment 2:

One-thousand 60-ml glass jars were filled with 40 ml APW and two small pieces of gravel, to provide some substrate for the animals to hold on to. There were 40 community treatments and 25 test vessels of each, although some were removed during the experiment due to the occurrence of uncontrollable events, e.g. animal mortality. The experiment was performed in three time blocks, starting sequentially every two days (i.e. 360 test vessels commenced on day zero, 320 on day two and 320 on day four). Test vessels were aerated though a syringe needle attached to an air supply.

5.2.5. Quantifying leaf processing.

After inoculating the leaf material (see Section 5.2.2.), leaf disks from each flask were divided into either 15 groups of 20 disks (Experiment 1) or 25 groups of 5 disks (Experiment 2) and placed into pre-weighed foil cups. Leaf disks were placed in an oven at 60 °C for a period of 8 days and then transferred to a desiccator overnight. Foil cups and leaf disks were then weighed together on either a Mettler AT261 Delta range Electrobalance (reading precision 10 µg; Experiment 1) or a Cahn 25: Automatic Electrobalance (reading precision 0.1 µg; Experiment 2). After weighing, groups of leaf disks were placed into each test vessel for four days to rehydrate in APW, prior to the addition of shredders.

In Experiment 1, five individuals of either *G. pulex* or *S. personatum* were added to every 2 out of 3 test vessels. Test vessels that did not contain shredders provided a

control treatment. Shredders were given 8 days to feed on leaf disks. In Experiment 2, a single *G. pulex* individual was added to 20 randomly chosen test vessels of the 25 test vessels for each fungal community treatment. The remaining 5 test vessels provided a control treatment. Shredders were given 7 days to feed on leaf disks. The control treatment was for mass loss due to any further leaching of nutrients from leaves and/or microbial processing (*F*: see Equation 5.1).

During the experiment, water levels were kept constant with the addition of distilled water, animal moults were removed from test vessels, and any mortality was recorded. After the required number of days of feeding, leaf disks were removed, placed back into their foil cups, oven-dried for 8 days at 60 °C, and reweighed. Mass loss was subsequently calculated. Shredders were also removed, oven-dried and weighed.

Leaf processing rates were expressed as a mass-specific consumption rate (*Cm*) (i.e. mg of leaf material/mg of shredder/day) using the following equation (Maltby *et al.* 2002):

$$Cm = \frac{((W_i \times F) - (W_z))}{S \times t} \qquad \text{Equation 5.1.}$$

where W_i is the start weight of leaf material (mg, oven-dried), W_z is the end weight of leaf material (mg, oven-dried), S is shredder dry mass (mg, oven-dried), and t is the number of days animals were fed for. F is a correction factor representing the mean proportional change in leaf mass for control leaf material in the non-shredder treatment (W_z/W_i).

5.2.6. Statistical Analyses.

5.2.6.1. Experiment 1.

The relationship between the rate of shredder leaf processing and fungal species richness (3 or 12 species) was tested for using ANOVA (implemented as a General Linear Model as the design was unbalanced). The response was the mean rate of leaf

processing (*Cm*) per fungal community treatment per time block (see Table 5.1) (i.e. each community treatment was a treatment replicate, rather than test vessels). 'Time blocks', 'species richness' and 'shredder treatment' were entered into the model as 'fixed' factors. 'Shredder treatment' was nested within 'time blocks', because there were only *S. personatum* and non-shredder treatments in time block 1, and only *G. pulex* and non-shredder treatments in time block 4. The following interactions were also entered into the model: 'time blocks' x 'fungal species richness', and 'fungal species richness' x 'shredder treatment' (nested within 'time blocks').

5.2.6.2. Experiment 2.

Richness effects were tested in two stages. First, the relationship between the rate of leaf processing and fungal species richness (1, 3 or 9 species). Data were analysed using ANOVA (implemented as a General Linear Model as the design was unbalanced). The response was the mean rate of leaf processing (*Cm*) per fungal community treatment per time block (see Table 5.1) (i.e. each community treatment was an individual replicate). The zero fungal species treatment was not included in this analysis because there was no replication in community treatment at this level of species richness. A Tukey Multiple Comparison test was used to identify differences between species richness treatments. Second, the differences among single species cultures and the zero species treatment, were tested (using ANOVA and Dunnett's test). The response was, once again, the rate of leaf processing per replicate mesocosm. 'Time blocks' and 'community identity' were both entered into the model as 'fixed' factors, rather than 'random' factors, because I was interested in examining the effects of these factors on the mean of *Cm* rather than effects on the variance-covariance structure of *Cm* (Crawley 2002).

Overyielding was used to test whether there were any positive effects of species' complementarity on the leaf processing rate of *G. pulex* on leaves inoculated with a multi-species polyculture, defined as (Loreau 1998a):

$$D_{Max} = \frac{Cm_{Poly} - Max(Cm_{Mono})}{Max(Cm_{Mono})} \qquad \text{Equation 5.2.}$$

where Cm_{Poly} is the observed mean mass-specific leaf consumption rate (Cm) of *G. pulex* on leaves inoculated with a polyculture of fungal species. $Max(Cm_{Mono})$ is the observed mean Cm of the highest monoculture species. Overyielding occurs when $D_{Max} > 0$.

Underyielding is defined as:

$$D_{Min} = \frac{Cm_{Poly} - Min(Cm_{Mono})}{Min(Cm_{Mono})} \qquad \text{Equation 5.3.}$$

$Min(Cm_{Mono})$ is the observed mean Cm of the lowest monoculture species. Underyielding occurs when $D_{Min} > 0$.

5.3. Results.

5.3.1. Objectives 1 and 3: Was there a relationship between fungal species richness and the mean rate of leaf processing by macroinvertebrate shredders, and did the relationships differ between the two shredder species?

5.3.1.1. Experiment 1.

There was no significant effect of fungal species richness on rates of shredder leaf processing (*Cm*) (Table 5.2; Figure 5.1). However, there were significant differences between the two shredder species, and between time blocks (Table 5.2). Rates of leaf processing by *S. personatum* were significantly lower than for *G. pulex*, and rates of leaf processing in time blocks 1 and 2 were significantly less than in time blocks 3 and 4 (Tukey Multiple Comparison test). There was no significant interaction between shredder treatment and fungal species richness.

Table 5.2: The effect of fungal species richness, time blocks and shredder treatment on mean mass-specific leaf consumption rate (*Cm*) (Experiment 1). Response = *Cm* per community treatment per time block. d.f. = degrees of freedom (treatment, error). Parentheses indicate nesting.

Factor	d.f.	F	p
Time block	3, 76	11.86	<0.001
Shredder treatment (time block)	1, 76	16.62	<0.001
Fungal species richness	1, 76	1.32	0.254
Time block x Fungal species richness	3, 76	0.33	0.807
Fungal species richness x Shredder treatment (time block)	2, 76	0.14	0.871

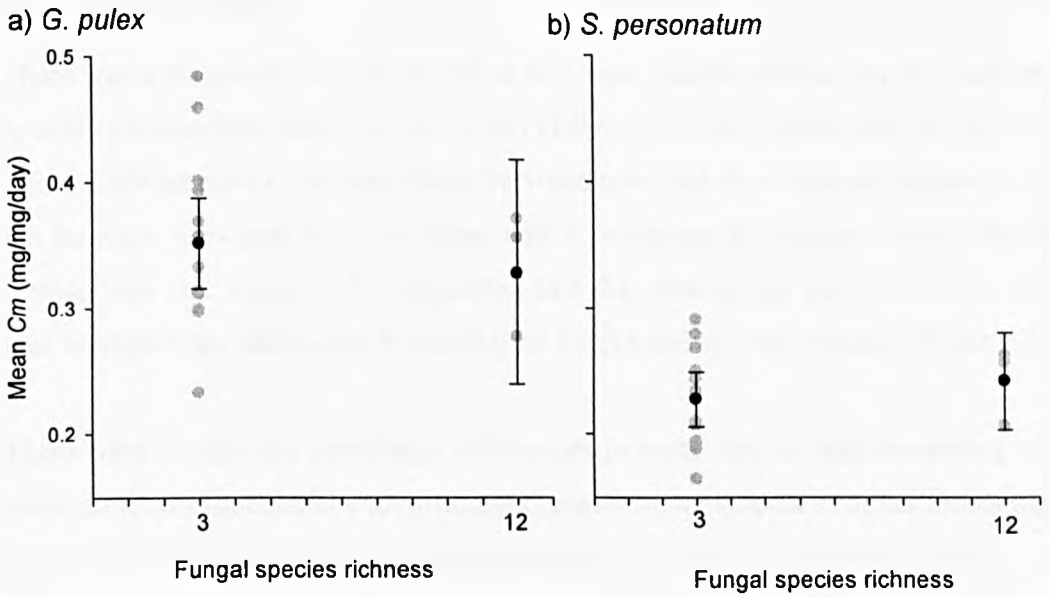

Figure 5.1: Mean (with 95% confidence intervals) mass-specific leaf processing rate (*Cm*) per fungal species richness treatment (3 or 12 species) (black dots) and per community treatment (grey dots) for a) *Gammarus pulex* and b) *Sericostoma personatum* in Experiment 1.

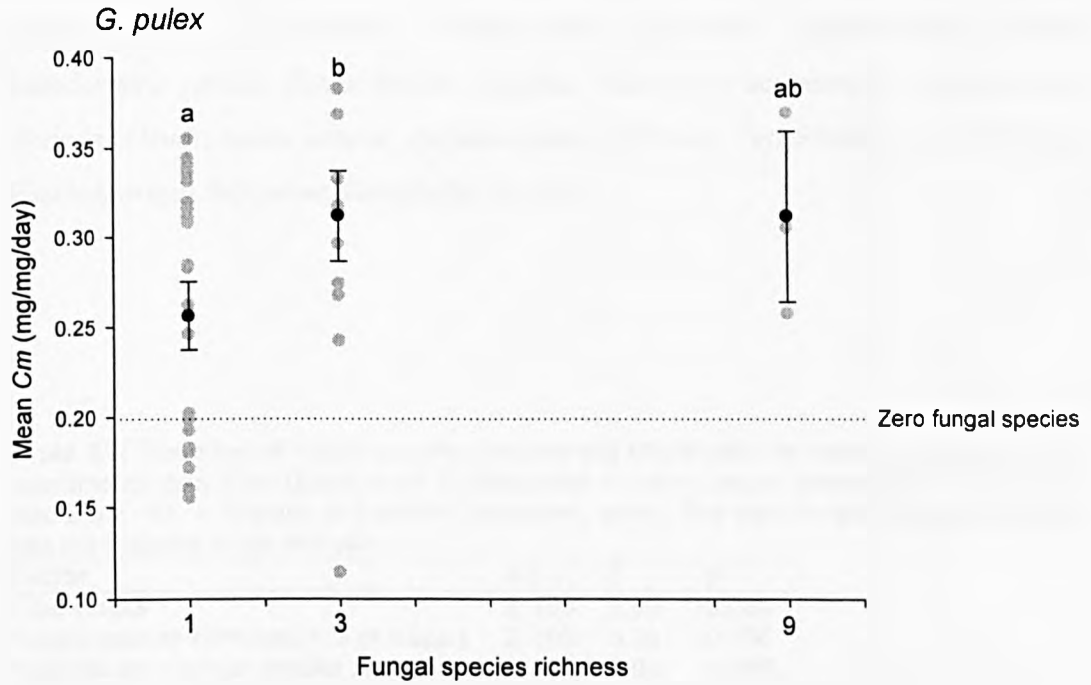

Figure 5.2: Mean (with 95% confidence intervals) mass-specific leaf processing rate (*Cm*) per fungal species richness treatment (1, 3 or 9 species) (black dots) and per community treatment (grey dots) (Experiment 2). Dotted line indicates the mean *Cm* of the zero species treatment. Letters above bars indicate differences between species richness treatments (Tukey Multiple Comparison test).

5.3.1.2. Experiment 2.

There was a marginally significant effect of fungal species richness on the mean mass specific consumption rate (*Cm*) ($p = 0.05$) (Table 5.3). Time blocks had no significant effect. Differences in *Cm* were found between one- and three-species treatments, but not between one- and nine-, or three- and nine-species treatments (Tukey Multiple Comparison test: Figure 5.2), suggesting that the relationship was non-linear. There was no significant interaction between time blocks and species richness (Table 5.3).

There were statistically significant differences in mean rate of leaf processing of *G. pulex* on leaves inoculated with different community treatments of either monoculture or zero fungal species (General Linear Model: $F = 8.44$, $p < 0.001$, d.f. = 27, 510) (Figure 5.3). Time blocks were also significant (General Linear Model: $F = 16.82$, $p < 0.001$, d.f. = 2, 510). Most monoculture treatments were not significantly different from the zero species treatment (Dunnett Simultaneous test). Those species whose performances in monoculture were significantly different from the zero species treatment were as follows: *Varicosporium gigantum, Anguillospora furtiva, Lunulospora cuvula, Tetrachaetum elegans, Alatospora acuminata, Varicosporium elodeae, Dendrospora erecta, Anguillospora filiformis, Tetracladium maxilliforme, Varicosporium delicatum, Dactylella aquatica.*

Table 5.3: The effect of fungal species richness and time blocks on mean mass-specific leaf consumption rate (*Cm*) (Experiment 2). Response = mean *Cm* per community treatment per time block. d.f. = degrees of freedom (treatment, error). The zero fungal species treatment was not included in the analysis.

Factor	d.f.	F	p
Time blocks	2, 108	2.59	0.080
Fungal species richness (1, 3 or 9 spp.)	2, 108	3.08	0.050
Time blocks x fungal species richness	4, 108	0.08	0.989

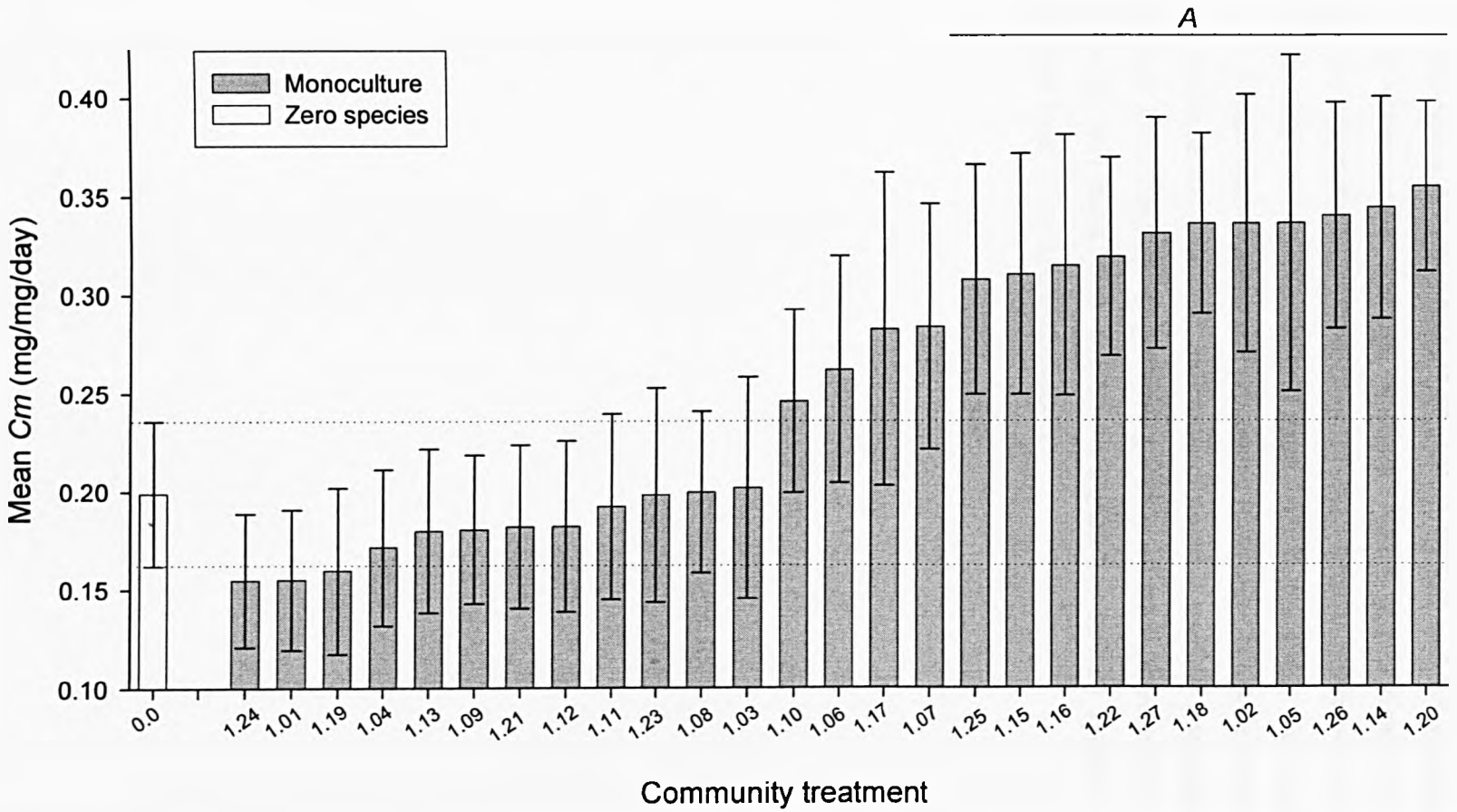

Figure 5.3: Mean (± 95 % confidence interval) mass-specific leaf consumption (*Cm*) across a zero species treatment and 27 monoculture treatments (for statistics see text) (Experiment 2). For details of community identity see Table 5.1. Dotted line indicates 95 % confidence intervals of the zero species treatment. Solid line and the letter *A* indicates those community treatments whose means were significantly different from the zero species treatment (Dunnett Simultaneous test).

5.3.2. Objective 2: Was there any evidence for species' complementarity?

In Experiment 2, polycultures 3.1", 3.2", 3.3", 3.7" and 9.1 yielded higher rates of mean leaf processing than their constituent highest species in monoculture (i.e. $D_{max} > 0$) (see Appendix I for presentation of D_{Max} values), indicative of positive species' interactions or complementarity (Figure 5.4 a, b, c, g and j). Conversely, polyculture 3.5" yielded rates of mean leaf processing lower than its lowest constituent species in monoculture (i.e. $D_{Min} > 0$), indicative of negative species' interactions or inhibition (Figure 5.4e). The rates of processing seen in all other polycultures were not greater than (or less than), their constituent highest (or lowest) species in monoculture (Figure 5.4d, f, h, i, k, l) (Appendix I).

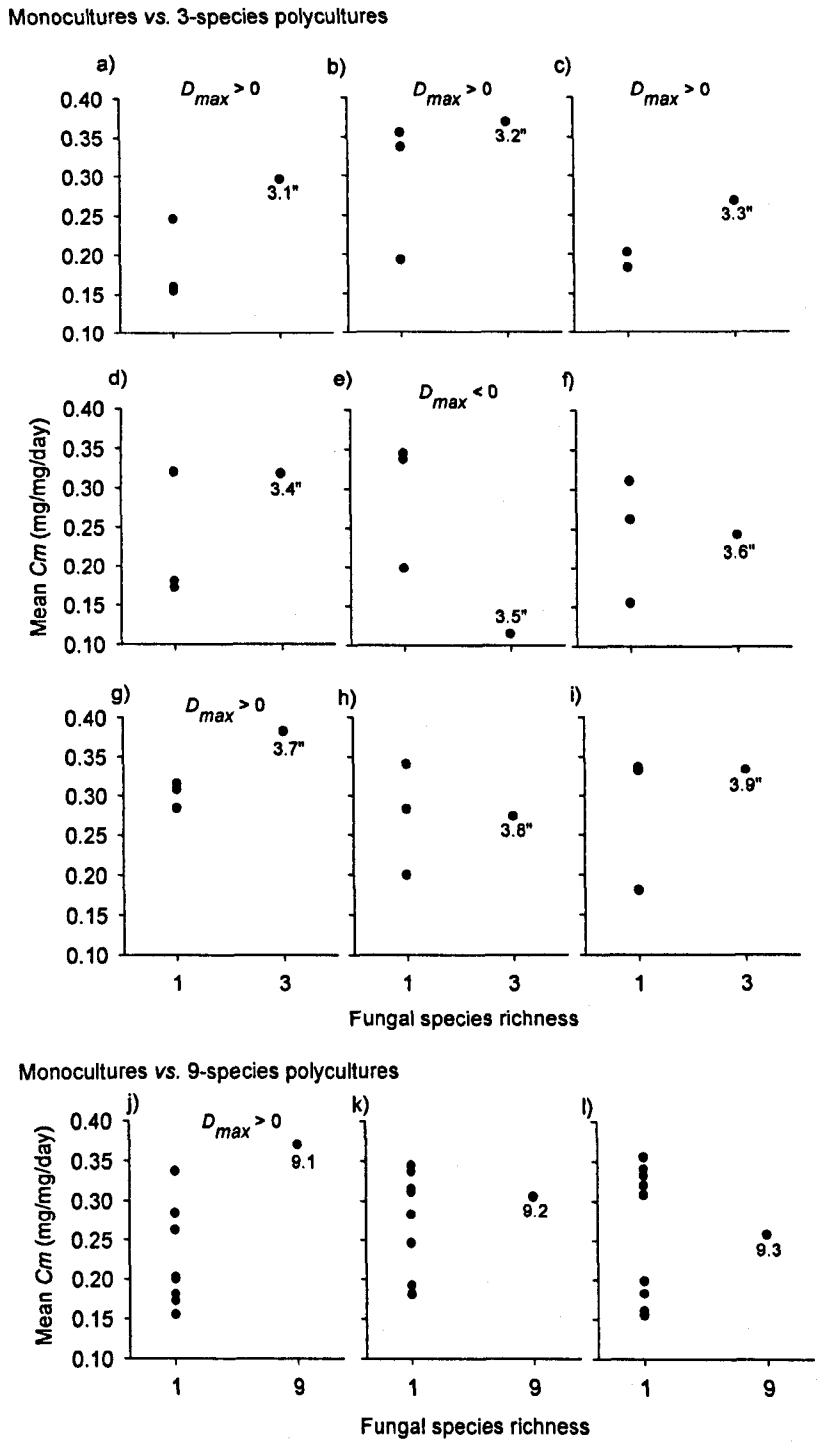

Figure 5.4: Mean mass-specific leaf processing rate (*Cm*) per fungal species richness treatment for polycultures vs. their respective monocultures (Experiment 2). Graphs a – i were three-species polycultures vs. respective monocultures. Graphs j – l were nine-species polycultures vs. respective monocultures. Numbers adjacent to data points indicate the polyculture identity (see Table 5.1). Polycultures 3.1", 3.2" 3.3", 3.7" and 9.1 overyielded (i.e. $D_{Max} > 0$) (see a, b, c, g and i). Polyculture 3.5" underyielded (i.e. $D_{Min} < 0$) (see e) (see Appendix I for values and identity of highest (or lowest) monoculture community treatment).

5.4. Discussion.

The aim of this study was to test whether leaf processing rates varied across a gradient of fungal species richness. The relationship was examined for two species of macroinvertebrate shredders, *G. pulex* and *S. personatum*, for a total fungal species pool of 37 native UK fungal species. Overall, there was no effect of increasing fungal species richness on rates of leaf processing by either shredder species. The aim was addressed through three objectives, and these are discussed in the following sections.

5.4.1. Objective 1: Was there relationship between fungal species richness and mean rates of leaf processing by macroinvertebrate shredders?

In neither of the two experiments were effects of species richness *per se* important in determining rates of shredder leaf processing rates (Figure 5.1 and 5.2). In Experiment 1, rates of shredder leaf processing were no different when shredders were fed leaves inoculated with either 3 or 12 fungal species (Figure 5.1). In Experiment 2, rates of shredder leaf processing were no different when shredders were fed leaves inoculated with either 3 or 9 fungal species, or 1 or 9 fungal species. However, there was a significant difference between 1 and 3 fungal species (Figure 5.2).

A positive effect of fungal species richness on rates of leaf processing by *Gammarus fossarum* has been shown by Lecerf *et al.* (2005). However, this positive effect only occurred in the absence of one particularly palatable fungal species, *Goniopila monticola*. Furthermore, in the absence of this species there was no difference between the mean rates of leaf consumption on leaves inoculated with 1 or 2 fungal species, and between 4, 6 and 8 fungal species, suggesting that if a positive relationship did exist it was non-linear. As previously discussed (Section 5.1.3.), the design of the previous study prohibits separation of the effects of species richness from those of community composition. The present study was designed with this in mind, and as a consequence, the pattern, or lack of pattern, in both experiments, means that I am confident that the number of fungal species *per se* was not important

in determining rates of shredder leaf processing in my experiment.

Contrasts between the community treatments at different levels of species richness indicate unpredictability in the direction of the effects of species richness on rates of leaf processing. This is consistent with the 'idiosyncratic hypothesis', of the relationship between species richness and ecosystem functioning (see Section 1.2.1.2.: Figure 1.1e). The idiosyncratic hypothesis proposes that "*ecosystem function changes when richness changes, but the magnitude and direction of change is unpredictable, because the roles of individual species are complex and varied*" (Lawton 1994). The idiosyncratic nature of increasing the number of species present on leaf disks can be seen in Figure 5.4. Various patterns exist as you move from 1 to 3 fungal species (Figure 5.4a-i): from an increase, above and beyond the processing rate of the highest performing monoculture (e.g. polycultures 3.1", 3.2", 3.3", 3.7"; Figure 5.4a, b, c & g respectively) to a decrease, below that of the processing of the lowest performing monoculture (e.g. polyculture 3.5"; Figure 5.4e). Polycultures 3.6" and 3.8" show rates of processing roughly equal to the mean of the performance of their respective monocultures (Figure 5.4f & h respectively). A similar pattern exists in the 9-species polycultures, with an absence of any polyculture performing worse than the lowest respective monoculture species (Figure 5.4j-l).

Variability among individual species responses (see Figure 5.3) is also consistent with a positive but idiosyncratic pattern of ecosystem function with increased richness (Emmerson *et al.* 2001). Several authors have recently argued that the consequences of biodiversity loss are likely to be idiosyncratic, differing quantitatively and qualitatively between trophic groups and ecosystems (Emmerson *et al.* 2001; Raffaelli *et al.* 2002; Covich *et al.* 2004). Such patterns mean that predicting the consequences of species loss for ecosystem processes is difficult, especially when there are simultaneous reductions in richness at more than one trophic level (Srivastava & Velland 2005).

Interestingly, two of the highest processed species in monoculture (1.14: *Varicosporium delicatum* & 1.05: *Anguillospora filiformis)* were inoculated into the lowest processed polyculture treatment (polyculture 3.5). The third species to have

been inoculated into polyculture 3.5, *Clavatospora longibrachiata* (monoculture treatment: 1.23) was as unpalatable as the zero fungal species treatment. This is also in support of the idea that rates of leaf processing of monoculture species are not reliable indicators of rates of leaf processing in polyculture.

That individual fungal species (monocultures) differ in their palatability to shredders (Figure 5.4) concurs with the results of previous fungi-shredder studies (Bärlocher & Kendrick 1973a; Arsuffi & Suberkropp 1984; 1988; Graça *et al.* 1994). Previous studies have also shown that *Lemonniera aquatica* (monoculture community treatment 1.19) is usually rejected in food preference studies by shredders (Suberkropp *et al.* 1993). Rates of leaf processing on this species in monoculture were nearly the lowest of all monoculture treatments. Only monoculture treatments 1.01 and 1.24 were lower, though not significantly so.

5.4.2. Objective 2: Was there any evidence for species' complementarity?

I found evidence for transgressive overyielding in 44 % of 3-species polycultures in Experiment 2 (Appendix I, Figure 5.4a-i), indicating that positive species' complementarity exists in this system, and this may be responsible for increased rates of leaf processing from 1 to 3 fungal species. This is in contrast with studies from other ecosystems which have found no evidence for transgressive overyielding, across ecosystems (Cardinale *et al.* 2006), in terrestrial plant systems (Špaèková & Lepš 2001; Hector *et al.* 2002; Fridley 2003; Hooper & Dukes 2004), and in coral reef ecosystems (Bruno *et al.* 2006). Transgressive overyielding is a very stringent test however, so it seems unlikely that the instances of overyielding here are spurious results. The existence of complementarity in this system suggests that particular combinations of species, or particular 'community identities', are important in determining rates of leaf processing.

In the majority of polycultures where there was no overyielding (polycultures 3.4", 3.5", 3.6", 3.9", 9.2, 9.3), all of the highest monoculture species were 'highly palatable' species (i.e. their mean rate of processing in monoculture was significantly greater than zero species (Figure 5.4)). The exception to this was polyculture 3.8",

whose highest processing monoculture was species 1.26, which was not significantly greater than zero in monoculture. This suggests that these 'highly palatable' species are associated with higher rates of processing in polyculture, but not consistently so. This provides some evidence consistent with the sampling effect hypothesis, such that there is the possibility that the inclusion of certain highly palatable species in polyculture might drive rates of processing in polyculture, although this might just be circumstantial evidence and is not tested for explicitly.

There was also evidence for one polyculture underyielding: 3.5" yielded lower rates of processing than any of its constituent monocultures (Figure 5.4e). This is indicative of negative species' interactions, or interference. Previous studies have shown that fungi have mechanisms for inducing inhibition, e.g. allellopathy, the release of inhibitive chemicals (Platas *et al.* 1998; Gulis & Stephanovich 1999). However, these kinds of effects were rare in the study system.

5.4.3. Objective 3: Were there differences between the shredder species?

Rates of leaf processing by shredder species were significantly greater for one species than for the other (*G. pulex* > *S. personatum*) (Figure 5.1). Previous studies have indicated that different shredder species have different niches, and feeding strategies, for example *G. pulex* and *Asellus aquaticus* (L.) (Isopoda: Asellidae) exhibit preferences for different species of fungi (Graça *et al.* 1993). While the mean rate of leaf processing differed between the two shredder species, the relationship between fungal species richness and the rate of leaf processing was similar across the two species (supported by the lack of a significant interaction between fungal species richness and the shredder treatments in the General Linear Model (Table 5.2)). The lack of an effect of fungal species richness is not dependent on shredder species identity.

5.4.4. Scaling up to natural systems.

Previous studies of leaf processing rates by fungal species in the absence of a shredder have indicated that fungal species richness was not related to rates of microbial leaf processing (Dang *et al.* 2005). Inclusion of a second trophic level in the present study indicates that the value of preserving the species richness of fungi may be because it provides "insurance" (Yachi & Loreau 1999) that some palatable species will be present to allow the most efficient transfer of energy through the system, though the threshold number needed to insure moderately high rates of shredder processing is relatively low.

Leaf processing rates in natural streams will also be affected by external abiotic factors. For example, rates of microbial leaf processing are determined by nutrient concentrations in streams (Gulis & Suberkropp 2003), and leaf litter quality (Leroy & Marks 2006). In the present study I saw no effects of changes in biotic factors, in terms of the effect of fungal species richness on rates of shredder leaf processing, but did see idiosyncratic effects of changes in fungal community identity. It remains to be seen how important these community identity effects are in relation to other external abiotic factors in determining rates of leaf processing in natural systems. A recent study (Ferreira & Graça 2006) revealed that shredders did not play a large role in structuring the fungal community, but abiotic factors (current velocity) did, suggesting that the influence of abiotic factors might be paramount in structuring fungal communities.

5.4.5. Conclusion.

There was no evidence for a positive relationship between fungal species richness and rates of leaf processing by macroinvertebrate shredder species, but rather evidence points to an idiosyncratic relationship. If this is the case, then predicting the consequences of species losses are difficult.

There was some evidence for positive species' interactions, which resulted in increased rates of leaf processing in some 3-species polycultures. This suggests that

certain combinations of species, 'community identities', are more able to support higher leaf process rates than others. Further study is needed to identify of the traits of these key species, such as tolerances to pollution, and rates of leaf decomposition independent of shredders. This information could potentially be used to establish 'key processing species' able to withstand the effects of anthropogenic stressors and insure rate of leaf processing in natural stream ecosystems.

6. General Discussion.

The central aim of this study was to investigate the relationships between stressors, macroinvertebrate community structure and the rate of leaf processing (as a measure of function) in stream ecosystems. There were four objectives (Section 1.7.). In this chapter, I summarize the key findings from each of these areas of investigation (Section 6.1.) and synthesise the results. I consider the implications for: a) the biodiversity – ecosystem function debate (Section 6.2.1.); b) managing and assessing freshwater ecosystems (Section 6.2.2.).

6.1. Results in relation to the objectives of the study.

6.1.1. Objective 1.

To perform a meta-analysis of published experimental and field studies to quantify the effects of anthropogenic stressors on the relationship between macroinvertebrate community structure and ecosystem function across streams (Chapter 2).

Systematic searches of the literature successfully yielded 97 studies which could be used to formally assess whether responses of structure and function to three distinct kinds of pollutant stressor (heavy metal contamination, acidification and organic pollution) were consistent across stressors and across streams. Freshwater ecosystem function was measured as the rate of leaf processing. The results indicate that stressors had strong and consistent effects on some aspects of macroinvertebrate community structure and function, specifically:

i) all three stressors reduced the number of taxa;

ii) heavy metal contamination and acidification reduced community density, while organic pollution had no effect;

iii) heavy metal contamination had no effect on the number of EPT taxa, while acidification and organic pollution reduced the number of EPT taxa;

iv) acidification increased the percentage of shredder taxa;

v) acidification reduced the rate of leaf processing, while organic pollution increased the rate of leaf processing (measured as the leaf breakdown coefficient *k*) (Table 2.3a).

While there was indication that other aspects of structure and function were also affected, the power to detect effects was often limited by low sample sizes (Table 2.3b). The analysis of aspects of structure and function independently indicates that across large geographical and temporal scales, the patterns of the effects of stressors on some aspects of structure and function are predictable and sizeable.

In order to ascertain whether or not structural responses were a good predictor of ecosystem function, I tested whether responses of structure and function to the three pollutant stressors were associated (Section 2.1.4.). There was no association between structure and function.

6.1.2. Objective 2.

To conduct field studies to document the effects of heavy metal contamination on the relationship between macroinvertebrate community structure and ecosystem function in streams (Chapter 3).

This role of this objective was to address the same problem as the previous objective (i.e. are structural responses a good predictor of ecosystem function?). Rather than rely on studies from the literature (as seen in Objective 1), I generated data on the impacts of both structure and function directly. Thus I was able to address the problem of lack of data on the similarity of responses of structure and function to stress.

I successfully documented structure and function downstream of multiple heavy metal contaminated and reference sites in Cornwall, south west England and in the Leadhills, Lanarkshire, Scotland. Macroinvertebrate community structure, the rate of

leaf breakdown and a range of abiotic variables were measured. The study design was to compare pairs of sites (contaminated *vs.* reference). Sites in Cornwall met these criteria (Section 3.2.1.) and were analysed as site pairs. Sites in the Leadhills did not meet the criteria and so sites were not analysed as pairs. At contaminated sites in Cornwall (n = 6) there were significant reductions in the:

- shredder community (number of taxa);
- non-shredder community (density, number of taxa);
- whole community (density, number of taxa and biomass);
- rates of leaf processing (measured as the percentage leaf mass loss).

Strong associations were found between structure and function across sites in Cornwall, but not in the Leadhills. Evidence suggests that the association between structure and function in Cornwall was driven by the direct effect of stressors on both structure and function (i.e. independently), rather than through indirect pathways (Section 3.3.2.5.; Figure 3.5) (see also Section 6.2 below), although the basis for this conclusion is weak, because of the limited sample size within the available study design. The disparity in the results between the two regions suggests that the association between structure and function may be sensitive to the specific context (community, location, stressor type) in which the impact occurs. If this is the case then future work might focus on the importance of context in determining rates of ecosystem processes.

6.1.3. Objective 3.

To use artificial stream mesocosms to test whether rates of leaf processing by mixed-species assemblages are predictable from the sum of their constituent parts (Chapter 4).

Objectives 1 and 2 addressed the relationship between macroinvertebrate community structure and the rate of leaf processing in natural streams, subjected to anthropogenic stressors. In Objective 3 I examined the relationship between macroinvertebrate

community structure and the rate of leaf processing in indoor stream mesocosms. Specifically I tested whether the rate of leaf processing of multiple assemblages of species, with varying composition, could be predicted from measurement of mean species-specific leaf processing rates in isolation. An experimental approach permitted control over biotic and abiotic factors which may have affected rates of leaf processing in the two previous studies (Objectives 1 and 2). Two separate experiments were undertaken: the first, to ascertain rates of leaf processing by species in isolation (Section 4.2.3.); the second, to ascertain rates of leaf processing by mixed-species assemblages (Section 4.2.4.).

The leaf processing rates in isolation of a variety of shredder species (n = six out of seven studied species) conformed to standard allometric scaling relationships (i.e. leaf processing rates were allometrically related to body size) (Section 4.3.1.). This is important because it indicates that a large portion of the mechanistic differences between shredder species can be explained through differences in body size. Individual leaf processing rates were not good predictors of the net consumption rates of assemblages of species (Section 4.3.4.). Rates of leaf processing by assemblages of shredders were greater than the sum of their constituent parts (Figure 4.7). This pattern may have been driven by biological mechanisms, e.g. facilitation between species, but was not explained by species composition (Figure 4.8). Variation in the rate of leaf processing by the different assemblages is indicative of strong species identity effects (Section 4.4.2.3.). Overall, the results of this study indicate that rates of shredder leaf processing are not predictable, even in controlled experimental systems and from robust knowledge of individual species' rates of processing in isolation.

6.1.4. Objective 4.

To assess the effect of fungal species richness on the rate of leaf processing mediated through macroinvertebrate shredders (Chapter 5).

In the previous three objectives, the leaf material fed to shredders was conditioned in natural streams (Section 1.5.2.2.). In this objective I experimentally manipulated the species richness of fungi on the leaf material, in a highly replicated and controlled experimental system. I tested whether there was an effect of changes in species richness on the rate of leaf processing by macroinvertebrate shredders. I performed two separate experiments. In the first experiment I created a diversity gradient of either 3 or 12 fungal species and fed leaves inoculated with fungi to two species of shredders (*Gammarus pulex* and *Sericostoma personatum*); in the second experiment I created a diversity gradient of either 0, 1, 3 or 9 species and focussed on refining the understanding of the relationship for a single shredder species, *G. pulex*. Both experiments showed that there was no effect of increasing fungal species richness on rates of shredder leaf processing (Figures 5.1 and 5.2). Importantly, the experimental design was such that the effect of species richness could not have been confounded by that of community composition. While there was some evidence for complementarity between fungal species, which resulted in increases in leaf processing between 1 and 3 fungal species, overall there was no effect of increasing fungal diversity. The lack of an effect is also not dependent on the identity of the shredder species (Section 5.3.1.1.). Leaf processing rates of the two shredder species were different when fed with the same fungi treatments (Figure 5.1) (*G. pulex* > *S. personatum*). This reinforces previous work, which has predominantly examined differences between shredder species in their preferences for single fungal species (Section 5.1.1.; e.g. Graça *et al.* 1993: *Asellus aquaticus* and *G. pulex*). The evidence indicates that the relationship between fungal species richness and rates of leaf processing by macroinvertebrate shredders is idiosyncratic (see also Section 6.2.1.).

6.2. Synthesis.

Let us consider the messages from each chapter as we scale from a relatively simple, controlled laboratory system seen in Chapter 5, to the more complex (but also controlled) laboratory stream system in Chapter 4, to even more complex natural stream ecosystems within regions the UK (Chapter 3) and across regions (Chapter 2).

At the simplest level considered in this thesis (i.e. the effect of changes in the diversity of aquatic hyphomycetes on the rate of leaf processing by single macroinvertebrate shredder species - Chapter 5) there was indication that fungal species identity and composition were more important than diversity *per se* in determining rates of macroinvertebrate leaf processing (Figure 1.3: Arrow H). Therefore the shape of the relationship between microbial community structure and macroinvertebrate leaf processing is likely to be described by an idiosyncratic relationship (Figure 1.1e).

Now consider increasing the complexity of the system to the inclusion of more than one shredder species in a system. In Chapter 4 I manipulated shredder community composition in three-species assemblages in laboratory stream mesocosms and measured the rate of leaf processing. The results indicate that changes in shredder community composition are likely to have unpredictable effects on rates of ecosystem processing (Figure 1.3: Arrow C). It is important to bear in mind that in the two controlled experimental study systems there was no stressor present, and both experiments indicate that even in these relatively simple systems the relationships between structure and function of both shredder and fungal communities are hard to describe. As such, structure cannot be used to predict function.

Now consider increasing the complexity of the system to examination of the relationship between structure and function in natural streams, subject to anthropogenic stressors. In Chapter 3 I surveyed stream sites within distinct two regions of the UK and in Chapter 2 I assessed responses of structure and function in streams across regions. Both studies showed that all three stressors had strong and predictable effects on structure (Figure 1.3: Arrow A). However, the effects on function were more complex, increasing in one case and decreasing in two other cases. These results strongly concur with the results of the experimental studies (Chapters 4 and 5): that structure cannot predict function.

6.2.1. The biodiversity - ecosystem function debate.

6.2.1.1. The shape of the relationship between structure and function.

The recent reviews by Balvanera *et al.* (2006) and Cardinale *et al.* (2006), summarized results from 446 and 111 'biodiversity – ecosystem function' studies respectively, and found that the majority of studies showed positive effects of increasing species diversity on function. However, these patterns are determined primarily from terrestrial grassland studies. The results of the present study in freshwater microbial decomposer assemblages (Chapter 5) indicate that the effects of species losses are likely to be idiosyncratic (see Section 5.4.1.). This has been argued of the aquatic ecosystem by several authors (Emmerson *et al.* 2001; Raffaelli *et al.* 2002; Duffy 2003; Covich *et al.* 2004). This highlights a need for caution when extrapolating the results of studies across ecosystems. The consequences of an idiosyncratic relationship between biodiversity and ecosystem functioning is that predicting the consequences of species' losses on the magnitude of ecosystem processing is difficult (Srivastava & Velland 2005) (see also Section 6.2.2.2.).

It would be useful to understand how much rates of leaf processing vary over time, and how the species losses affect this variability. Theory predicts that losses in biodiversity may increase the variability in process rates (Doak *et al.* 1998; Cottingham *et al.* 2001; Loreau *et al.* 2001b). A useful next step, which has already begun in part (Dang *et al.* 2005), is to consider the consequences of species losses on the variability of leaf process rates. A recent study by Dang *et al.* (2005) experimentally tested for the mechanisms through manipulation of fungal species richness and measuring rates of leaf decomposition. Dang *et al.* found that variability in rates of decomposition increased with the loss of species, even though the magnitude of the process remained unaffected. Whether these same patterns are true in shredder assemblages and the extent to which these patterns reflect those in natural streams, remains to be investigated.

6.2.1.2. Species identity and composition effects.

Many authors have argued that species composition may be more important in determining the rates of ecosystem processes than the number of species (Naeem *et al.* 1996; Aarssen 1997; Hooper & Vitousek 1997; Huston 1997; Tilman *et al.* 1997a; Wardle *et al.* 1997c; Symstad *et al.* 1998; Ruesink & Srivastava 2001; Stampe & Daehler 2003; Wardle *et al.* 2004; Bruno *et al.* 2005; Bruno *et al.* 2006; Straub & Snyder 2006) (Section 1.3.1.). This is because the mechanisms underpinning diversity effects relate strongly to the functional attributes of species (Section 1.2.2.). In addition, very few studies have manipulated the diversity of a primary consumer community, and measured changes in ecosystem process rates. The experiments undertaken in Chapter 4 were designed to address this gap. I demonstrated that rates of leaf processing by multiple combinations of three-species shredder assemblages can vary in magnitude by up to five times across assemblages (Chapter 4; Figure 4.7). This is indicative of strong species identity effects (Section 4.4.2.3.). This supports the contention of several previous studies which have manipulated species diversity in shredder assemblages and concluded that species identity effects are important drivers of process rates in these freshwater shredder decomposer systems (Jonsson *et al.* 2001; Jonsson *et al.* 2002). In addition, complementarity between shredder species was indicated, although this was not tested for formally.

The possible existence of complementarity in this system (Chapter 4) and also the strong indication of complementarity in the microbial system in Chapter 5 (Section 5.4.2.), suggests that particular combinations of species, or particular community identities are important in determining rates of leaf processing. Future research would benefit from generating a better understanding of:

1. the roles which individual species play in communities, in particular, in identifying:
    a. which the key shredder species and community identities are;
    b. which the key fungal species and community identities are, in terms of their contribution both to rates of shredder leaf processing and also to rates of microbial processing;
2. the relative sensitivities and tolerances of these important species;

3. whether the traits which determine vulnerability to extinction are related to functional dominance in communities (i.e. are the dominant species most sensitive to stress?)

Furthering our understanding of species' traits in natural stream assemblages may be the key to our understanding of the relationship between structure and function. There is current interest in developing an understanding of the functional roles which individual species play in freshwater ecosystems (Lancaster 2000; Bady *et al.* 2003; Heino 2005), moving away from assessing the numbers and densities of species as equals in ecosystems and toward considering the individual identities, traits and roles which species play in ecosystems (Bis & Usseglio-Polantera 2004).

6.2.2. Implications for managing and assessing freshwater ecosystems.

6.2.2.1. For the preservation of stream ecosystem function.

Overall, my studies suggest that the relationship between macroinvertebrate shredder community structure and function cannot be described by any one simple relationship. Thus, I suggest that for the purpose of conservation of freshwater ecosystems, maintaining biodiversity becomes important due to the unpredictable nature of what will happen if a species is lost. Both increases and decreases in biodiversity, as well as changes in community composition may elicit effects of varying magnitude on ecosystem function. The precautionary approach, from the point of view of wanting to preserve ecosystem functioning, is to preserve the system as it is, in order to avoid unpredictable changes in the magnitude of ecosystem functioning. In the situation where species loss is inevitable, knowledge of individual species traits (as described in the previous section) might allow us to manage against the loss of key species and make an informed judgement as to which species are 'better' or 'worse' to lose.

6.2.2.2. Assessing freshwater functional ecosystem status.

There is increasing recognition that an important component of monitoring and assessing the integrity of ecosystems is the evaluation of their functional status (Section 1.5.1.). Implementation of the Water Framework Directive (WFD) (E.C. 2000/60/E) requires assessment of the functional status of aquatic flowing-water ecosystems (Article 2, Annex V, Section 1.2.1.), although, at present, an appropriate methodology has not been developed. Leaf processing has been proposed as an indicator of functional ecosystem integrity in freshwaters (Gessner & Chauvet 2002; Hagen *et al.* 2006). The RIVFUNCTION project aims to determine the performance of leaf processing in response to two types of anthropogenic impacts, eutrophication and changes in riparian vegetation (see also Section 1.5.2.2.). The results of the present study extend this work to the inclusion of the effects of heavy metal contamination and acidification. The results of the meta-analysis in Chapter 2 indicate that leaf processing may respond predictably to anthropogenic stress (Table 2.3a) (Section 6.1.1.). The direction of the response can be either negative or positive; organic pollution will tend to increase rates of leaf processing, probably through increases in the nutrients available to microbes (Section 2.4.2.1.). Whereas acidification and heavy metal contamination (see also Chapter 2) tend to decrease the rate of leaf processing. These differences in the direction of the trend suggest that if leaf processing rates were used to assess impacts, simply looking at function would not tell you anything useful. Information would be improved if it could be put into context (e.g. accompanied by water chemistry data which indicated an impact). A reference state would be needed, in order to determine what the rate of processing might be in the absence of stress, in order to understand that there has been a change in the rate of leaf processing. This suggests that, if developed, the rate of leaf processing may work well as an accompaniment to existing methods of ecological assessment.

The results of objectives 2 and 3 show that although structure and function can be related (e.g. across sites in Cornwall, Chapter 3), the more frequent response is that they are not (e.g. across sites in Scotland, Chapter 3 and across the many studies incorporated into the meta-analysis, Chapter 2). This indicates that the only way to

assess function effectively in natural streams may be to make direct assessment of functional aspects of the system, in addition to structural assessment, because there is no one simple relationship between the two (see also Section 6.2.2.). Simply, structure does not reveal function, and current assessment procedures can only tell us a limited amount with regard to the functional status of freshwater stream ecosystems.

Future work should consider:

1. the effects of multiple impacts on rates of leaf breakdown.
2. whether assessments of leaf processing are useful indicators of functional status in parts of the watercourse which are not the headwaters?
3. the extent to which the rate of leaf processing actually informs the status of the provision of freshwater ecosystem goods and services.

6.3. Conclusion.

Both macroinvertebrate community structure and rates of leaf processing must be monitored in stream ecosystems in order to protect against the effects of anthropogenic stressors.

Appendix A: Studies included in the meta-analysis (Chapter 2).

Allard, M. & Moreau, G. (1986). Leaf decomposition in an experimentally acidified stream channel. *Hydrobiologia* **139:** 109-118.

Armitage, P.D. (1980). The effects of mine drainage and organic enrichment on benthos in the river Nent system, northern Pennines. *Hydrobiologia* **74**: 119-128.

Barton, D.R. & Metcalfe-Smith, J.L. (1992). A comparison of sampling techniques and summary indices for assessment of water quality in the Yamaska River, Quebec, based on benthic macroinvertebrates. *Environmental Monitoring and Assessment* **21**: 225-244.

Beltman, D.J., Clements, W.H., Lipton, J. & Caecela, D. (1999). Benthic invertebrate metals exposure, accumulation, and community-level effects downstream from a hard-rock mine site. *Environmental Toxicology and Chemistry* **18:** 299-307.

Bernard, D.P., Neill, W.E. & Rowe, L. (1990). Impact of mild experimental acidification on short term invertebrate drift in a sensitive British Columbia stream. *Hydrobiologia* **203:** 63-72.

Brooker, M.P. & Morris, D.L. (1980). A survey of the macro-invertebrate riffle fauna of the rivers Ystwyth and Rheidol, Wales. *Freshwater Biology* **10:** 459-474.

Brown, C.A. (1996). Macroinvertebrate community patterns in relation to physico-chemical parameters measured at two land-based trout farms affecting streams in the south-western Cape, South Africa. *Archiv Für Hydrobiologie* **138**: 57-76.

Burton, T.M., Stanford, R.M. & Allan, J.W. (1985). Acidification effects on stream biota and organic-matter processing. *Canadian Journal of Fisheries and Aquatic Sciences* **42:** 669-675.

Camargo, J.A. (1992). New diversity index for assessing structural alterations in aquatic communities. *Bulletin of Environmental Contamination & Toxicology* **48:** 428-434.

Camargo, J.A. (1994). The importance of biological monitoring for the ecological risk assessment of freshwater pollution. *Environmental International* **20:** 229-238.

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Carlisle, D.M. & Clements, W.H. (1999). Sensitivity and variability of metrics used in biological assessments of running waters. *Environmental Toxicology and Chemistry* **18:** 285-291.

Carlisle, D.M. & Clements, W.H. (2003). Growth and secondary production of aquatic insects along a gradient of Zn contamination in Rocky Mountain streams. *Journal of the North American Benthological Society* **22:** 582-597.

Carlisle, D.M. and Clements, W.H. (2005). Leaf litter breakdown, microbial respiration and shredder production in metal-polluted streams. *Freshwater Biology* **50:** 380-390.

Chadwick, M.A. and Huryn, A.D. (2003). Effect of whole-catchment *N* addition on stream detritus processing. *Journal of the North American Benthological Society* **22:** 194-206.

Chaffin, J.L., Valett, H.M. and Webster, J.R. (2005). Influence of elevated As on leaf breakdown in an Appalachian headwater stream. *Journal of the North American Benthological Society* **24:** 553-568.

Clements, W.H. (1994). Benthic invertebrate community responses to heavy metals in the Upper Arkansas River Basin, Colorado. *Journal of the North American Benthological Society* **13:** 30-44.

Clements, W.H. (1999). Metal tolerance and predator-prey interactions in benthic macroinvertebrate stream communities. *Ecological Applications* **9:** 1073-1084.

Clements, W.H. (2004). Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. *Ecological Applications* **14:** 954-967.

Clements, W.H., Cherry, D.S. and Cairns, J. (1988). Impact of heavy metals on insect communities in streams: A comparison of observational and experimental results. *Canadian Journal of Fisheries and Aquatic Sciences* **45:** 2017-2025.

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Courtney, L.A. and Clements, W.H. (2002). Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach. *Freshwater Biology* **47:** 1766-1778.

Dangles, O.J. and Guerold, F.A. (2000). Structural and functional responses of benthic macroinvertebrates to acid precipitation in two forested headwater streams (Vosges Mountains, northeastern France). *Hydrobiologia* **418:** 25-31.

Dangles, O. and Guerold, F. (2001a). Influence of shredders in mediating breakdown rates of beech leaves in circumneutral and acidic forest streams. *Archiv Für Hydrobiologie* **151:** 649-666.

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Appendix B: Heavy metal contaminated regions (Chapter 3).

Four geographic regions in the UK, known to be affected by heavy metal contamination due to mining, were considered for the purpose of the study in Chapter 3. Of these, 2 regions were selected for study and 2 regions were considered to be unsuitable. The reasons behind choices were as follows:

a) West Wales.

Previous studies in this region have reported the effects of heavy metal contamination (primarily zinc) on the macroinvertebrate community, focussing on the River Ystwyth, Cardiganshire (Carpenter 1924; Jones 1948; 1958). Examination of the physico-chemical conditions of streams in this area revealed there to be a multitude of stream sites present all of which were contaminated by heavy metals. However, these streams were considered to be unsuitable for the present study because pH values of the water tended to fall below pH 6. There was evidence to suggest that these streams were impacted by acidic deposition (Martin & Walley 2000, data from the Environment Agency; Monteith 2005) and therefore stream sites in this region were considered to be unsuitable for inclusion in the present study (see selection criteria in Section 3.2.2.1).

b) Northern England.

i) The North Pennine Orefields, Northumbria.

In this region, many spoil heaps remain today from mines which produced substantial quantities of zinc ore in the past. Streams in this area (e.g. the West Allen NGR: NY800452) (Abel 1996) were not acidic (Green 1984 unpublished; Martin & Walley 2000) and fulfilled many of the site selection criteria (see Section 3.2.2.1). However, only one independent site pair could be identified for which abiotic and biotic data were available *a priori*: contaminated site NGR: NY803461, site 9+ (Green 1984 unpublished); reference site NGR: NY804461, site 9a (Green 1984 unpublished). Therefore, because I needed replicate site pairs

ii) South and West Yorkshire and Derbyshire.

Sites in this region were affected by coal mine effluent (e.g. Ashton 1997; Maltby *et al.* 2002; Wilding 2004), and were therefore considered to be unsuitable for inclusion in the present study (see Section 3.2.2.1).

c) Leadhills, South Lanarkshire, Scotland.

This region of Scotland was mined for lead for over 200 years. Metalliferous run-off from old mines and mining slopes has caused heavy metal contamination of many streams in the region (Grieg 1971). A survey in the region in 2000, by an undergraduate student at the University of Sheffield, documented abiotic data for 34 streams. From this, four sites contaminated with cadmium (Table B.1) and four reference sites were selected for the present study. For a dossier of the selected sites in this region see Appendix C.

d) Cornwall, South West England.

Cornwall is heavily mineralised as a result of tectonic processes associated with the granite bedrock which underlies the region. Ores are mainly non-ferrous and tin (Sn) and copper (Cu) are deposited near to the granite. The mining history of Cornwall extends back to the 16th Century, and many of the underground mining galleries continue to discharge groundwater containing heavy metals (Gower *et al.* 1994). Six catchments, highlighted by Gower *et al.* (1994), were considered and selected for study. Hirst *et al.* (2002) confirmed that these catchments were contaminated with heavy metals (Table B.2). For a dossier of the selected sites in the region see Appendix D.

Table B.1: Abiotic data, and heavy metal (zinc, cadmium and aluminium) concentrations for 4 streams in the Leadhills region of Scotland (Scholes 2000 unpublished). NGR = National Grid Reference. Temp. = temperature. Cond. = conductivity.

Site	NGR	pH	Temp. (°C)	Dissolved oxygen (mg/l)	Cond. (µS/l)	Flow rate (m/s)	Width (cm)	Depth (cm)	Alkalinity (ml $CaCO_3$/l)	Zn (mg/l)	Cd (µg/l)	Al (µg/l)
Glengonnar Water	NS877177	6.1	7.9	11.4	145	216	286	20	81.4	0.1	5	68
Wanlock Water A	NS873129	6.1	7.6	11.3	138	132	183	18	57.5	0.06	3	81
Wanlock Water B	NS855146	6.9	7.9	11.3	125	156	36	1	52.8	0.24	11	59
Mennock Water	NS843103	7.6	8	12.2	84	62	490	30	32.5	0.02	2	66

Table B.2: Abiotic and chemical data from 6 contaminated catchments in Cornwall (Hirst *et al.* 2002). NGR = National Grid Reference.

Stream	NGR	Catchment	Altitude (m)	Slope (m/100m)	Distance From source (km)	pH	Al (µg/l)	Fe (µg/l)	Mn (µg/l)	Cd (µg/l)	Cu (µg/l)	Pb (µg/l)	Zn (µg/l)
Haye Valley	SX346701	LYNHER	85	0.025	1.25	7.42	26.3	78.1	50.2	2.5	18.6	4.1	638.4
Seaton	SX263696	SEATON	180	0.040	2.10	6.56	252.0	17.9	56.1	0.8	468.7	3.3	309.0
Carnon	SW762419	FAL	20	0.008	4.80	6.55	24.8	48.1	58.1	1.2	43.4	0.3	663.2
Hayle	SW611327	HAYLE	40	0.004	6.50	5.27	22.9	118.0	184.6	2.1	148.2	0.3	1006.3
Porthtowan	SW695475	PORTHTOWAN	25	0.013	3.30	6.77	18.2	66.5	185.4	3.3	267.0	0.8	1684.6
Gannel	SW834552	GANNEL	50	0.014	1.30	4.86	318.4	325.0	635.2	5.7	59.5	302.2	1102.6

Appendix C: Site dossiers for sites in the Leadhills, SW Scotland (Chapter 3).

The Leadhills area of Scotland was mined for lead for over 200 years. Run-off from old mines and mining slopes has caused heavy metal contamination of many streams in the area (Grieg 1971; Roberts 1996 unpublished; Spicer *et al.* 1998). Abiotic and biotic data were compiled on sites within the region and from this the best 4 site pairs were selected (using selection criteria: see Section 3.2.2). Detailed information on each of the site pairs was evaluated *a priori* to the field study in Chapter 3 and follow in individual site dossiers. Limited data existed for some sites, which were not routinely monitored by the Scottish Environment Protection Agency (SEPA). Half of the sites flow into the River Nith (sites 3 & 4, 5 & 6), and half into the River Clyde (sites 1 & 2, 7 & 8).

C.1. Dossier for site pair 1 (sites 1 & 2).

C.1.1. Contaminated site (1): Glengonnar Water

National Grid Reference: NS887177

Site code: LC-GW

- SEPA monitor Glengonnar Water at two sites close to my sample site. SEPA call the sites 'Below Leadhills' (NGR: NS91922164) and 'Glencaple Bridge' (NGR: NS88621571). For physico-chemical measurements from these sites see Tables C.2 & C.3. In 2003 both of these sites were classed as D, seriously polluted, in SEPA's water quality classification scheme in 2003. Annual average for 2003:
    - 'Below Leadhills': Pb = 15.82 µg/l , Zn = 94.71 µg/l.
    - 'Glencaple': Pb = 24.28 µg/l , Zn = 39.49 µg/l.
- In 2000 Glengonnar Water had elevated levels of Cd (Table C.1; Figure C.1A) (Scholes).
- Ecological data from SEPA indicate that despite the fact that the watercourse was heavy metal contaminated, it still has excellent biological community present at Glencaple Bridge (Table C.2).

Table C.1: Abiotic data from the sites in the Leadhills (Scholes 2000 unpublished). Odd numbered sites were contaminated with elevated Cd concentrations. Even numbered sites act as reference sites. NGR = National National Grid Reference; DO = dissolved oxygen. Alk = alkalinity. Temp. = temperature. Cond. = conductivity.

Site Pair	Site no	Site name	NGR	pH	DO (mg/l)	Temp. (°C)	Cond. (µS/l)	Flow rate (m/s)	Width (cm)	Depth (cm)	Alk. (ml $CaCO_3$/l)	Zn (ml/l)	Cd (µg/l)	Al (µg/l)
1	1	Glengonnar Water	NS887177	6.13	11.4	7.9	145	216	286	20	81.4	0.1	5	68
	2	Tr. Elvan Water	NS901157	7.42	11.7	6.9	138	106	195	8	78.1	0.02	1	56
2	3	Wanlock Water A	NS873129	6.1	11.3	7.6	138	132	183	18	57.5	0.06	3	81
	4	Allershaw Burn	NS963119	6.64	11.5	8.7	94	76	220	20	56.1	0.04	<1	117
3	5	Wanlock Water B	NS855146	6.87	11.3	7.9	125	156	156	36	52.8	0.24	11	59
	6	Tr. Camps Water	NS973224	6.8	11.0	8.8	121	132	110	15	59.1	0.0	<1	119
4	7	Mennock Water	NS843103	7.61	12.2	8.0	84	62	490	30	32.5	0.02	2	66
	8	Tr. Mennock Water	NS853102	7.58	11.6	7.4	89	132	240	18	39.6	<0.004	<1	60

Table C.2: Biotic analyses of macroinvertebrate samples from Glengonnar Water and Elvan Water (downstream of reference site 2), Daer Water (upstream of reference site 4) and from Camps Water (downstream of reference site 6) (SEPA). BMWP = Biological Monitoring Working Party score. BMWP Scores: 71-100 = indicates that water was clean but slightly impacted, > 100 indicates excellent unpolluted and unimpacted water. ASPT = Average Score Per Taxon. ASPT Scores > 4 indicate good water quality.

Nearest site	Watercourse	Site Name	NGR	Comments	Date	BMWP	ASPT
1	Glengonnar Water	Glencaple	NS919216	Excellent biological quality.	06/11/03	133	6.65
					26/05/03	104	6.50
					15/06/95	139	6.62
					12/04/95	145	6.59
					17/02/92	134	7.05
					16/02/82	113	7.06
2	Elvan Water	Elvanfoot	NS950173	Excellent water quality.	29/10/03	161	6.71
					27/03/03	130	6.50
					15/06/95	154	6.70
					12/04/95	122	6.78
4	Daer Water	d/s Road Bridge	NS956132	Good diverse sample.	29/10/03	122	5.81
					27/03/03	132	6.29
6	Camps Water	u/s Road Bridge	NS957214	Good diverse sample.	06/11/03	179	6.39
					26/05/03	142	6.76

C.1.2. Reference site (2): Tributary Elvan Water

National Grid Reference: NS901157

Site code: LR-TEW

The reference site (2) was sampled by Scholes (2000 unpublished) (Table C.1, Figure C.1B). It was approximately 2.5 km south-west of contaminated site 1. A nearby SEPA monitored site at Elvan Water indicates a good diverse community in this watercourse (Table C.2), and was classed as A1, 'excellent', on SEPA's water quality classification scheme in 2003.

Table C.3: Physico-chemical measurements of sites on Glengonnar Water monitored by SEPA. Data were mean values from sampling conducted approximately every 2 months between November 1998 - November 2003. NGR = National Grid Reference. BOD ATU refers to biological oxygen demand measured when nitrification in the sample was suppressed by adding allylthiourea (ATU).

Variable	Glencaple Bridge (NGR: NS91922164)	Below Leadhills (NGR: NS88621571)
Ammonia (mg/L)	0.02	0.03
BOD ATU (mg/L)	0.96	1.07
O_2 as Dissolved Oxygen (mg/L)	11.42	11.11
0_2 as % saturation (%)	98.29	94.91
Pb (µg/L)	64.29	33.88
Pb <0.45 µm (µg/L)	23.49	15.84
Sample Temperature (°C)	8.52	8.24
Suspended solids (mg/l)	3.15	1.96
Zn (µg/L)	45.77	104.44
Zn <0.45 µm (µg/L)	37.86	96.65
o-Phosphate (mg/L)	0.02	0.01
pH	7.45	7.37

Figure C.1: Photograph of the sites at A) Glengonnar Water; B) Tributary Elvan Water (Scholes 2000 unpublished).

A)

B)

C.2. Dossier for site pair 2 (sites 3 & 4).

C.2.1. Contaminated site (3): Wanlock Water A

National Grid Reference: NS873129

Site code: LC-WWA

- Wanlock Water A is not monitored by SEPA.
- Wanlock Water A had elevated Cd concentrations, 3 µg/l in 2000 (Table C.1).

C.2.2. Reference site (4): Allershaw Burn

National Grid Reference: NS955132

Site code: LR-AB

The reference site, a tributary of Daer Water, was approximately 8.5km to the East of Wanlockhead. Downstream of the reference site was a site routinely monitored by SEPA, which has a good diverse community of macroinvertebrates (Table C.2).

C.3. Dossier for site pair 3 (sites 5 & 6).

C.3.1. Contaminated site (5): Wanlock Water B

National Grid Reference: NS855146

Site code: LC-WWB

- Wanlock Water B is not monitored by SEPA.
- Wanlock Water B has elevated Cd concentrations, 11 µg/l in 2000 (Table C.1).

Wanlock Water B was actually located on a tributary to the Wanlock Water, on Sowen Burn, located to the West of the mining town of Leadhills. It was spatially independent from the site Wanlock Water A.

C.3.2. Reference site (6): Tributary Camps Water

National Grid Reference: NS973224

Site code: LR-TCW

The reference site (6), a tributary of Camps Water, was approximately 14 km to the north east of the contaminated site (5). Data from SEPA indicate that there was a good diverse community of macroinvertebrates downstream of the reference site (Table C.2).

C.4. Dossier for site pair 4 (sites 7 & 8).

C.4.1. Contaminated site (7): Mennock Water

National Grid Reference: NS 843103

Site code: LC-MW

- SEPA do not monitor the Mennock Water.
- Mennock Water had elevated Cd concentrations, 2 µg/l in 2000 (Table C.1).
- Scholes sampled it in 2000 (Figure C.2)

C.4.1. Reference site (8): Tr. Mennock Water

National Grid Reference: NS 853102

Site code: LR-TMW

Tributary Mennock Water was located upstream of the contaminated site (7), a couple of kilometres South West of the mining town of Leadhills (Figure C.2B).

Figure C.2: Photograph of the sampling site at A) contaminated site (7) Mennock Water; B) reference site (8) Tributary Mennock Water (Scholes 2000 unpublished).

A)

B)

Appendix D: Site dossiers for sites in Cornwall, SW England (Chapter 3).

Following Gower *et al.* (1994) 6 catchments in this region were identified as being contaminated by heavy metals (elevated zinc concentrations). Abiotic and biotic information was compiled on sites within each of these catchments and the best 6 site pairs were selected (see Section 3.2.2.). Detailed information on each of the six site pairs was obtained and evaluated *a priori* to the field study (Chapter 3) and follow in individual site dossiers. Where reference is made to 'shredder families' this refers to the fact that some or all of the species within these families are leaf eating species (Merritt & Cummins 1996; Bis & Usseglio-Polantera 2004).

D.1: Dossier for site pair 5 (sites 9 & 10).

Contaminated site (9):	***Twelveheads***
Catchment:	Fal
River:	**Carnon**
National Grid Reference:	SW76154206
Site code:	CC-TH

Summary of site

- The site was highly contaminated with heavy metals, with zinc being the highest in concentration, though other metals were also present (Table D.1).
- Ecological data from Environment Agency and from previous studies (Hirst *et al.* 2002) indicate that the macroinvertebrate community was sparse and few families have been recorded (Tables D.2 and D.3).

Reference site (10):	***Trenarth Bridge***
Catchment:	Helford River
Stream:	Porth Navas Stream
National Grid Reference:	SW75772830
Site code:	CR-TB

The reference site was on the nearby Porth Navas Stream, in the Helford River Catchment, approximately 14 km away from the contaminated site at Twelveheads. Chemical data indicate that the site had a small amount of zinc in it, 10.46 μg/l (Table D.1), but there were no perceived stressors at the site (Martin & Walley 2000). The sites were paired based on proximity, alkalinity, and broadly matching abiotic features (Table D.4). The reference site had good diversity of macroinvertebrate families present; more than 30 families were present in 2000 (Table D.5). The following shredder families were present at the reference site, indicating that there was the potential for leaf processing: Nemouridae, Leuctridae, Sericostomatidae, Odontoceridae, Tipulidae (Table D.5).

Table D.1: Mean chemical data for the contaminated site (9) at Twelveheads and the reference site (10) at Trenarth Bridge, from monthly sampling between January 2003 and May 2004 (Environment Agency). - = No Data. BOD ATU refers to biological oxygen demand measured when nitrification in the sample is suppressed by adding allylthiourea (ATU).

Factor	Contaminated Site 9 Mean	Reference Site 10 Mean
pH	6.76	7.20
Water temp (°C)	11.68	11.35
BOD ATU as O_2 (mg/l)	1.13	1.5
Mercury as Hg (µg/l)	0.01	-
Cadmium as Cd (µg/l)	0.91	-
Ammonia as N (mg/l)	0.06	0.07
Nitrogen total oxidised – as N (mg/l)	5.03	6.40
Nitrate – as N (mg/l)	5.02	6.39
Nitrite – as N (mg/l)	0.02	0.0074
Ammonia as un-ionised (calculated mg/l)	7.818×10^{-5}	0.0003
Hardness Total as $CaCO_3$ (mg/l)	71.56	53.47
Alkalinity pH 4.5 – as $CaCO_3$ (mg/l)	23.5	24.4
Orthophosphate – as P (mg/l)	0.14	0.035
Potassium as K (mg/l)	4.00	3.08
Magnesium as Mg (mg/l)	6.91	5.22
Calcium – as Ca (mg/l)	17.28	12.8
Aluminium dissolved as Al (mg/l)	0.042	-
Tellorium – as Te (mg/l)	<0.001	-
Chromium – as Cr (µg/l)	<0.5	-
Chromium dissolved – as Cr (µg/l)	0.8	-
Nickel dissolved - as Ni (µg/l)	11.88	-
Nitrogen total inorganic (calculated) (µg/l)	5.09	6.46
Silver – as Ag (µg/l)	<1	-
Tin – as Sn (µg/l)	<2.5	-
Arsenic dissolved – as As (µg/l)	35.21	-
Arsenic dissolved – as As (µg/l)	45.36	-
Selenium - as Se (µg/l)	<1	-
Manganese – as Mn (µg/l)	45.88	-
Iron – as Fe(µg/l)	171	-
Cobalt dissolved – as Co (µg/l)	5.74	-
Cobalt - as Co (µg/l)	5.67	-
Aluminium – as Al (µg/l)	190.47	-
Antimony – as Sb (µg/l)	<1	-
Boron – as B (µg/l)	<100	-
Titanium – as Ti (µg/l)	4	-
Vanadium – as V (µg/l)	<2	-
Barium – as Ba (µg/l)	<10	-
Copper dissolved – as Cu (µg/l)	54.54	<2.5
Copper – as Cu (µg/l)	72.05	-
Zinc – as Zn (µg/l)	563.35	10.47
Manganese dissolved – as Mn (µg/l)	43.88	-
Iron dissolved – as Fe (µg/l)	69.46	-
Nickel – as Ni (µg/l)	11.73	-
Uranium – as U (µg/l)	<1	-
Molybdenum – as Mo (µg/l)	<3	-
Beryllium – as Be (µg/l)	<1	-
Oxygen dissolved (instrumental) – as % saturation (%)	93.53	96
Oxygen dissolved (instrumental – *in situ*) as O (mg/l)	10.15	10.51

Table D.2: Macroinvertebrate species counts from the contaminated site (9) at Twelveheads, from a one-minute kick sample (Hirst *et al.* 2002). Bold text = taxonomic order.

Taxon Name	Count	Percentage of total
Diptera		
Chironomidae	7	20.6
Plecoptera		
Chloroperla torrentium	3	8.8
Leuctra hippopus	21	61.8
Tricoptera		
Hydropsyche siltalai	3	8.8

Table D.3: Families present at the contaminated site (9) at Twelveheads on 02/03/2000 (Environment Agency). Abundance categories were as follows: A = 1-9 individuals; B = 10-99 individuals; C = 100-999 individuals (Murray-Bligh *et al.* 1997).

Taxon Name	Abundance
PLANARIIDAE	A4
SPHAERIIDAE (PEA MUSSELS)	A3
BAETIDAE	A5
LEUCTRIDAE	C
CORDULEGASTERIDAE	A4
DYTISCIDAE	A2
DRYOPIDAE	A1
POLYCENTROPODIDAE	A3
HYDROPSYCHIDAE	A3
LIMNEPHILIDAE	A9
LEPTOCERIDAE	A1
TIPULIDAE	A2
CHIRONOMIDAE	C

Table D.4: Abiotic and biotic comparisons between the contaminated site (9) at Twelveheads and the reference site (10) at Trenarth Bridge, from samples taken in spring 1995 (Environment Agency).

Feature	Contaminated site 9	Reference site 10
Altitude (m above sea level)	23	10
Distance from source (km)	4.62	3.77
Slope (m/km)	12.5	20.0
Width (m)	0.5	2.1
Depth (cm)	150	18
Boulders (%)	10	40
Pebbles (%)	25	45
Sand (%)	45	10
Silt (%)	20	5
Alkalinity	15.7	21.8
Number of families present	8	29

Table D.5: Families present at the reference site (10) at Trenarth Bridge on the Porth Navas Stream on 02/03/2000 and 21/09/2000 (Environment Agency). Abundance categories were as follows: A = 1-9 individuals; B = 10-99 individuals; C = 100-999 individuals (Murray-Bligh *et al.* 1997).

TAXON NAME	02/03/2000	21/09/2000
PLANARIIDAE	A9	B
HYDROBIIDAE	B	B
ANCYLIDAE	A6	B
SPHAERIIDAE (PEA MUSSELS)		A1
OLIGOCHAETA	B	B
HYDRACARINA		A2
OSTRACODA		A2
BAETIDAE	B	B
HEPTAGENIIDAE	B	A1
EPHEMERELLIDAE		A1
TAENIOPTERYGIDAE	A3	
NEMOURIDAE	A8	B
LEUCTRIDAE	B	C
PERLODIDAE	A9	
CHLOROPERLIDAE	B	A2
CALOPTERYGIDAE		A3
CORDULEGASTERIDAE	A4	A9
GYRINIDAE	A3	A1
HYDROPHILIDAE	A1	B
SCIRTIDAE	A2	B
ELMIDAE	B	B
RHYACOPHILIDAE	A5	A5
PHILOPOTAMIDAE	A3	B
POLYCENTROPODIDAE		A1
HYDROPSYCHIDAE	B	B
LEPIDOSTOMATIDAE	A5	A3
LIMNEPHILIDAE	A3	A1
GOERIDAE	B	B
SERICOSTOMATIDAE	A4	A7
ODONTOCERIDAE		A4
TIPULIDAE	A3	A9
PSYCHODIDAE		A4
DIXIDAE		B
CERATOPOGONIDAE		A1
SIMULIIDAE	A3	B
CHIRONOMIDAE	A3	B
RHAGIONIDAE	A1	

D.2: Dossier for site pair 6 (sites 11 & 12).

Contaminated site (11):	*Crow's Nest*
Catchment:	Seaton
River:	Seaton
National Grid Reference:	SX26406938
Site code:	CC-CN

Summary of site

- The site was highly contaminated with heavy metals, with copper and zinc being highest in concentration (Table D.6).
- Ecological data from the Environment Agency and from previous studies (Hirst *et al.* 2002) indicate that the macroinvertebrate community was sparse and few families have been recorded (Tables D.7 and D.8).

Reference site (12):	*Harrowbridge*
Catchment:	Fowey
River:	Fowey
National Grid Reference:	SX20667440
Site code:	CR-HB

The reference site was on the nearby River Fowey, less than 8 km away from the contaminated site at Crow's Nest. Chemical data indicate that the site had a small amount of zinc in it, 5.2 μg/l (Table D.6), but there were no perceived stressors at the site (Martin & Walley 2000). After these considerations, the sites were paired based on proximity, alkalinity, and broadly matching abiotic features (Tables D.6, D.9). The reference site had good diversity of macroinvertebrate families present (Table D.10). The following shredder families were present at the reference site, indicating that there was the potential for leaf processing: Nemouridae, Leuctridae, Limnephilidae, Sericostomatidae, Odontoceridae (Table D.10).

Table D.6: Mean chemical data for the contaminated site (11) at Crow's Nest and reference site (12) at Harrowbridge. The sites were sampled monthly between January 2003 and May 2004 (Environment Agency). BOD ATU refers to biological oxygen demand measured when nitrification in the sample is suppressed by adding allylthiourea (ATU).

Factor	Contaminated site 11	Reference site 12
pH	6.50	6.73
Water temp (°C)	11.11	10.49
BOD ATU as O_2 (mg/l)	1.23	1.19
Ammonia as N (mg/l)	0.062	0.036
Nitrogen total oxidised – as N (mg/l)	1.21	0.6
Nitrate – as N (mg/l)	1.20	0.593
Nitrite – as N (mg/l)	0.009	<0.008
Ammonia as un-ionised (calculated mg/l)	5.17×10^{-5}	$<2e^{-005}$
Hardness Total as $CaCO_3$ (mg/l)	19.34	12.83
Alkalinity pH 4.5 – as $CaCO_3$ (mg/l)	<20	<20
Orthophosphate – as P (mg/l)	0.35	<0.02
Potassium as K (mg/l)	1.62	1.09
Magnesium as Mg (mg/l)	1.57	1.16
Calcium – as Ca (mg/l)	5.15	3.23
Nitrogen total inorganic (calculated) (µg/l)	1.25	0.63
Copper dissolved – as Cu (µg/l)	578.81	<2.5
Zinc – as Zn (µg/l)	251.5	5.2
Oxygen dissolved (instrumental) – as % saturation (%)	96.5	95.44
Oxygen dissolved (instrumental – *in situ*) as O (mg/l)	10.59	10.68

Table D.7: Macroinvertebrate species present at contaminated site (11) at Crow's Nest, from one-minute kick samples (Hirst *et al.* 2002).

Taxon Name	Count	Percentage of total
Amphinemura sulcicollis	1	50
Plectrocnemia conspersa	1	50

Table D.8: Families present at the contaminated site (11) at Crow's Nest on the River Seaton on 12/03/2002 and 23/09/2002 (Environment Agency). Abundance categories were as follows: A = 1-9 individuals; B = 10-99 individuals (Murray-Bligh *et al.* 1997).

Taxon Name	12/03/2002	23/09/2002
PLANARIIDAE	A	B
OLIGOCHAETA	A	
HYDRACARINA		A
NEMOURIDAE	B	
PERLODIDAE	A	
VELIIDAE		A
RHYACOPHILIDAE	A	A
POLYCENTROPODIDAE	A	
CERATOPOGONIDAE	A	
CHIRONOMIDAE	B	B
EMPIDIDAE	B	B

Table D.9: Abiotic and biotic comparisons between the contaminated site (11) at Crow's Nest and the reference site (12) at Harrowbridge, from samples taken in spring 1995 (Environment Agency).

Feature	Contaminated site 11	Reference site 12
Altitude (m above sea level)	95	210
Distance from source (km)	1.8	8.8
Slope (m/km)	40	8.89
Width (m)	1.4	6.1
Depth (cm)	16.6	17.3
Boulders (%)	55	15
Pebbles (%)	30	60
Sand (%)	10	20
Silt (%)	5	5
Alkalinity	10.5	10.9
Number of families present	6	24

Table D.10: Species present at the reference site (12) at Harrowbridge on the river Fowey on 26/03/2003 and 02/09/2003 (Environment Agency). Abundance categories were as follows: A= 1-9 individuals; B = 10-99 individuals; C = 100-999 individuals (Murray-Bligh *et al.* 1997).

TAXON NAME	26/03/2003	02/09/2003
PLANARIIDAE		
Polycelis felina	B	B
Crenobia alpina	B	A
ANCYLIDAE		
Ancylus fluviatilis	A	A
Pisidium	A	A
OLIGOCHAETA	C	B
HYDRACARINA	A	A
BAETIDAE		
Baetis	B	A
Baetis niger	B	
Baetis rhodani	B	A
Baetis vernus	B	
HEPTAGENIIDAE		A
Rhithrogena	A	
Rhithrogena semicolorata	A	
Heptagenia	B	
EPHEMERELLIDAE		
Ephemerella ignita		A
TAENIOPTERYGIDAE		
Brachyptera risi	A	
NEMOURIDAE		
Protonemura meyeri	B	C
Amphinemura sulcicollis	A	
LEUCTRIDAE		
Leuctra	B	B
Leuctra fusca	B	B
Leuctra inermis	B	
PERLODIDAE	B	
Perlodes microcephala	A	A
Isoperla grammatica	B	A
CHLOROPERLIDAE		
Chloroperla	A	
Chloroperla torrentium	A	A
CORDULEGASTERIDAE		
Cordulegaster boltonii		A
GYRINIDAE		
Orectochilus villosus	A	A
HYDRAENIDAE		
Hydraena gracilis	A	A
ELMIDAE		
Elmis aenea	B	B
Limnius volckmari	B	C
Oulimnius	B	B
Oulimnius tuberculatus	A	B
RHYACOPHILIDAE		

Table D.10 continued:

TAXON NAME	26/03/2003	02/09/2003
Rhyacophila	A	B
Rhyacophila dorsalis	A	B
Rhyacophila munda	A	A
HYDROPTILIDAE		
Ithytrichia	C	
POLYCENTROPODIDAE		
Plectrocnemia	A	
Polycentropus	A	A
Polycentropus flavomaculatus		A
HYDROPSYCHIDAE		
Hydropsyche	B	A
Hydropsyche pellucidula		A
Hydropsyche siltalai	C	B
BRACHYCENTRIDAE		
Brachycentrus subnubilus		A
LEPIDOSTOMATIDAE	C	A
Lepidostoma hirtum	C	
LIMNEPHILIDAE	B	A
Drusus annulatus	B	
Halesus radiatus	A	
Chaetopteryx villosa	A	A
GOERIDAE		
Silo	A	A
Silo pallipes	B	A
SERICOSTOMATIDAE		
Sericostoma personatum	B	B
ODONTOCERIDAE		
Odontocerum albicorne		A
LEPTOCERIDAE		
Mystacides		A
Oecetis testacea	A	
LIMONIINAE		
Pedicia	A	
Dicranota	A	B
LIMNOPHILA (ELOEOPHILA)	A	A
SIMULIIDAE	A	B
CHIRONOMIDAE	C	C
RHAGIONIDAE		
Atherix marginata	A	B
EMPIDIDAE	A	B

D.3: Dossier for site pair 7 (sites 13 & 14).

Contaminated site (13):	*Porthtowan Stream*
Catchment:	Porthtowan
River:	Porthtowan Stream
National Grid Reference:	SW69544740
Site code:	CC-PTB

Summary of site

- The site was highly contaminated with heavy metals, with zinc being highest in concentration, but other metals (e.g. copper) were also present (Table D.11).
- Ecological data from the Environment Agency and from previous studies (Hirst *et al.* 2002) indicate that the macroinvertebrate community was sparse and few families have been recorded (Tables D.12. & D.13).

Reference site (14):	*Polwheveral Bridge*
Catchment:	Helford
River:	Lestraines River
National Grid Reference:	SW73772900
Site code:	CR-PBL

The reference site was on the South coast, in the Helford River Catchment, approximately 18 km away from the contaminated site at Porthtowan. Chemical data indicate that the site had a small amount of zinc in it, 10.46 µg/l (Table D.11), but there were no perceived stressors at the site (Martin & Walley 2000). After these considerations, the sites were paired based on proximity, alkalinity, and broadly matching abiotic features (Table D.14). The reference site had good diversity of macroinvertebrates present (Table D.15). The following shredder families were present at the reference site, indicating that there was the potential for leaf processing: Nemouridae, Leuctridae, Limnephilidae, Sericostomatidae, Leptoceridae, Tipulidae (Table D.15).

Table D.11: Mean chemical data for the contantaminated site (13) at Porthtowan and the reference site (14) at Polwheveral Bridge. The stream was sampled monthly between January 2003 and May 2004 (Environment Agency). BOD ATU refers to biological oxygen demand measured when nitrification in the sample is suppressed by adding allylthiourea (ATU).

	Contaminated site 13	Reference site 14
Factor	**Mean**	**Mean**
Lead as Pb (µg/l)	2.7	-
Lead dissolved as Pb (µg/l)	2.7	-
pH	6.69	7.21
Water temp (°C)	12.09	10.47
BOD ATU as O_2 (mg/l)	1.1	1.37
Mercury as Hg (µg/l)	0.03	-
Cadmium as Cd (µg/l)	3.37	-
Ammonia as N (mg/l)	0.07	0.12
Nitrogen total oxidised – as N (mg/l)	7.29	5.24
Nitrate – as N (mg/l)	7.27	5.22
Nitrite – as N (mg/l)	0.03	0.02
Ammonia as un-ionised (calculated mg/l)	0.000099	0.00053
Hardness Total as $CaCO_3$ (mg/l)	107.53	45.45
Alkalinity pH 4.5 – as $CaCO_3$ (mg/l)	28	16
Orthophosphate – as P (mg/l)	0.36	0.12
Potassium as K (mg/l)	3.66	3.46
Magnesium as Mg (mg/l)	11.41	3.37
Calcium – as Ca (mg/l)	25.25	12.64
Aluminium dissolved as Al (mg/l)	0.091	-
Tellorium – as Te (mg/l)	<0.001	-
Chromium – as Cr (µg/l)	<0.5	-
Chromium dissolved – as Cr (µg/l)	0.8	-
Nickel dissolved - as Ni (µg/l)	25.09	-
Nitrogen total inorganic (calculated) (µg/l)	7.34	5.35
Silver – as Ag (µg/l)	<1	-
Tin – as Sn (µg/l)	<2.5	-
Arsenic dissolved – as As (µg/l)	5.89	-
Arsenic dissolved – as As (µg/l)	7.59	-
Selenium - as Se (µg/l)	<1	-
Manganese - as Mn (µg/l)	232.88	-
Iron – as Fe(µg/l)	172.94	-
Cobalt dissolved – as Co (µg/l)	15.19	-
Cobalt -as Co (µg/l)	16.68	-
Aluminium – as Al (µg/l)	171.13	-
Antimony – as Sb (µg/l)	<1	-
Boron – as B (µg/l)	<100	-
Titanium – as Ti (µg/l)	2	
Vanadium – as V (µg/l)	<2	-
Barium – as Ba (µg/l)	13.6	-
Thallium – total as Tl (µg/l)	1.3	-
Copper dissolved – as Cu (µg/l)	320.75	16.21
Copper – as Cu (µg/l)	362.25	-
Zinc – as Zn (µg/l)	1745	10.39
Manganese dissolved – as Mn (µg/l)	214.06	-
Iron dissolved – as Fe (µg/l)	110.25	-
Nickel – as Ni (µg/l)	26.33	-
Uranium – as U (µg/l)	<1	-
Molybdenum – as Mo (µg/l)	<3	-
Beryllium – as Be (µg/l)	<1	-
Oxygen dissolved (instrumental) – as % saturation (%)	92.44	96.47
Oxygen dissolved (instrumental – *in situ*) as O (mg/l)	9.93	10.79

Table D.12: Macroinvertebrate species present at the contaminated site (13) at Porthtowan, from one-minute kick samples (Hirst *et al.* 2002).

Taxon Name	Count	%
Limnius volckmari (adults)	1	0.35
Chironomidae	247	85.76
Pupae	12	4.17
Empididae	2	0.69
Simuliidae	17	5.90
Baetis rhodani	8	2.78
Plectrocnemia conspersa	1	0.35

Table D.13: Families present at the contaminated site (13) at Porthtowan on 22/03/2002 and 16/09/2002 (Environment Agency). Abundance categories were as follows: A = 1-9 individuals; B = 10-99 individuals; C = 100-999 individuals (Murray-Bligh *et al.* 1997)

Taxon Name	22/03/2002	16/09/2002
PLANARIIDAE	C	C
SPHAERIIDAE (PEA MUSSELS)	B	
OLIGOCHAETA	A	
HYDRACARINA	A	B
ASELLIDAE	A	
BAETIDAE	C	C
LEUCTRIDAE	A	
RHYACOPHILIDAE	A	A
TIPULIDAE	A	A
CERATOPOGONIDAE	A	
SIMULIIDAE	A	
CHIRONOMIDAE	C	C
EMPIDIDAE	B	
EPHYDRIDAE		A
MUSCIDAE	A	

Table D.14: Abiotic and biotic comparisons between contaminated site (13) at Porthtowan and reference site (14) at Polwheveral Bridge, from samples taken in spring 1995 (Environment Agency).

Feature	Contaminated site 13	Reference site 14
Altitude (m above sea level)	25	33
Distance from source (km)	5.85	6.02
Slope (m/km)	6.4	7.1
Width (m)	4.1	4.8
Depth (cm)	36	33.6
Boulders (%)	35	30
Pebbles (%)	55	55
Sand (%)	5	10
Silt (%)	5	5
Alkalinity	29.3	15
Number of families present	12	23

Table D.15: Families present at the reference site (14) at Polwheveral Bridge on the Lestraines River on 17/03/2003 and 17/10/2003 (Environment Agency). Abundance categories were as follows: A= 1-9 individuals; B = 10-99 individuals; C = 100-999 individuals (Murray-Bligh *et al.* 1997).

TAXON NAME	17/03/2003	17/10/2003
PLANARIIDAE	B	B
HYDROBIIDAE	A	
ANCYLIDAE	A	B
SPHAERIIDAE (PEA MUSSELS)	A	
OLIGOCHAETA	B	B
HYDRACARINA		A
GAMMARIDAE	A	A
BAETIDAE	C	B
EPHEMERELLIDAE	A	
TAENIOPTERYGIDAE	C	
NEMOURIDAE	B	A
LEUCTRIDAE	A	A
CALOPTERYGIDAE		A
CORDULEGASTERIDAE	A	A
HYDROPHILIDAE		A
HYDRAENIDAE	A	
ELMIDAE	C	C
RHYACOPHILIDAE	B	B
GLOSSOSOMATIDAE	B	
HYDROPSYCHIDAE	B	B
LEPIDOSTOMATIDAE	C	B
LIMNEPHILIDAE	A	A
GOERIDAE	A	A
BERAEIDAE	A	
SERICOSTOMATIDAE	B	B
LEPTOCERIDAE	B	B
TIPULIDAE	B	
PSYCHODIDAE	A	A
SIMULIIDAE	B	B
CHIRONOMIDAE	B	B
EMPIDIDAE	A	A

D.4: Dossier for site pair 8 (sites 15 & 16).

Godolphin Stream: Contaminated site (15)

Catchment:	Hayle
National Grid Reference:	SW60433208
Site code:	CC-GS

Summary of site

- Godolphin Stream was highly contaminated with heavy metals, with zinc being the highest in concentration (Table D.16).
- Ecological data from the Environment Agency indicate that the macroinvertebrate community was more diverse than the other contaminated sites (Table D.17).

Reference site (16):	***Tregolls Bridge***
Catchment:	Fal
River:	Kennal River
National Grid Reference:	SW72953605
Site code:	CR-KTB

The reference site was on the river Kennal, approximately 13 km away from the contaminated site on the Godolphin Stream. The Environment Agency do not monitor the River Kennal at Tregolls Bridge any more, but they do monitor a site further downstream called Ponsanooth Gauging Station, which had a small amount of zinc in it, 12.22 µg/l (Table D.16). There was no obvious mining activity upstream of the reference site (Martin & Walley 2000). The sites were paired based on proximity, alkalinity, and broadly matching abiotic features (Table D.17). The reference site had good diversity of macroinvertebrates present (Table D.18). The following shredder families were present at the reference site, indicating that there was the potential for leaf processing: Nemouridae, Leuctridae, Limnephilidae, Sericostomatidae, Leptoceridae, Tipulidae (Table D.18).

Table D.16: Mean chemical data for the contaminated site (13) on Godolphin stream and a site downstream of the reference site (14) on the River Kennal. The streams were sampled monthly between January 2003 and May 2004 (Environment Agency). BOD ATU refers to biological oxygen demand measured when nitrification in the sample is suppressed by adding allylthiourea (ATU).

	Contaminated site 15	D/S of the reference site 16
Factor	**Mean**	**Mean**
pH	7.02	7.13
Water temp (°C)	11.71	11.48
BOD ATU as O2 (mg/l)	1.66	1.22
Ammonia as N (mg/l)	0.21	0.04
Nitrogen total oxidised – as N (mg/l)	5.79	4.42
Nitrate – as N (mg/l)	5.75	4.41
Nitrite – as N (mg/l)	0.039	0.012
Ammonia as un-ionised (calculated mg/l)	0.00065	0.0000875
Hardness Total as $CaCO_3$ (mg/l)	72.58	40.83
Alkalinity pH 4.5 – as $CaCO_3$ (mg/l)	23	11.53
Orthophosphate – as P (mg/l)	0.044	0.023
Potassium as K (mg/l)	4.87	3.046
Magnesium as Mg (mg/l)	7.43	11.21
Calcium – as Ca (mg/l)	16.83	11.21
Nitrogen total inorganic (calculated) (µg/l)	5.99	4.45
Copper dissolved – as Cu (µg/l)	106.95	3.275
Zinc – as Zn (µg/l)	442.71	12.22
Oxygen dissolved (instrumental) – as % saturation (%)	91.47	95.5
Oxygen dissolved (instrumental – *in situ*) as O (mg/l)	9.92	10.436

Table D.17: Abiotic and biotic comparisons between contaminated site (15) at Godolphin Stream and reference site (16) at Tregolls Bridge, from samples taken in spring 1995 (Environment Agency).

Feature	Contaminated site 15	Reference site 16
Altitude (m above sea level)	38	120
Distance from source (km)	1.15	5.6
Slope (m/km)	9.52	9.1
Width (m)	1.5	3.3
Depth (cm)	38.6	32.3
Boulders (%)	5	20
Pebbles (%)	40	50
Sand (%)	30	20
Silt (%)	25	10
Alkalinity	19	12.8
Number of families present	7	25

Table D.18: Families present at contaminated site (15) on Godolphin Stream on 22/03/2002 and 16/09/2002 and reference site (16) at Tregolls Bridge on the River Kennal (Environment Agency). Abundance categories were as follows: A = 1-9 individuals; B = 10-99 individuals; C=100-999 individuals (Murray-Bligh *et al.* 1997).

	Contaminated site 15		Reference site 16	
TAXON NAME	**05/09/2000**	**08/03/2000**	**01/03/2000**	**12/07/2000**
PLANARIIDAE	A1	A1	B	B
HYDROBIIDAE			A4	A2
ANCYLIDAE			A6	B
SPHAERIIDAE (PEA MUSSELS)		A1	A3	A1
OLIGOCHAETA		A2	B	B
HYDRACARINA	A1		A3	B
OSTRACODA				A1
GAMMARIDAE			C	C
BAETIDAE			B	B
HEPTAGENIIDAE	C	A6	A1	A1
EPHEMERELLIDAE				B
NEMOURIDAE				B
LEUCTRIDAE			B	B
CHLOROPERLIDAE	A4	B	A2	A1
CALOPTERYGIDAE		A6		
CORDULEGASTERIDAE	A3			
DYTISCIDAE	A2	A4		
GYRINIDAE			A2	
HYDROPHILIDAE			A5	B
SCIRTIDAE			A3	A1
ELMIDAE	A8		B	B
RHYACOPHILIDAE			B	A6
HYDROPTILIDAE			B	
PHILOPOTAMIDAE				B
POLYCENTROPODIDAE			A2	A1
HYDROPSYCHIDAE			B	B
LEPIDOSTOMATIDAE		A1	A1	A1
LIMNEPHILIDAE			B	
GOERIDAE			A4	A1
BERAEIDAE	B	B	A1	
SERICOSTOMATIDAE			A6	B
LEPTOCERIDAE	A1			
DIPTERA	B	B		
TIPULIDAE			B	B
PSYCHODIDAE	A1	A6		
CERATOPOGONIDAE				A1
SIMULIIDAE			B	A3
CHIRONOMIDAE	B	B	C	B
EMPIDIDAE				A2

D.5: Dossier for site pair 9 (sites 17 & 18).

Contaminated site (17):	***East Wheal Rose Bridge***
Catchment:	Gannel
Stream:	East Wheal Rose Stream
National Grid Reference:	SW834552
Site code:	CC-EWR

Summary of site

- East Wheal Rose Stream was highly contaminated with heavy metals, zinc being the highest in concentration (Table D.19).
- Ecological data from the Environment Agency and from previous studies (Hirst *et al.* 2002) indicate that the macroinvertebrate community was sparse and few families have been recorded (Table D.20).

Reference site (18):	***Rosecliston***
Catchment:	Gannel
Stream:	Newlyn East Stream
National Grid Reference:	SW81715877
Site code:	CR-NES

The reference site was on the nearby Newlyn East Stream, approximately 4 km away from the contaminated site at East Wheal Rose Bridge. The Newlyn East Stream flows into the Gannel less than 1 km upstream of the reference site location. Chemical data indicate that the site had a small amount of zinc in it, 8.68 µg/l, but there were no perceived stressors at the site (Table D.19). After these considerations the sites were paired based on proximity, and broadly matching abiotic features (Table D.21). There was no information available on the macroinvertebrate community at the reference site.

Table D.19: Mean chemical data for the contaminated site (17) at East Wheal Rose stream site and the reference site (18) at East Wheal Rose Bridge. The stream was sampled monthly between January 2003 and May 2004 (Environment Agency). BOD ATU refers to biological oxygen demand measured when nitrification in the sample is suppressed by adding allylthiourea (ATU).

	Contaminated site 17	Reference site 18
Factor	**Mean**	**Mean**
pH	6.34	7.55
Water temp (°C)	11.69	11.68
BOD ATU as O_2 (mg/l)	1.18	1.41
Ammonia as N (mg/l)	0.0395	0.08
Nitrogen total oxidised – as N (mg/l)	2.13	6.11
Nitrate – as N (mg/l)	2.12	6.10
Nitrite – as N (mg/l)	0.004	0.014
Ammonia as un-ionised (calculated mg/l)	0.00022	0.0011
Hardness Total as $CaCO_3$ (mg/l)	49.12	132.83333
Alkalinity pH 4.5 – as $CaCO_3$ (mg/l)	35	84.75
Orthophosphate – as P (mg/l)	0.06	0.074
Potassium as K (mg/l)	1.29	2.79
Magnesium as Mg (mg/l)	6.95	11.73
Calcium – as Ca (mg/l)	8.23	33.81
Nitrogen total inorganic (calculated) (µg/l)	2.16	6.15
Copper dissolved – as Cu (µg/l)	18.71	<2.5
Zinc – as Zn (µg/l)	739.47	8.68
Oxygen dissolved (instrumental) – as % saturation (%)	94.18	95.14
Oxygen dissolved (instrumental – *in situ*) as O (mg/l)	10.23	10.36

Table D.20: Biological family-level macroinvertebrates present from a one-minute kick sample at the contaminated site (17) at East Wheal Rose Bridge (Hirst *et al.* 2002).

Taxon name	Count	Percentage of total
Annelida		
Oligochaeta	1	5.26
Coleoptera		
Elmis aenea (larvae)	1	5.26
Hydraena gracilis	1	5.26
Collembola	1	5.26
Diptera		
Chironomidae	2	10.53
Tipulidae	1	5.26
Emphemeroptera		
Baetis muticus	2	10.53
Baetis rhodani	2	10.53
Plecoptera		
Chloroperla torrentium	3	15.79
Leuctra hippopus	3	15.79
Leuctra nigra	1	5.26
Polycentropodidae	1	5.26

Table D.21: Abiotic and biotic comparisons between the contaminated site (17) at East Wheal Rose Bridge and reference site (18) at Rosecliston, from samples taken in spring 1995 (Environment Agency).

Feature	Contaminated site 17	Reference site 18
Altitude (m above sea level)	51	30
Distance from source (km)	1	3.62
Slope (m/km)	16	19.1
Width (m)	1.7	2.1
Depth (cm)	23.3	14.3
Boulders (%)	30	40
Pebbles (%)	50	50
Sand (%)	10	10
Silt (%)	10	0
Alkalinity	15.7	72.1
Number of families present	23	31

D.6: Dossier for site pair 10 (sites 19 & 20).

Contaminated site (19):	*Haye Farm*
Catchment:	Lynher
Stream:	Kelly Stream
National Grid Reference:	SW346701
Site code:	CC-HF

Summary of site

- The Kelly Stream was contaminated with heavy metals, zinc being the highest in concentration (Hirst *et al.* 2002).
- The Environment Agency monitor the stream downstream at Cadderpit (SW 337 686).
- Macroinvertebrate data from Hirst *et al*'s study (2002) show that there were 29 families of macroinvertebrates present in the stream (Table D.23)

Reference site (20):	*Trebartha Road Bridge*
Catchment:	Lynher
River:	Lynher
National Grid Reference:	SX26297782
Site code:	CR-TRB

The reference site was on the nearby River Lynher, approximately 11 km away from the contaminated site at Haye Farm. Chemical data indicate that the site had a small amount of zinc in it (Table D.22), but there were no perceived stressors at the site (Martin & Walley 2000). After these considerations, the sites were paired based on proximity and broadly matching abiotic features (Table D.24). Table D.25 shows that the macroinvertebrate community at Trebartha was taxonomically more diverse than the contaminated site on the Kelly Stream. The following shredder families were present at the reference site, indicating that there was the potential for leaf processing: Nemouridae, Leuctridae, Limnephilidae, Sericostomatidae, Odontoceridae, Leptoceridae, Tipulidae (Table D.25).

Table D.22: Mean chemical data for Cadderpit House, an EA monitored site downstream from the contaminated site (19) at Haye Farm and for the reference site (20) at Trebartha Road Bridge. The stream was sampled monthly between January 2003 and May 2004 (Environment Agency). BOD ATU refers to biological oxygen demand measured when nitrification in the sample is suppressed by adding allylthiourea (ATU).

	D/s of Contaminated site 19	Reference site 20
Factor	**Mean**	**Mean**
pH	7.35	7.04
Water temp (°C)	11.96	11.21
BOD ATU as O_2 (mg/l)	2.11	1.19
Ammonia as N (mg/l)	0.31	0.05
Nitrogen total oxidised – as N (mg/l)	4.49	2.79
Nitrate – as N (mg/l)	4.41	2.78
Nitrite – as N (mg/l)	0.09	0.0079
Ammonia as un-ionised (calculated mg/l)	0.0016	0.00018
Hardness Total as $CaCO_3$ (mg/l)	59.88	35.73
Alkalinity pH 4.5 – as $CaCO_3$ (mg/l)	55.03	18.67
Orthophosphate – as P (mg/l)	0.68	0.028
Potassium as K (mg/l)	6.014	1.64
Magnesium as Mg (mg/l)	5.04	3.28
Calcium – as Ca (mg/l)	15.68	8.9
Nitrogen total inorganic (calculated) (µg/l)	4.80	2.82
Copper dissolved – as Cu (µg/l)	8.36	6.1
Zinc – as Zn (µg/l)	231.69	15.47
Oxygen dissolved (instrumental) – as % saturation (%)	93.02	96.11
Oxygen dissolved (instrumental – *in situ*) as O (mg/l)	10.04	10.56

Table D.23: Macroinvertebrate families and species present from a one-minute kick sample taken at the contaminated site (19) at Haye Farm (Hirst *et al.* 2002).

Taxon Name	Count	Percentage of total
Coleoptera		
Limnius volckmari (larvae)	1	0.22
Hydraena gracilis	1	0.22
Diptera		
Ceratopogonidae	1	0.22
Chironomidae	120	26.79
Pupae	3	0.67
Empididae	1	0.22
Simuliidae	6	1.34
Tipulidae	1	0.22
Emphemeroptera		
Baetis rhodani	94	20.98
Plecoptera		
Chloroperla torrentium	50	11.16
Chloroperla tripunctata	23	5.13
Leuctra hippopus	31	6.92
Amphinemura sulcicollis	85	18.97
Isoperla grammatica	12	2.68
Trichoptera		
Agapetus fuscipes (cased)	1	0.22
Hydropsyche siltalai	12	2.68
Limnephilidae	1	0.22
Potamophylax cingulatus	1	0.22
Rhyacophila dorsalis	3	0.67
Sericostoma personatum (cased)	1	0.22

Table D.24: Abiotic and biotic comparisons between a site downstream of the contaminated site (19) at Cadderpit and reference site (20) at Trebartha Road Bridge, from samples taken in spring 1995 (Environment Agency).

Feature	D/S of Contaminated site 19	Reference site 20
Altitude (m above sea level)	50	130
Distance from source (km)	2.6	9.12
Slope (m/km)	15.3	7.1
Width (m)	3.5	5.8
Depth (cm)	17.3	48.3
Boulders (%)	25	20
Pebbles (%)	45	45
Sand (%)	20	20
Silt (%)	10	15
Alkalinity	35.2	18.2
Number of families present	29	31

Table D.25: Biological data, species and families present at contaminated site (19) at Haye on the Kelly Stream and the reference site (20) at Trebartha Road Bridge, on the River Lynher (Environment Agency). Abundance categories were as follows: A = 1-9 individuals; B = 10-99 individuals; C=100-999 individuals (Murray-Bligh *et al.* 1997).

	Contaminated site 19		Reference site 20	
Taxon Name	**19-08-94**	**06-10-94**	**19-08-94**	**06-10-94**
Hydrobiidae			B	B
Lymnaeidae			A	
Ancylidae		A	A	A
Sphaeriidae (Pea mussels)	A	A	A	
Oligochaeta	A	A	B	B
Hydracarina	A		A	
Gammaridae			B	A
Baetidae	A	B	B	B
Heptageniidae			A	
Leptophlebiidae				
Ephemerellidae			B	
Caenidae				A
Taeniopterygidae				
Nemouridae		A	B	A
Leuctridae	B	B	C	B
Perlodidae			A	A
Perlidae			A	
Chloroperlidae	A	B	A	
Cordulegasteridae		A		
Gyrinidae	A	A		A
Hydrophilidae	B	A	A	A
Elmidae	A	A	B	B
Rhyacophilidae	A		B	B
Hydroptilidae				A
Polycentropodidae	A	A	A	B
Hydropsychidae	B	B	B	A
Brachycentridae			B	A
Lepidostomatidae	A		B	B
Limnephilidae		A	A	B
Goeridae			B	B
Sericostomatidae	A		B	A
Odontoceridae			B	
Leptoceridae		A	A	B
Tipulidae	A	A	B	B
Psychodidae	A		B	
Ceratopogonidae				
Simuliidae		A	B	B
Simulium				
Chironomidae	B	B	B	B
Rhagionidae			A	A
Atherix				
Empididae				A
Muscidae				
Planariidae	A	A	B	
No Of Taxa	**16**	**19**	**30**	**25**

Appendix E: Mean total heavy metal concentrations (Chapter 3).

Mean (SE) total heavy metal concentration collected across twenty stream sites in Chapter 3. Data are mean (+/- SE) values from 6 replicate samples taken on two separate visits to site, three weeks apart, except for sites 1-8 which were only sampled on one visit (i.e. mean from 3 replicate samples). Minimum detectable concentrations were: Mn < 0.007 mg/l, Fe < 0.01 mg/l, Pb < 0.02 mg/l, Zn < 0.004 mg/l, Cu < 0.014 mg/l, Sn < 0.011mg/l, Al < 0.08 mg/l, Ni < 0.02 mg/l, Cd < 0.5 µg/l, Cr < 0.011 mg/l. - = No data. Site 1-8 were in the Leadhills, and sites 9-20 in Cornwall. Sites in Cornwall were paired (contaminated vs. reference): odd numbered sites were contaminated sites and even numbered sites were reference sites.

Site no.	Total metals									
	Mn (mg/l)	Fe (mg/l)	Pb (mg/l)	Zn (mg/l)	Cu (mg/l)	Sn (mg/l)	Al (mg/l)	Cd (µg/l)	Ni (mg/l)	Cr (mg/l)
1	0.05 (<0.01)	0.40 (0.17)	0.36 (<0.01)	0.11 (<0.01)	<0.014 (0.00)	<0.11 (0.00)	0.10 (0.00)	4.87 (0.84)	0.02 (<0.01)	<0.011 (0.00)
2	0.02 (<0.01)	0.10 (0.04)	0.06 (<0.01)	0.09 (<0.01)	<0.014 (0.00)	<0.11 (0.00)	0.09 <0.01)	2.83 (1.70)	0.02 (<0.01)	<0.011 (0.00)
3	<0.007 (0.00)	0.03 (<0.01)	0.04 (<0.01)	0.02 (0.00)	<0.014 (0.00)	0.14 (0.03)	<0.08 (0.00)	1.42 (0.41)	0.02 (<0.01)	<0.011 (0.00)
4	*0.04 (0.02)*	*0.09 (0.03)*	*<0.02 (0.00)*	*0.004 (<0.01)*	*<0.014 (0.00)*	*<0.11 (0.00)*	*0.10 (0.00)*	*1.25 (0.58)*	*<0.02 (0.00)*	*<0.011 (0.00)*
5	<0.007 (0.00)	0.08 (<0.01)	<0.02 (0.00)	0.005 (<0.01)	<0.014 (0.00)	0.14 (0.03)	<0.08 (0.00)	6.23 (3.46)	0.02 (<0.01)	<0.011 (0.00)
6	0.01 (<0.01)	0.11 (<0.01)	0.02 (<0.01)	0.49 (0.12)	<0.014 (0.00)	2.10 (0.10)	0.10 (0.00)	2.13 (0.76)	<0.02 (0.00)	<0.011 (0.00)
7	<0.007 (0.00)	0.02 (<0.01)	<0.02 (0.00)	0.01 (<0.01)	<0.014 (0.00)	<0.11 (0.00)	<0.08 (0.00)	0.88 (0.15)	<0.02 (0.00)	<0.011 (0.00)
8	<0.007 (0.00)	0.02 (<0.01)	<0.02 (0.00)	<0.004 (0.00)	<0.014 (0.00)	0.17 (0.03)	<0.08 (0.00)	8.97 (3.80)	<0.02 (0.00)	<0.011 (0.00)
9	0.01 (<0.01)	0.07 (0.02)	0.03 (<0.01)	0.45 (0.05)	0.02 (<0.01)	<0.011 (0.00)	0.08 (<0.01)	4.63 (0.81)	0.02 (<0.01)	<0.011 (0.00)
10	0.01 (<0.01)	0.09 (0.03)	0.03 (<0.01)	0.006 (<0.01)	0.014 (0.00)	<0.011 (0.00)	0.08 (0.00)	1.48 (0.66)	<0.02 (0.00)	0.011 (<0.01)
11	0.06 (<0.01)	0.02 (0.00)	0.03 (<0.01)	0.31 (0.03)	0.71 (0.06)	<0.011 (0.00)	1.54 (1.12)	4.24 (0.22)	<0.02 (0.00)	<0.011 (0.00)
12	0.01 (<0.01)	0.08 (0.01)	0.03 (<0.01)	0.004 (0.01)	0.014 (0.00)	<0.011 (0.00)	0.08 (0.02)	0.60 (0.06)	<0.02 (0.00)	<0.011 (0.00)
13	0.26 (0.01)	0.27 (0.02)	0.03 (<0.01)	1.64 (0.11)	0.40 (<0.01)	<0.011 (0.00)	0.23 (0.02)	13.95 (1.93)	0.03 (<0.01)	<0.011 (0.00)
14	0.01 (0.00)	0.02 (0.00)	0.03 (0.01)	0.01 (<0.01)	0.04 (<0.01)	<0.011 (0.00)	0.08 (0.01)	1.13 (0.48)	<0.02 (0.00)	<0.011 (0.00)
15	0.09 (0.01)	0.18 (0.04)	0.03 (<0.01)	0.45 (0.03)	0.13 (<0.01)	<0.011 (0.00)	0.32 (0.04)	11.17 (3.35)	0.02 (<0.01)	<0.011 (0.00)
16	0.07 (0.05)	0.09 (0.04)	0.03 (<0.01)	0.01 (<0.01)	0.014 (0.00)	<0.011 (0.00)	0.11 (0.02)	1.18 (0.33)	<0.02 (0.00)	<0.011 (0.00)
17	0.81 (0.06)	0.48 (0.06)	0.35 (0.03)	0.85 (0.09)	0.02 (<0.01)	<0.011 (0.00)	0.27 (0.02)	21.12 (2.78)	0.05 (0.00)	<0.011 (0.00)
18	0.39 (0.11)	1.99 (0.54)	0.03 (<0.01)	0.03 (0.01)	0.014 (0.00)	<0.011 (0.00)	0.43 (0.11)	1.07 (0.25)	<0.02 (0.00)	<0.011 (0.00)
19	0.24 (0.02)	0.30 (0.12)	0.03 (<0.01)	0.74 (0.05)	0.03 (0.01)	<0.011 (0.00)	0.08 (0.02)	15.62 (0.98)	0.02 (<0.01)	<0.011 (0.00)
20	0.02 (<0.01)	0.12 (0.01)	0.03 (<0.01)	0.04 (<0.01)	0.014 (0.00)	<0.011 (0.00)	0.10 (0.00)	3.50 (2.82)	<0.02 (0.00)	0.012 (<0.01)

Appendix F: Species abundances for sites in the Leadhills (Chapter 3).

Data are from 10 Surber samples (i.e. ind/m^2), with the exception of site 4, which had 8 Surber samples (ind/0.8 m^2). Odd numbered sites were contaminated sites and even numbered sites were reference sites. For identity of sites see Appendix E.

	Site number							
Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Shredders								
Amphipoda								
Gammaridae spp.	0	0	0	0	0	0	26	0
Gammarus pulex	410	68	18	42	3	92	0	53
Plecoptera								
Amphinemura standfussi	0	0	0	0	2	0	7	0
Amphinemura sulcicollis	0	1	0	0	5	0	2	0
Nemurella picteti	0	0	0	1	0	0	0	0
Nemoura spp.	1	0	0	0	0	0	0	0
Leuctra inermis	317	50	36	0	13	6	16	29
Leuctra moselyi	0	3	14	0	0	51	26	24
Leuctra nigra	0	0	0	0	0	0	1	0
Tricoptera								
Phryganidae spp.	0	1	0	0	0	0	0	0
Drusus annulatus	182	0	28	0	0	0	0	0
Halesus radiatus	0	0	1	1	0	0	2	1
Limnephilidae spp 1	14	0	0	0	0	0	0	0
Limnephilidae spp 2	0	1	0	0	0	0	0	0
Limnephilidae spp 3	0	0	3	0	0	0	0	0
Potamophylax cingulatus	0	0	0	0	0	1	0	0
Chaetopteryx villosa	2	0	0	0	0	0	0	0
Sericostoma personatum	0	5	1	0	2	1	0	1
Odontocerum albicorne	0	1	0	1	5	5	1	9
Diptera								
Tipula spp.	0	0	0	0	0	1	0	0
Tipula oleracea	0	0	0	1	0	0	0	0
Non-shredders								
Oligochaeta	1	9	19	2	6	20	19	6
Hydrachnae spp.	0	0	0	0	1	0	0	0
Ephemeroptera								
Baetidae spp.	0	0	0	0	0	0	0	19
Baetis muticus	0	0	0	0	0	0	14	40
Baetis rhodani	0	0	0	0	0	0	9	16
Rhithrogena semicolorata	1	4	27	0	0	0	2	18
Ecdyonurus spp.	0	124	0	0	0	0	146	7
Electrogena lateralis	0	0	29	39	0	123	0	37
Paraleptophlebia spp.	0	0	0	0	0	0	1	0
Serratella ignita	0	660	34	0	0	423	0	208

Appendix F continued:

Taxa	Site number 1	2	3	4	5	6	7	8
Caenis rivulorum	0	200	0	0	0	3	30	0
Plecoptera								
Diura bicaudata	2	0	0	0	0	0	0	0
Isoperla grammatica	3	1	0	0	0	0	0	0
Dinocras cephalotes	0	54	0	0	0	1	8	11
Perla bipunctuata	0	21	5	0	0	5	10	0
Chloroperla torrentium	2	1	4	0	38	1	1	4
Hemiptera								
Mesovelia furcata	1	0	0	0	0	0	0	0
Coleptera								
Dytiscidae	0	0	0	0	0	14	0	2
Hygrotus novemlineatus	0	21	0	0	0	0	0	1
Oreodytes spp.	0	1	2	0	0	0	0	0
TRICOPTERA	48	3	10	0	1	0	4	8
Rhyacophila dorsalis	6	2	12	6	7	8	0	0
Rhyacophila obliterata	0	0	0	0	0	0	1	0
Glossosoma spp.	2	0	0	0	0	0	0	1
Hydroptilidae spp.	0	1	0	0	0	0	0	0
Plectrocnemia conspersa	18	13	8	0	0	4	2	1
Hydropsyche siltalai	0	4	0	4	0	7	19	13
Silo pallipes	9	1	0	0	0	1	2	15
Diptera								
Limnophila spp.	0	0	1	0	0	0	1	0
Limnophila (Eloephila) spp.	0	0	0	0	0	0	0	2
Pediciidae	3	2	0	0	2	0	1	0
Pedicia spp.	0	0	0	0	0	2	0	0
Pedicia rivosa	0	0	5	0	0	0	0	0
Dicranota bimaculata	4	2	5	5	22	12	10	5
Pericoma spp.	0	0	12	0	0	0	0	0
Ceratopogonidae spp.	0	0	0	0	0	1	0	0
Simuliidae	1	23	39	34	7	97	1	0
Chironomidae	200	286	356	279	35	1917	155	25
Orthocladiinae	0	0	0	0	0	0	1	0
Chironomini	0	0	0	0	0	0	5	0
Empididae spp.	0	0	2	0	0	0	0	0

Appendix G: Species abundances for sites in Cornwall (Chapter 3).

Species abundances and presence/absence in across twelve stream sites in Cornwall for Chapter 3. Data are from 10 Surber samples. Odd numbered sites were contaminated sites and even numbered sites were reference sites. Data are from 10 Surber samples (i.e. ind/m^2)For identity of sites see Appendix E.

	Site number											
Taxa	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Shredders												
Basommatophora												
Lymnaeidae spp.	0	2	0	0	0	0	0	0	0	0	0	0
Planorbidae spp.	0	0	0	0	0	0	2	0	0	0	0	0
Isopoda												
Asellus aquaticus	0	1	0	0	0	0	0	0	0	1	1	0
Amphipoda spp.	0	0	0	0	0	2	0	0	0	0	0	0
Gammarus pulex	0	1514	0	0	0	2	3	1258	0	0	0	18
Plecoptera												
Nemouridae spp.	0	0	0	0	0	0	0	0	0	1	0	0
Protonemura spp.	0	0	0	0	0	0	0	0	0	1	0	0
Protonemura meyeri	0	0	0	0	0	0	0	0	0	0	0	9
Protonemura montana	0	0	0	0	0	10	0	0	0	0	0	0
Amphinemura sulcicollis	0	0	0	0	0	0	0	0	0	0	8	0
Nemoura cambrica	0	0	0	0	0	0	0	0	0	2	0	0
Leuctridae spp.	0	0	0	0	0	0	0	0	0	23	0	0
Leuctra geniculata	0	0	0	2	0	0	0	0	0	70	0	2
Leuctra hippopus	0	0	0	0	0	0	0	2	0	0	0	0
Leuctra inermis	10	0	0	9	0	5	0	3	0	261	0	2

Appendix G: continued

Taxa	Site number											
	9	10	11	12	13	14	15	16	17	18	19	20
Leuctra moselyi	0	3	0	35	0	0	0	0	0	0	0	16
Leuctra nigra	0	0	0	0	0	0	0	0	0	1	0	0
Coleoptera												
Hydrophilidae spp.	0	0	0	0	0	0	0	0	0	2	0	0
Tricoptera												
Phryganeidae spp.	0	0	0	0	0	1	0	0	0	0	0	0
Brachycentrus subnubilis	0	0	0	26	0	0	0	0	0	0	0	1
Lasiocephala basalis	0	0	0	0	0	0	0	1	0	0	0	0
Lepidostoma hirtum	0	0	0	32	0	23	0	0	0	3	0	7
Limnephilidae spp 1	0	0	0	1	0	0	0	0	1	0	4	0
Limnephilidae spp 2	0	0	0	0	0	0	0	0	0	1	0	0
Limnephilidae spp 3	0	0	0	0	0	0	0	0	0	0	0	1
Limnephilidae spp 4	0	0	0	0	0	1	0	0	0	1	0	0
Drusus annulatus	0	0	0	2	0	0	0	0	0	0	0	0
Allogamus auricollis	0	0	0	0	0	0	0	0	0	0	0	1
Halesus spp.	0	1	0	0	0	0	0	0	0	0	0	0
Halesus digitatus	0	0	0	0	0	0	0	0	0	0	2	0
Halesus radiatus	0	0	0	0	0	0	0	0	0	0	4	0
Potamophylax cingulatus	0	1	0	4	0	2	0	15	0	6	0	2
Stenophylax permistus	0	0	0	0	0	0	0	0	0	1	0	0
Grammotaulius nigropunctuatus	0	0	0	0	0	0	0	1	0	0	0	0
Limnephilus lunatus	0	0	0	0	0	0	0	0	0	0	0	1
Sericostoma personatum	0	0	0	9	0	14	0	14	0	5	0	30
Odontocerum albicorne	0	1	0	0	0	0	0	0	0	12	0	8
Athripsodes albifrons	0	0	0	0	0	0	0	0	0	0	0	1

Appendix G: continued

Taxa	Site number 9	10	11	12	13	14	15	16	17	18	19	20
Lepidoptera spp.	0	0	0	0	0	0	0	0	0	2	0	0
Acentria ephemeralla	1	0	0	0	0	0	0	0	0	0	0	0
Diptera												
Tipulidae spp.	0	0	2	0	0	0	0	0	0	0	0	0
Tipulinae	3	0	0	0	2	0	0	0	0	0	0	0
Tipula	0	0	0	0	0	0	0	0	1	0	1	0
Tipula (Yamoto tipula)	0	0	1	0	0	0	0	0	0	0	0	0
Tipula subcunctans	0	0	0	0	2	0	0	0	0	0	0	0
Non-shredders												
Nematoda spp.	0	1	0	0	0	0	0	0	0	0	0	0
Mesogastropoda												
Hydrobiidae spp.	0	0	0	0	0	0	0	0	0	1	0	0
Veneroida												
Sphaeriidae spp.	0	0	0	0	0	0	0	2	0	0	0	0
Oligochaeta spp.	4	24	0	11	0	0	7	39	2	66	0	4
Hydrachnae	0	0	3	0	0	1	0	0	0	3	0	0
Collembola spp.	0	0	0	0	0	0	0	0	0	0	1	0
Proistoma spp.	0	0	0	0	1	0	0	0	0	0	0	0

Appendix G: continued

Taxa	Site number 9	10	11	12	13	14	15	16	17	18	19	20
Ephemeroptera												
Siphlonuridae spp.	0	0	0	0	0	0	0	0	0	0	0	1
Baetidae spp.	0	0	0	11	0	0	0	0	0	6	0	0
Baetis muticus	0	0	0	3	0	0	0	0	0	0	0	0
Baetis rhodani	150	85	0	0	327	12	1	0	1	18	35	32
Baetis scambus	0	0	0	3	0	0	0	0	0	0	0	0
Heptageniidae spp.	0	0	1	0	0	0	0	0	0	13	0	0
Rhithrogena semicolorata	0	0	0	0	0	0	0	0	0	1	0	0
Leptophlebiidae spp.	1	0	0	0	0	0	0	0	0	0	0	0
Ephemera danica	0	0	0	0	0	0	0	0	0	71	0	0
Serratella ignita	0	1	0	132	0	14	0	20	0	23	0	106
Isoperla grammatica	0	0	0	1	0	1	0	0	0	3	0	0
Perla bipunctuata	0	0	0	3	0	0	0	4	0	0	4	3
Chloroperla torrentium	0	0	0	7	0	1	0	0	0	1	14	0
Calopteryx virgo	0	0	0	0	0	2	0	0	0	0	0	0
Cordulegaster boltoni	14	2	0	4	0	4	1	0	0	19	2	0
Hemiptera												
Mesovelia furcata	0	0	0	0	0	0	6	0	0	0	4	0
Hydrometridae spp.	0	1	0	0	0	0	0	0	0	0	0	0
Veliidae	0	1	5	0	0	0	0	0	0	0	0	0
Microvelia	1	0	0	0	0	0	0	1	0	0	0	1
Gerris odontogaster	0	0	0	0	0	0	0	0	0	1	0	0
Notonectidae spp.	0	0	0	0	0	0	1	0	0	0	0	0
Coleoptera spp.	0	0	0	0	0	0	0	0	0	0	1	0
Dytiscidae	2	0	15	0	2	2	0	0	0	0	0	0
Hygrotus spp.	0	0	0	0	0	0	0	0	2	0	0	0

Appendix G: continued

Taxa	Site number 9	10	11	12	13	14	15	16	17	18	19	20
Hydroporus spp.	0	0	0	0	0	0	0	0	1	0	0	0
Oreodytes sanmarkii	0	0	0	0	0	0	0	0	0	0	0	2
Agabus bipustulatus	1	0	0	0	0	0	0	0	0	0	0	0
Colymbetes fuscus	0	0	0	0	0	0	0	0	0	0	0	1
Gyrinidae	1	0	0	0	0	0	2	0	0	0	0	0
Helophorus arvernicus	1	0	0	0	1	0	0	0	1	0	0	0
Hydraenidae	0	0	0	0	0	0	0	0	0	0	4	1
Hydraena gracilis	0	0	0	1	0	0	0	0	0	0	0	2
Dryopidae spp.	0	0	0	0	0	0	0	0	0	1	0	0
Elmis aenea	0	3	0	33	0	3	0	103	0	6	0	20
Limnius volckmari	0	32	0	21	0	25	0	93	0	51	1	33
Oulimnius spp.	0	0	0	0	0	0	0	0	0	0	0	4
Curculionidae spp.	0	0	0	1	0	0	0	0	0	0	0	0
Tricoptera	0	2	0	7	0	0	0	8	0	0	0	5
Rhyacophila dorsalis	0	3	0	4	0	5	0	0	0	8	0	7
Rhyacophila munda	0	0	0	0	0	0	0	0	0	0	0	9
Rhyacophila obliterata	0	0	0	1	0	0	0	0	0	0	0	0
Agapetus	0	14	0	0	0	0	0	4	0	0	0	0
Agapetus fuscipes	0	0	0	0	0	0	0	0	0	0	0	4
Psychomyiidae spp.	0	0	0	0	0	1	0	0	0	0	0	5
Polycentropodidae spp.	0	0	0	0	0	0	1	0	0	0	0	0
Plectrocnemia conspersa	0	0	3	12	3	10	0	18	12	0	3	1
Polycentropus kingi	4	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche siltalai	0	5	0	2	0	4	0	14	0	2	8	54
Diplectrona felix	0	0	0	0	0	0	0	107	0	0	0	0
Goeridae spp.	0	0	0	0	0	0	0	1	0	0	0	0
Silo pallipes	0	0	0	0	0	0	0	1	0	0	0	1

Appendix G: continued

Taxa	Site number 9	10	11	12	13	14	15	16	17	18	19	20
Silo nigricornis	0	2	0	0	0	0	0	0	0	0	0	0
Oecetis spp.	0	0	0	0	0	0	0	0	0	0	5	0
Diptera	0	0	1	17	9	47	0	7	8	4	11	8
Dicranota bimaculata	0	3	0	9	0	0	3	0	1	57	1	3
Psychodidae	0	0	0	0	0	1	0	0	0	2	0	0
Pericoma spp.	0	0	0	0	0	0	0	2	0	2	0	0
Psychoda spp.	0	0	0	0	0	0	0	0	0	3	0	0
Ptychoptera spp.	0	0	0	0	0	0	0	0	0	2	0	0
Dixa spp.	0	0	0	0	0	0	0	0	0	0	0	1
Culicidae spp.	0	0	0	0	0	0	0	0	0	5	0	0
Thaumalicidae spp.	3	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	31	1	0	0	4	2	1	0	0	2	1	0
Simuliidae	76	2	1	5	0	8	0	44	0	45	8	27
Chironomidae	322	57	124	1236	118	657	656	692	96	669	384	1060
Empididae	0	1	33	4	19	3	3	0	0	8	1	9
Hemerodromiinae spp.	0	0	0	0	0	0	0	0	0	0	0	5
Clinocera spp.	0	0	0	0	0	0	0	0	0	0	0	2
Ephydridae spp.	0	0	0	0	1	0	0	0	0	0	0	0

Appendix H: Species replacement into artificial stream mesocosms (Chapter 4).

In this appendix, animal biomass change over the course of the 21-day experimental period in the artificial stream mesocosms (community compartments: see Figure 4.3) is examined (Chapter 4).

H.1. Animal biomass at the start and end of the experiment.

By visual inspection, the mass of animals present at the end of the experiment was sometimes of the same magnitude as the mass of animals at the start of the experiment (Figure H.1), e.g. stream mesocosms: 1, 2, 5, 13: 14, 16, 21. While other mesocosms saw a large reductions in assemblage biomass over the course of the experiment including streams: 3: 6, 7, 12, 15, 19, 20, 22, 28, 29, 30.

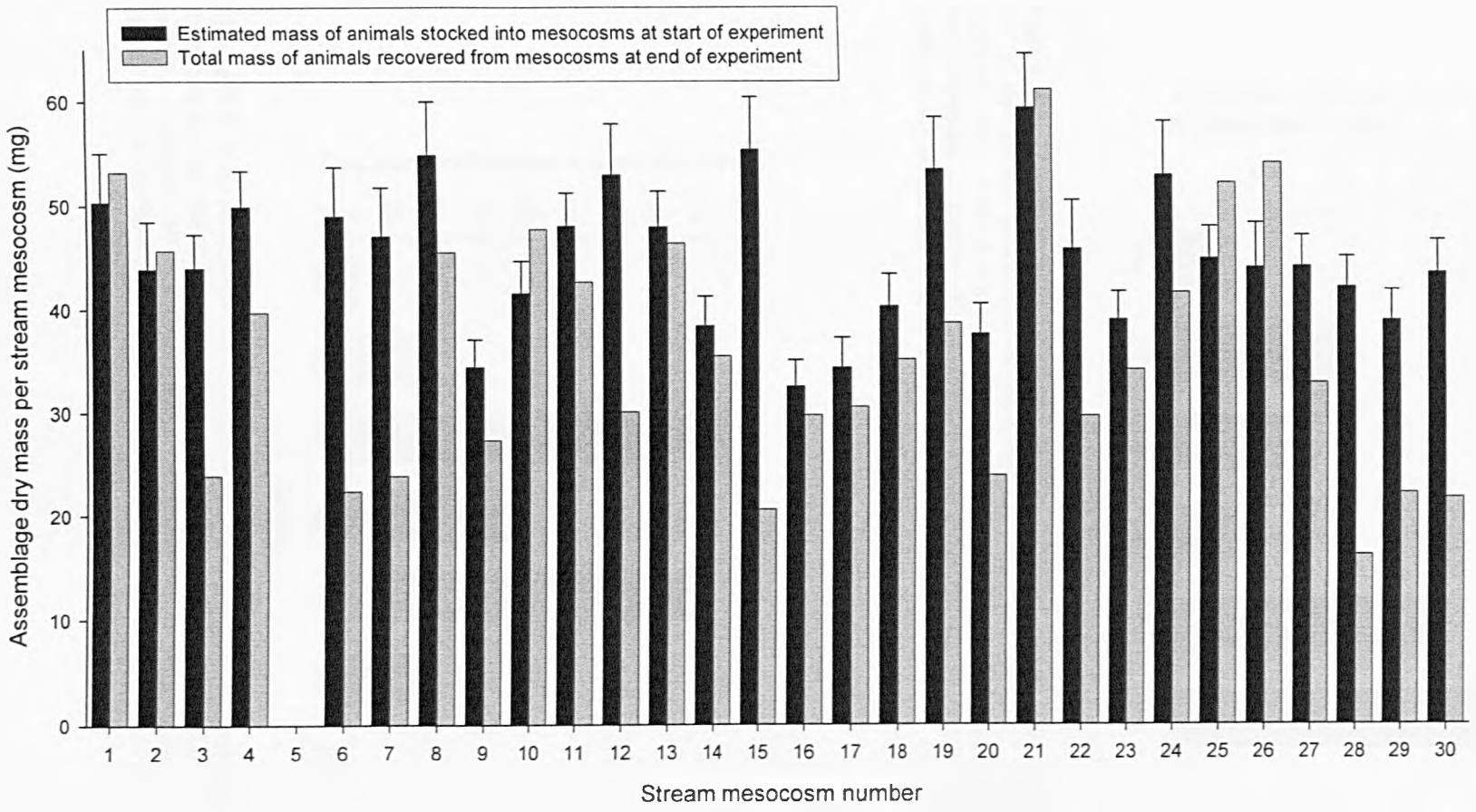

Figure H.1: Dry mass of each stream mesocosm assemblage between the start and end of the 21 day experimental period. Black bars are the estimated dry mass of each assemblage at the start of the experiment ($M_{Assemblage}$) (see Section 4.2.3.6). Error bars are +/- 1 SE. Grey bars are the actual mass of each assemblage recovered from mesocosms at the end of the experiment.

H.2. Replacement of animals into stream mesocosms.

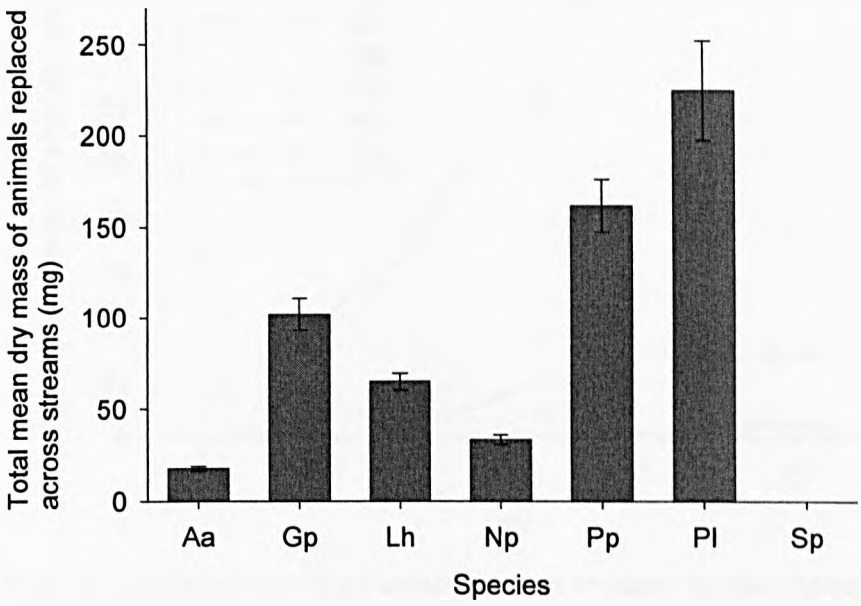

Figure H.2: Total dry mass of shredder species replaced into all stream mesocosms over the 21 day period. Calculated as the mean dry mass per species * number of animals replaced. Aa = *Asellus aquaticus*, Gp = *Gammarus pulex*; Lh = *Leuctra hippopus*, Pl = *Potomophylax latipennis*, Pp = *Protonemura praecox*, Np = *Nemurella picteti*, Sp = *Sericostoma personatum.* Error bars are +/- 1 SE.

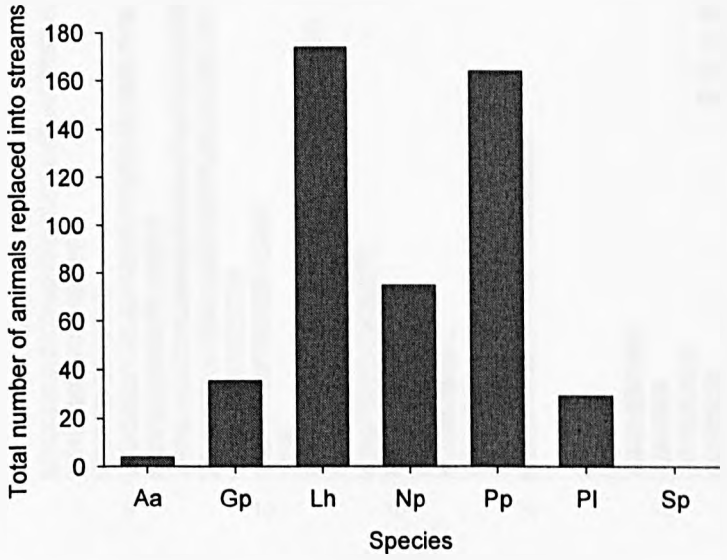

Figure H.3: Total number of individuals of each shredder species replaced across stream mesocosms over the 21 day period. Aa = *Asellus aquaticus*, Gp = *Gammarus pulex*; Lh = *Leuctra hippopus*, Pl = *Potomophylax latipennis*, Pp = *Protonemura praecox*, Np = *Nemurella picteti*, Sp = *Sericostoma personatum.* Error bars are +/- 1 SE.

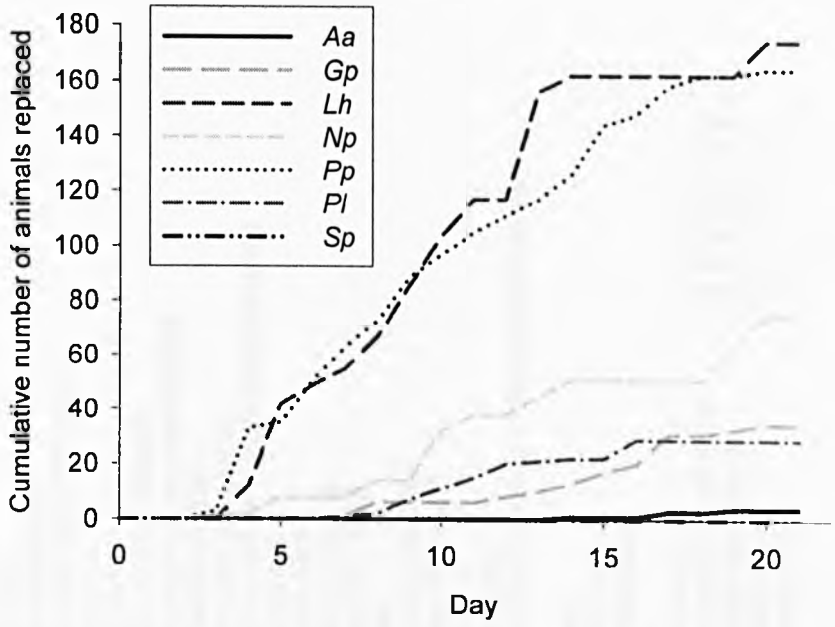

Figure H.4: Cumulative number of individuals of each shredder species replaced across stream mesocosms over the 21 day period. Aa = *Asellus aquaticus*, Gp = *Gammarus pulex*, Lh = *Leuctra hippopus*, Pl = *Potomophylax latipennis*, Pp = *Protonemura praecox*, Np = *Nemurella picteti*, Sp = *Sericostoma personatum*.

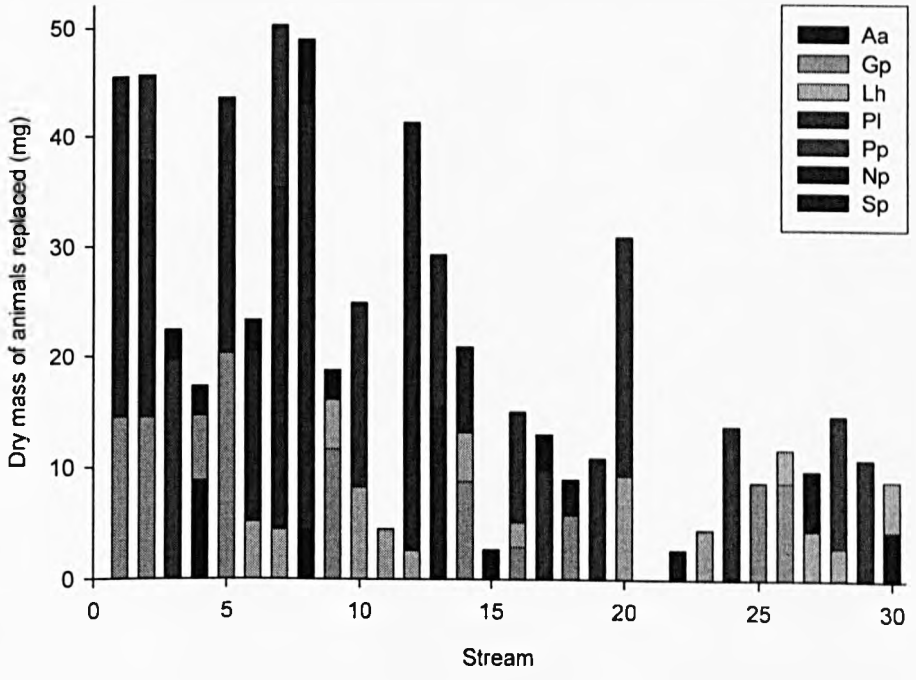

Figure H.5: Estimated dry mass of shredders replaced into each stream mesocosm over the 21 day period. Estimated from mean dry mass per species * number of individuals replaced. Aa = *Asellus aquaticus*, Gp = *Gammarus pulex*, Lh = *Leuctra hippopus*, Pl = *Potomophylax latipennis*, Pp = *Protonemura praecox*, Np = *Nemurella picteti*, Sp = *Sericostoma personatum*.

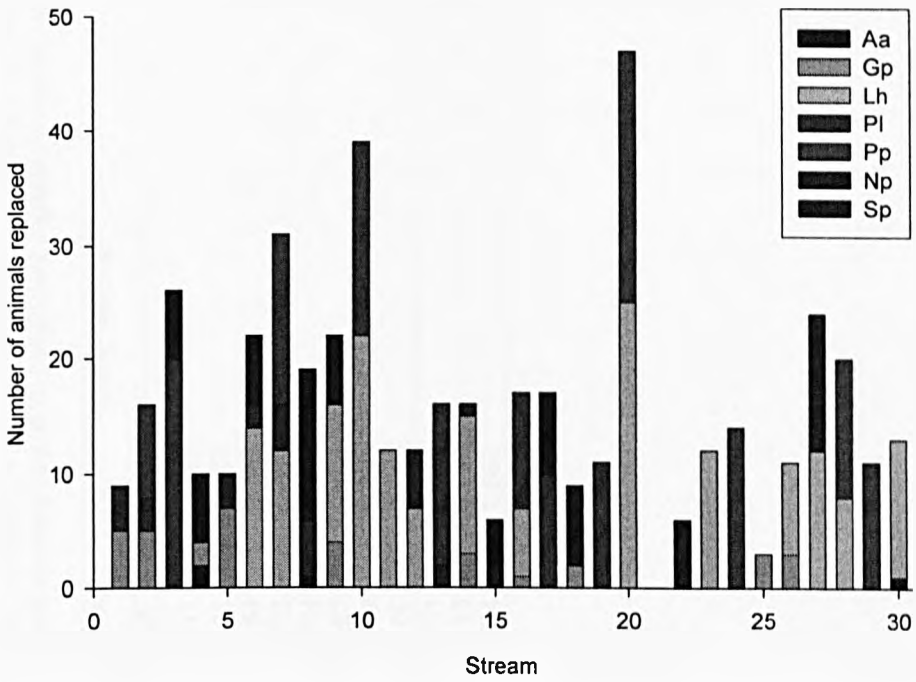

Figure H.6: Number of shredders replaced into each stream mesocosm over the 21 day period. Aa = *Asellus aquaticus*, Gp = *Gammarus pulex*, Lh = *Leuctra hippopus*, Pl = *Potomophylax latipennis*, Pp = *Protonemura praecox*, Np = *Nemurella picteti*, Sp = *Sericostoma personatum*.

Appendix I: Results of $D_{max(or\ Min)}$ tests for transgressive over- (or under-) yielding for each polyculture (C_{Poly}) (Chapter 5: experiment 2). Asterisks indicate that the polyculture was over- (or under-) yielding rates of processing greater than (or less than) that of its constituent highest (or lowest) species in monoculture (*Max- or* $MinC_{Mono}$). For identity of communities see Table 5.1.

Species richness of polyculture	Identity of Cm_{Poly}	Identity of $MaxCm_{Mono}$	D_{Max}	Over yielding	Identity of $MinCm_{Mono}$	D_{Min}	Under yielding
3	^{2}3.1	^{2}1.10	0.203	*	^{2}1.01	0.910	
	^{2}3.2	^{2}1.20	0.038	*	^{2}1.11	0.916	
	^{2}3.3	^{2}1.03	0.323	*	^{2}1.21	0.467	
	^{2}3.4	^{2}1.22	-0.008		^{2}1.04	0.847	
	^{2}3.5	^{2}1.14	-0.669		^{2}1.23	-0.425	*
	^{2}3.6	^{2}1.15	-0.220		^{2}1.24	0.566	
	^{2}3.7	^{2}1.16	0.213	*	^{2}1.07	0.301	
	^{2}3.8	^{2}1.26	-0.196		^{2}1.08	0.371	
	^{2}3.9	^{2}1.18	-0.011		^{2}1.09	0.840	
9	^{2}9.1	^{2}1.05	0.020	*	^{2}1.01	1.381	
	^{2}9.2	^{2}1.14	-0.115		^{2}1.13	0.693	
	^{2}9.3	^{2}1.20	-0.275		^{2}1.24	0.663	

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