ECOLOGY OF ACIDIFICATION AND RECOVERY IN WELSH UPLAND STREAMS

by

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ABSTRACT

This study investigates the current impact of acid deposition on the structure and function of benthic communities in streams of contrasting pH in mid-Wales, UK, and examines barriers to biological recovery across the sites. The results of field surveys across 30 streams revealed sustained effects of acid stress on macroinvertebrate assemblages. Acid streams supported fewer grazers and filter-feeders than circumneutral waters but other functional groups were less affected by low pH. Field experiments tested the effects of acidification on two key processes in stream ecosystems, benthic algal grazing and leaf decomposition. Grazer impacts on algal abundance were generally feeble and largely unaffected by acid stress. Leaf litter decomposition was impaired by acidification, due largely to reduced microbial breakdown. Prospects for biological recovery in chemically restored streams are discussed, and field experiments were undertaken to test two hypotheses (biotic resistance and resource limitation) proposed to explain the observed delays in faunal recolonisation. A resident-colonist competition experiment revealed no evidence in support of biotic resistance as mediated through interspecific competition, whereas growth experiments revealed that the quality or palatability of algal resources in chemically 'restored' streams limits growth and survival of colonist mayfly nymphs (*Baetis*).

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 INTRODUCTION

1.1.1 Causes of surface water acidification

Freshwater acidification occurs when oxides of sulphur (SO_2) and nitrogen (NO_x) produced through industrial processes react with moisture in the atmosphere to form acids, which are deposited into rivers and lakes during rain or snowfall (Mason, 2002). In addition to this wet deposition, acids reach the ground in gaseous or particulate form during dry weather following a series of complex photochemical reactions with powerful oxidising agents such as ozone (Mason, 2002). In the UK, the majority of SO_2 is derived from power stations and industrial plants, whereas NO_x is produced predominantly by motor vehicles (Mason, 1989). Certain river catchments with local geology and soils with a low capacity to buffer acid anions (SO_4^{2-}, NO_3^-) may be particularly susceptible to the influence of acid deposition (Hornung *et al.*, 1990). Such regions, where underlying geology consists of granite and acid igneous rocks or gneiss (Hornung *et al.*, 1990), are located primarily in North America, Scandinavia and the UK, and are termed ‘acid sensitive’ as they have insufficient base cations available in their soils to neutralise acidic pollutants (Mason, 2002). Acid deposition typically drives stream pH below 6.0, with spikes of acidity, termed ‘acid episodes’, following heavy rainfall or snowmelt (Lepori & Ormerod, 2005). In acid streams, leaching of cations such as Na^+, K^+, Mg^{2+} and Ca^{2+} occurs. Furthermore, Al^{n+} is mobilised in severely acidic streams ($pH < 5$), which is of particular significance due to the toxicity of this metal to aquatic animals (Herrmann, 2001), and concentrations of other heavy metals (e.g. Fe) may also increase (Mason, 1989; Mason, 2002).

In some areas, the problem of acid deposition has been exacerbated by changes in land-use. Large areas of acid-sensitive river catchments in Wales and Scotland, for example, have been afforested with conifers, which have accelerated the process of surface water acidification. Tree canopies trap and accumulate acidifying pollutants (Harriman *et al*., 1994), which can be washed out by heavy rainfall. In addition, tree roots facilitate removal of base cations from the soil (Harriman *et al*., 1994), resulting in reduced acid neutralising capacity and increased aluminium leaching (Kay & Stoner, 1988; Ormerod *et al*., 1989). Coniferous trees also have a greater acidifying capacity than deciduous trees due to their rapid growth rate and slow-decaying litter (Friberg *et al*., 1998).

1.1.2 Consequences of acidification for stream communities

Acid deposition has profoundly affected the biodiversity of freshwater ecosystems across the northern hemisphere (Mason, 2002), with impacts reported for biota at every trophic level in riverine food webs (Haines, 1981; Dillon *et al*., 1984; Hildrew & Ormerod, 1995). Salmonid fish, including brown trout (*Salmo trutta* L.) and salmon (*Salmo salar* L.), are particularly sensitive to the high aluminium concentrations in acid waters, and are typically absent where pH is less than 5.5 (Howells *et al*., 1983; Turnpenny *et al*., 1987; Kroglund *et al*., 2008; Monette & McCormick, 2008). In more moderately acidic streams, reduced density (Lancaster *et al*., 1996) and diversity (Turnpenny *et al*., 1987) of fish communities is often reported. Other top predators, such as the dipper (*Cinclus cinclus* L.) are negatively impacted by the reduced quality and quantity of their insect prey (Ormerod *et al*., 1985; Ormerod & Tyler, 1987). Furthermore, the decline in British amphibian populations has been attributed to the acidification of riparian breeding areas (Pough, 1976). Profound changes in the structure and function of stream ecosystems also occur at lower trophic levels. For example, below pH 5.0 sensitive bacteria, fungi (Haines, 1981) and macrophytes (Ormerod *et al*., 1987) are lost, and there is a shift in community structure towards more acidophilic species (Dillon *et al*., 1984). Shifts in community composition have also been observed amongst epilithic algae, with dominance of filamentous species occurring in some acid watercourses (Stokes, 1986; Herrmann *et al*., 1993; Kinross *et al*., 1993), coupled with a rapid decrease in diatom species richness (Hirst *et al*., 2004).

The response of biota to surface water acidification is perhaps most fully documented for the macroinvertebrates (e.g. Allard & Moreau, 1985; Friberg *et al*., 1998; Guérold *et al*., 2000), with substantial losses of sensitive groups, including snails (Sutcliffe & Carrick, 1973; Økland, 1980), crustaceans (Fryer, 1980) and mayflies (Peterson & Eeckhaute, 1992; Rundle *et al*., 1995) recorded at low pH. Historically, research has focused on determining pH tolerance limits of specific taxa (e.g. Hämäläinen & Huttunen, 1996; Davy-Bowker *et al*., 2003), and describing the shifts in community composition that occur as pH declines (e.g. Weatherley *et al*., 1989; Peterson & Eekhaute, 1992; Guérold *et al*., 2000; Petrin *et al*., 2007), whereas less attention has been given to the impact these shifts have on important ecosystem processes such as herbivory and leaf litter decomposition. The recognition that these processes are central to energy cycling and overall ecosystem health has added fresh impetus to research examining functional responses to acidification (Gessner & Chauvet, 2002; Simon *et al.*, 2009) (Chapter 2). Research assessing functional responses to acid stress is still relatively new, and a number of research gaps remain, some of which form the basis for this thesis. For example, the observed loss of macroinvertebrate grazers at low pH, and the decrease in grazing pressure caused by their absence, is thought to result in the proliferation of algae (Hendrey, 1976; Hall *et al.*, 1980; Stokes, 1986; Planas *et al.*, 1989). However, the 'grazer-release' hypothesis is yet to be tested formally in natural stream systems (see Chapter 3). Furthermore, loss of macroinvertebrate shredders in acid streams (Griffith & Perry, 1993; Dangles & Guérold, 2000; 2001; Thomsen & Friberg, 2002; Tixier & Guérold, 2005) could have a profound impact on detrital breakdown rates (Jonsson & Malmqvist, 2000; Jonsson *et al.*, 2001; Huryn *et al.*, 2002; Boyero *et al.*, 2007), but current evidence for this is inconsistent (Mason, 2002; Dangles *et al.*, 2004), and a clear picture is yet to emerge (Chapter 4).

1.1.3 Solutions to surface water acidification

Between 1986 and 2001, emissions of sulphur dioxide in the UK decreased by 71% (Fowler *et al.*, 2005), due to changes in industrial practices resulting from implementation of the 1956 and 1986 Clean Air Acts and subsequent pollution control legislation (RGAR, 1997; Mason, 2002). Emissions of nitrogen oxides also fell by 40% during this time (Fowler *et al.*, 2005), helped by the compulsory fitting of catalytic converters to all new motor vehicles (RGAR, 1997). As expected, reduced emission of acidifying pollutants has equated to reduced deposition of such compounds (Reynolds *et al.*, 1999; Fowler *et al.*, 2005). This trend is not unique to the UK, with studies in North America (Shannon, 1999; Driscoll *et al*., 2001) and mainland Europe (Schöpp *et al*., 2003) reporting similar decreases in acid deposition since the 1980s. As a result of the reduced deposition of acidifying pollutants, the pH of surface waters has steadily increased over the last decade in many sensitive regions (for example, Davies *et al*., 2005). In some areas, the process of chemical recovery from acidification has been accelerated by the addition of lime ($CaCO_3$) to hydrological source areas (Fjellheim & Raddum, 1992; Bradley & Ormerod, 2002; Raddum & Fjellheim, 2003). The river Audna, Norway, has been limed so that stream pH during snowmelt is maintained above 6.0 (Fjellheim & Raddum, 1992; Raddum & Fjellheim, 2003). In Wales, extensive liming programmes have been effective in increasing base flow pH (> 6.0) and decreasing aluminium concentrations in the upper Tywi (Bradley & Ormerod, 2002) and upper Wye (Lewis *et al*., 2007) catchments, although acid episodes still occur during high flow (Bradley & Ormerod, 2002). Increasingly however, full recovery of the chemical status of streams and lakes is now being reported in parts of Scandinavia (Skjelkvale *et al*., 2001a; Moldan *et al*., 2001), mainland Europe (Stoddard *et al*., 1999; Evans *et al*., 2001) and North America (Skjelkvale *et al*., 2001b).

1.1.4 Current status of biological recovery

Despite improvements to the acid-base status of many acid-sensitive river catchments, either naturally or as a result of liming, many studies report that the recovery of biological communities has been delayed (Alewell *et al*., 2000; Driscoll *et al*., 2001; Moerke & Lamberti, 2003; Pretty *et al*., 2003; Yan *et al*., 2003; Monteith *et al*., 2005). In mid-Wales, liming of acid streams in the upper Tywi catchment has resulted in steady improvements in water chemistry since the late 1980s, whereas the response of macroinvertebrates has not followed predicted trends (Weatherley & Ormerod, 1992; Rundle *et al*., 1995; Ormerod & Durance, 2009). A similar response to liming has been observed in the nearby upper Wye catchment (Lewis *et al*., 2007), where recolonisation of acid-sensitive macroinvertebrate species is occurring over a prolonged timescale. In some catchments there is currently no convincing evidence to suggest that biological recovery is underway (Soulsby *et al*., 1997; Gee, 2001). The uncertainty as to whether damaged communities are responding to chemical improvements makes it essential that recovery of watercourses from acidification is tracked over coming decades. The failure of acid-sensitive macroinvertebrate grazers to return to chemically restored streams (Bradley & Ormerod, 2002) is of particular concern due the important role this group of organisms plays in nutrient cycling (Wallace & Webster, 1996), and a number of key hypotheses have been put forward in explanation. The leading suggestions are discussed in detail in Chapter 5, but this thesis will focus on the two ideas that have received the least attention.

These hypotheses, which are not mutually exclusive, are first, that shifts in macroinvertebrate community structure resulting from the loss of the grazer functional group in acid streams renders it closed to reinvasion once water chemistry has been restored, and that this ‘community closure’ is mediated primarily through strong competitive interactions with acid-tolerant generalist species (Chapter 5), and second, that algal resources in limed streams are of reduced quality in comparison to those found in circumneutral streams, and are therefore unable to support the nutritional needs of reinvading macroinvertebrate grazers (Chapter 6). Despite persuasive evidence to suggest that both these mechanisms could be at work in circumstances where recovery of macroinvertebrate grazers has been delayed, neither has been tested explicitly in stream environments. For example, evidence supporting the *biotic resistance* hypothesis has been found in lakes, where delayed recovery of algal and zooplankton communities following chemical restoration has been attributed to strong competition from persistent acid-tolerant species (Keller & Yan, 1998; Shurin, 2000; Holt & Yan, 2003; Vinebrooke *et al*., 2003; Frost *et al*., 2006). In streams, the potential for competition among acid-tolerant generalists over shared food resources has been demonstrated (Ledger & Hildrew, 2000), but the existence of competition for food among residents and recolonists, and its impact on the success of the macroinvertebrate grazer community is yet to be tested (Chapter 5). With regard to the *resource limitation* hypothesis, hydrochemical analysis has raised the possibility that there is a discrepancy in the water quality of limed and naturally circumneutral streams (Rosseland *et al*., 1992; Kullberg *et al*., 1993; Teien *et al*., 2004), but the likely consequences for algal nutritional quality, and its significance in delaying the recovery of acid-sensitive grazers remains unclear (Chapter 6). It is imperative that these research gaps are addressed however, so that the biological damage to streams and rivers caused by acidification can be reversed.

1.2 THESIS AIMS

The two main aims of the research presented in this thesis were, first, to examine the effects of surface water acidification on the structure and function of upland stream ecosystems, and second, to establish whether specific biological barriers exist that retard biological recovery in streams recovering chemically from acidification. My specific objectives, addressed in consecutive chapters, were to:

1. Describe the changes in macroinvertebrate community structure and functional feeding group composition across streams of contrasting water quality (Chapter 2);
2. Compare algal growth and the intensity of herbivory along an acidity gradient (Chapter 3);
3. Examine the impact of stream pH and shredder identity on leaf litter breakdown rates in upland sites (Chapter 4);
4. Determine whether competition from acid-tolerant species affects the growth and invasion success of an acid-tolerant recolonist (Chapter 5);
5. Establish whether low algal food quality limits performance of recolonising grazers in limed streams (Chapter 6).

All chapters are presented in the format of self-contained papers for possible submission to journals, and have their own reference lists. As such, some overlap in methodologies between chapters may exist.

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CHAPTER TWO

STRUCTURAL AND FUNCTIONAL COMPOSITION OF MACROINVERTEBRATE COMMUNITIES IN STREAMS OF CONTRASTING pH

2.1 SUMMARY

1. A substantial literature reports the effect of surface water acidification on biodiversity in streams, but less is known about the functional consequences of biodiversity loss in acid-stressed aquatic ecosystems. The research presented in this chapter addresses the current status of streams subject to varying degrees of acid stress and relates shifts in macroinvertebrate assemblage structure to the functional feeding group composition of the community.
2. The structure and functional composition of macroinvertebrate assemblages was traced along an acidity gradient in thirty headwater streams in mid-Wales. Sites were divided into three groups (acid, intermediate and circumneutral) based on minimum and mean annual pH. Acid streams had a mean pH < 6.0 (minimum < 5.5), intermediate streams had a pH of between 6.0 and 6.5, and circumneutral streams had a mean pH > 6.5 (minimum > 6.0).
3. Acid streams were characterised by high dissolved Na^+ (> 4.2 mg L^{-1}), Cl^- (> 7.5 mg L^{-1}), Mn^{2+} (> 60 µg L^{-1}) and Al^{n+} (> 240 µg L^{-1}). Conversely, circumneutral streams generally contained higher dissolved Ca^{2+} (> 2.2 mg L^-

^{1}) and Mg^{2+} (> 1 mg L^{-1}) concentrations. The mean Pfankuch Index of stream stability across the thirty study sites was 55, indicating a high level of streambed instability.

4. Macroinvertebrate assemblages in acid streams were relatively impoverished, supporting low numbers of species (mean taxon richness spring 21 taxa, summer 25 taxa) relative to intermediate (spring 27 taxa, summer 31 taxa) and circumneutral streams (spring 31 taxa, summer 35 taxa). Macroinvertebrate density did not differ significantly between the three pH groups, but was significantly lower in spring than summer across sites.
5. In spring, substantial species turnover within macroinvertebrate assemblages across sites was related predominantly to stream pH and labile Al. In summer, variation in macroinvertebrate community composition between sites correlated strongly with streambed stability, as evidenced by the Pfankuch Index.
6. Macroinvertebrate communities in circumneutral streams were dominated by collector-gatherers, grazers and predators. As pH decreased, a shift in functional feeding group composition of communities was observed, with a loss of grazer and filter-feeder taxon richness, and a decrease in density of grazing macroinvertebrates. Macroinvertebrate predators and shredders were generally unaffected by pH. Loss of filter-feeders and grazers at low pH could have a profound impact on organic matter processing rates and nutrient cycling, and could potentially result in impaired ecosystem function.

2.2 INTRODUCTION

The effects of acidification on freshwater biota are perhaps most fully explored among the macroinvertebrates, and a substantial body of literature has accumulated over the past 20-30 years demonstrating the relationship between surface water pH and faunal assemblage structure (e.g. Allard & Moreau, 1985; Friberg *et al*., 1998; Guérold *et al*., 2000). Species-specific pH tolerance limits have been reported for stream macroinvertebrate fauna (Hämäläinen & Huttunen, 1996; Davy-Bowker *et al*., 2003), and form the basis of biotic indices used to monitor the severity of acidification in streams (Davy-Bowker *et al*., 2005; Ormerod *et al*., 2006; Clews & Ormerod, 2009). Molluscs (Bivalvia and Gastropoda) and crustaceans (e.g. Amphipoda and Isopoda) are particularly sensitive to surface water acidification since the calcium required for formation of their body tissues (e.g. shell, carapace) is scarce at low pH (Fryer, 1980). Consequently, the presence and abundance of these taxa in streams is often positively correlated with pH and total hardness of stream water. Økland (1980a) reported that in Norway, a prominent decline in gastropod taxon richness occurred in sites where pH is less than 6.0, and the group as a whole was absent in profoundly acid waters with pH less than 5.2 (Økland, 1980a). Similarly, in the River Duddon, England, the limpet *Ancylus* was absent below pH 5.7 (Sutcliffe & Carrick, 1973). Crustaceans such as *Gammarus pulex* (Linnaeus) and *Gammarus lacustris* (Sars) only inhabit watercourses with a pH greater than 5.7 (Sutcliffe & Carrick, 1973), or 6.0 (Økland, 1980b), respectively, and *Asellus aquaticus* (Linnaeus) is rarely found below pH 5.2 (Økland, 1980b). The Ephemeroptera larvae (mayflies), including those characteristic of upland streams such as *Electrogena lateralis* (Curtis), *Rhithrogena semicolorata* (Curtis) and *Baetis rhodani* (Pictet), are renowned for their acid-sensitivity (Rundle *et al.*, 1995), and are only found where pH is greater than 6.0 (Peterson & Eeckhaute, 1992). Under acidic conditions, restricted ion absorption (e.g. sodium, chloride, potassium) impairs osmoregulation in mayflies, causing physiological stress and mortality (Sutcliffe & Hildrew, 1989). Aluminium hydroxide is also rendered soluble at low pH and can accumulate on gill surfaces thereby limiting oxygen uptake (Herrmann & Andersson, 1986). Other acid-sensitive macroinvertebrate species occur within the Trichoptera and Coleoptera, although the reasons for their sensitivity are not fully understood (Sutcliffe & Carrick, 1973; Smith *et al.*, 1990).

The notable acid-sensitivity within the Crustacea, Ephemeroptera, Mollusca, and Trichoptera does not extend across all orders of the macroinvertebrate fauna. Plecoptera are well known for their tolerance to low pH (Peterson & Eeckhaute, 1992; Petrin *et al.*, 2007), and an abundance of nymphs of the Leuctridae (e.g. *Leuctra inermis* Kempny, *Leuctra nigra* Olivier) and Nemouridae *(e.g. Nemurella picteti* Klapálek, *Amphinemoura sulcilcollis* Stephens) typically characterise benthic macroinvertebrate assemblages in many acid streams (Dobson & Hildrew, 1992; Friberg *et al.*, 1998; Guérold *et al.*, 2000).

Although the impact of low pH on sensitive aquatic macroinvertebrates is well known, the significance of their absence for the functioning of acid streams has yet to be fully explored (Locke, 1992; Hildrew & Ormerod, 1995; Planas, 1996). The functional consequences of biodiversity loss driven by acidification are thought to depend on the diet and ecological role of sensitive species, as well as their interactions with other organisms in the food web (Rosenfeld, 2002). For example, where niche overlap exists with acid-tolerant species, and overall biomass is maintained, loss of function may not occur (Lawton & Brown, 1993; Petchey & Gaston, 2002). However, where the role of sensitive species within the community is demonstrably unique, losses could lead to functional impairment, regardless of shifts in community biomass (Ehrlich & Ehrlich, 1982).

Cummins (1973; 1974) was the first to formally classify macroinvertebrate species according to their 'function', according to the mouthpart morphology of individual species. Moog (1995) has since adapted these functional groupings for macroinvertebrates in Europe based on recent dietary analyses, and these assignments have been used extensively to aid assessment of a species' importance in maintaining ecosystem function (e.g. Ledger & Hildrew, 2005; Bergfur *et al.*, 2007; Schmera *et al.*, 2009). Macroinvertebrate assemblages in streams contain species belonging to one of several functional feeding groups, including grazers (periphyton consumers), shredders (large particle detritivores), collector-gatherers (small particle detritivores), predators (engulfers and suctorial feeders) and filter-feeders (suspended organic matter consumers) (Cummins, 1973). According to Cummins (1973), taxa in these functional groups process organic matter in different ways, and thus perform different roles in stream ecosystems. Any impact of acidification on the presence and abundance of fauna within these groups could therefore have profound consequences for the rate of key ecosystem processes.

The importance of assessing the functional composition of macroinvertebrate communities is now evident, and research has recently begun to focus on the extent to which acidification affects ecosystem processes (Gessner & Chauvet, 2002; Simon *et al.*, 2009). To date however, relatively few studies have examined the effect of acid stress on the taxonomic composition and abundance of species within and among functional feeding groups (but see Dangles & Guérold, 2001; Ledger & Hildrew, 2005), and debate still exists among published studies as to the likely effects of acid stress on well-researched functional groups (e.g. Dangles & Guérold, 2001; Petrin *et al.*, 2007; 2008b). For example, in the case of the shredder functional feeding group, decreased shredder diversity under acid conditions (Dangles & Guérold, 2000; Tixier & Guérold, 2005; Petrin *et al.*, 2008a) is associated with reduced rates of leaf litter decomposition (Burton *et al.*, 1985; Griffth & Perry, 1993; Jonsson *et al.*, 2001b; Huryn *et al.*, 2002). Similarly, the observed dramatic increases in algal biomass and primary production in some acid watercourses (Hall *et al.*, 1980; Allard & Moreau, 1985; Schindler *et al.*, 1985; Planas *et al.*, 1989) may be due to reduced herbivory (Hall *et al.*, 1980; Stokes, 1986; Planas *et al.*, 1989) resulting from the absence of acid-sensitive grazers. However, other studies have observed no such reduction in grazing pressure in acidified streams (Geelen & Leuven, 1986; Winterbourn *et al.*, 1992; Junger & Planas, 1993; Kinross *et al.*, 1993), possibly due to feeding plasticity by macroinvertebrates in other functional groups, which may consume algae even where grazers are completely absent (e.g. Dangles, 2002). It has also been suggested that large-bodied macroinvertebrate predators may proliferate in acid streams (Hildrew *et al.*, 1984; Muniz, 1990) due to the absence of fish (Howells *et al.*, 1983; Turnpenny *et al.*, 1987), although this phenomenon has only been proven experimentally in lakes (Eriksson *et al.*, 1980; Zaret, 1980). In contrast to the research undertaken on shredder and grazers, effects of acidity on collector-gatherers and filter-feeders are rarely reported and deserve further investigation.

The primary aim of the research reported in this chapter was to evaluate the functional feeding group composition of the macroinvertebrate assemblages in thirty headwater streams with varying acid-base status. Previously, extensive research in the three study catchments in mid-Wales has reported the effects of acidification on community structure and, more recently, assessed the success of subsequent chemical and biological recovery (e.g. Stoner *et al*., 1984; Rundle *et al*., 1995; Ormerod & Durance, 2009). By comparison, less is known about the consequences of shifts in community structure for the functional integrity of systems exposed to low pH (but see Merrix *et al*., 2006). The objectives of the research presented here were therefore to:

1. Assess the current status of macroinvertebrate community structure in streams across a pH gradient.
2. Evaluate corresponding changes in the functional structure of the biota by analysing trends in macroinvertebrate functional feeding group composition.
3. Assess the temporal variation of acid effects on macroinvertebrate communities using data collected in two different seasons (spring and summer).

My *a priori* expectations were that species richness and abundance in the grazer and shredder functional groups would diminish as pH declines, and that changes in predator community composition would follow the opposite trend. Shifts in the functional composition of macroinvertebrate communities along the acidity gradient are discussed in terms of potential impacts on ecosystem function.

2.3 METHODS

2.3.1 Study catchments

2.3.1.1 Geography and geology

Thirty streams from three catchments in mid-Wales were selected on the basis of historical stream water chemistry (S. Ormerod, unpublished data) and field collections made during this study. Six streams were located in the Llyn Brianne catchment (Figure 2.1, Table 2.1), which covers approximately 300 km^2, and is fed by two main rivers, the Afon Tywi (coded LI) and the Afon Camddwr (coded CI). Fifteen streams were located in upper River Wye (catchment 174 km^2), which is fed by the Tarenig and Bidno rivers at this location (coded WY). A further nine streams were located in the Irfon catchment (244 km^2), which is fed by both the Afon Irfon (coded IF) and Afon Cammarch (coded AC). The two latter catchments (Wye and Irfon) form part of the River Wye SSSI.

Geology underlying the catchments is predominately of base-poor Ordovician and Silurian shales (Edwards & Brooker, 1982). Overlying soils are brown podzols, stagnopodzols and peats (Weatherley and Ormerod, 1987). The poor buffering capacity of local bedrock and soils combine to render streams and rivers draining the three catchments particularly susceptible to the effects of acidification (Hornung *et al.*, 1990). At Llyn Brianne, mean total hardness of runoff is generally low (< 8 mg $CaCO_3$ L^{-1}), although in some streams this is alleviated by the presence of calcite veins (Durance and Ormerod, 2007).

2.3.1.2 Physical characteristics of the study streams

The thirty study streams were located between 230 and 410 m above sea level, with sites in the upper Wye and Llyn Brianne catchments located at higher altitude than those in the Irfon catchment. Stream gradient varied between 1 and 12° (mean ~ 4°, Table 2.2). The majority of sites drained a small area ($< 5\ km^2$), with the exception of the main channels of the Rivers Wye, Irfon, Bidno and Tarenig (WY34, IF8, WY31, WY48). Mean annual rainfall is approximately 1900 mm at Llyn Brianne (Weatherley & Ormerod, 1990), and approximately 2500 mm at Plynlimon, the source of the Wye (Neal *et al.*, 2001).

Stream order is a simple method of describing the size of a stream based on a hierarchy of tributaries (Strahler, 1957), and stream link magnitude is defined as the number of unbranched source streams upstream from a given segment in the drainage network (Shreve, 1966). Examination of topographical maps of the three catchments (O.S. 1:50000) revealed that the stream order and stream link magnitude of the 30 study streams was low (between 1 and 4, and between 1 an 10, respectively) with the exception of some of the more major rivers, such as the main River Wye and Irfon (Table 2.2). Riparian land use, classified according to River Habitat Survey protocol (Environment Agency, 1997), revealed that the majority of sites were located alongside rough/unimproved pasture (n = 10), improved/semi-improved grassland (n = 9) or coniferous plantation (n = 8), with only three sites located alongside broadleaf/mixed woodland.

The physical stability of the stream bed at each site was assessed using the Pfankuch Index (Pfankuch, 1975). Originally, fifteen variables (weighted in relation to their perceived importance) were scored across three regions of the stream channel (upper banks, lower banks and stream bed) and summed to produce an index reflecting the overall physical stability of the channel, with streams with high scores considered to have unstable channels at the reach scale. In this study, only the last seven components of the index (i.e. undercutting, rock angularity, bed-surface brightness, particle packing, per cent stable materials, scouring and aquatic vegetation), relating predominantly to the stream bottom were used, as these factors have the greatest influence on benthic macroinvertebrates (see Winterbourn & Collier, 1987; Death & Winterbourn, 1995; Lods-Crozet *et al.*, 2001). Measurements revealed that the study sites had a Pfankuch Index ranging between 20 and 74, and a mean Index of 55. These values are similar to those recorded for glacial Alaskan and Alpine streams (Maiolini & Lencioni, 2001; Milner *et al.*, 2006), indicating that they have highly unstable channels.

2.3.1.3 Catchment research and management

Research on acidification in the Llyn Brianne experimental catchments has been ongoing for 25 years (Ormerod & Durance, 2009). In 1983, the Welsh Water authority began a short-term monitoring programme of the reservoir's tributaries, in order to gather information on the extent of acid deposition in upland Wales, and increase understanding of the impacts of acidification on freshwater ecosystems (Stoner *et al.*, 1984). More detailed and intensive investigations followed in 1984, led by the Department of Environment and the Welsh Office. Since then, Cardiff University's Catchment Research Group, under the direction of Prof. Steve Ormerod, has continued sampling at key sites in the catchment (Ormerod and Durance, 2009). Liming of hydrological source areas at Llyn Brianne occurred at some sites in

1987/88 (CI2), although many limed streams are still prone to spikes of low pH following heavy rain and snowmelt (Kowalik and Ormerod, 2006). In 2002, the Wye and Usk Foundation instigated the Powys Habitat Improvement Scheme (pHish) to improve the salmonid fishery in the upper Wye and Irfon catchments, primarily by reversing the effects of acidification. Since 2003, calcium carbonate powder has been deposited on hydrological source areas of several streams in the upper Wye catchment (WY31, WY33, WY47, WY48, WY52 had been treated with lime by 2006).

2.3.2 Sampling design

2.3.2.1 Water chemistry

Stream water pH and temperature were measured monthly between March and August 2006, using a handheld pH meter (HANNA HI9024C). Water sampled from the thirty study sites in April and July 2006 was filtered (Whatman cellulose nitrate filter membrane, 0.45 μm pore size, 47 mm diameter), transferred to sterile plastic bottles and refrigerated (< 48 hours) prior to analysis. Samples for cation and metal determination were acidified (pH 2.0) using 70% Aristar nitric acid. Anion concentrations (Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-}) were determined by ion chromatography (Dionex, ICS-2000) using the gradient method. Cation (Na^+, K^+), metal (Ca^{2+}, Mg^{2+} Mn^{2+}, Fe^{2+}, Al^{n+}) and metalloid (Si) concentrations were determined using an Agilent 7500ce ICP-MS. Non-purgeable organic carbon (NPOC) was measured using a TOC analyser (Shimadzu TOC-V CSH). Dissolved oxygen concentrations (YSI 550A DO Meter), total dissolved solids and conductivity (Myron Utrameter II 6P) were measured *in situ* at the time of water sample collection. In addition, historical water chemistry data (2003 to 2005) for most sites were obtained from the Catchment

Research Group at Cardiff University (S. Ormerod, unpublished data). Sampling frequency differed among sites, but in general, water chemistry data were obtained monthly from Llyn Brianne and quarterly from the Wye and Irfon.

2.3.2.2 Macroinvertebrate sampling

Macroinvertebrates were collected in April and July 2006 using a standard Surber sampler (mesh aperture 250 µm, 0.0625 m^2 area) (Surber, 1970). At each site, five samples were collected at random from a representative riffle. During sampling, large stones and boulders were inspected and any macroinvertebrates attached to surfaces were collected. The remaining substratum was then manually disturbed (10 cm depth) by hand, upstream of the net. Macroinvertebrate samples were transferred to labelled plastic bags and preserved immediately in 4% formaldehyde.

In the laboratory, samples were washed to remove fixative, and all macroinvertebrates were sorted from debris and preserved in 70% Industrial Methylated Spirit (IMS). Macroinvertebrates were then identified to the lowest practicable taxonomic level (generally species) using a binocular (Zeiss Stemi 2000) or compound microscope (Nikon Optiphot-2), and counted. All nomenclature conforms to the CEH Coded Checklist of Animals Occurring in Fresh Water in the British Isles, Version 6 (October 2008).

2.3.2.3 Data analysis

The thirty study sites were divided in to three groups (acid pH, intermediate pH and circumneutral pH) on the basis of their mean and minimum pH (after Ormerod & Durance, 2009) between 2003 and 2006. Two-way analysis of variance (ANOVA) was used to test the main effects of pH group and season (spring, summer), and their interaction, on key water chemistry variables. *Post hoc* Tukey HSD tests were used identify significant differences among treatment means where the results of two-way ANOVA were significant at $P < 0.05$.

Macroinvertebrate community composition was related to environmental variables by canonical correspondence analysis (CCA) using CANOCO 4.5 (ter Braak and Šmilauer, 2002), following preliminary analysis by detrended correspondence analysis (DCA). CCA was used to manually forward select environmental variables accounting for variation in the macroinvertebrate data. Variables were only selected if they were significant ($P < 0.05$; using 999 unrestricted Monte Carlo permutations), and the ordination was constrained solely to the variables that were selected in the model.

Macroinvertebrate taxa were assigned to functional feeding groups (*sensu* Cummins & Klug, 1979) by reference to Moog (1995). Two-way analysis of variance (ANOVA) was used to test the main effect of stream pH and functional feeding group, and their interaction, on macroinvertebrate taxon richness, density (m^{-2}) and relative abundance (% of total density). One-way ANOVA was used to separate the effect of pH on each individual functional feeding group in each season.

2.4 RESULTS

2.4.1 Stream physicochemistry

‘Acid pH’ sites had a mean pH below 6.0 and a minimum pH below 5.5; ‘intermediate pH’ sites had a mean pH between 6.0 and 6.5 and a minimum pH between 5.5 and 6.0; sites in the ‘circumneutral pH’ group had a mean pH greater than 6.5 and a minimum pH greater than 6.0 (Figure 2.2). One-way ANOVA revealed that overall group mean and minimum pH values were significantly different ($P < 0.05$, Figure 2.3). Variation in stream pH was generally less in summer, both within sites and across the three study catchments, and minimum recorded pH values were higher.

Mean phosphate and nitrate concentrations across all sites were 0.033 and 0.814 mg L^{-1}, respectively, in spring, and 0.001 and 0.987 mg L^{-1}, respectively, in summer. Concentrations of Na^+, Mn^+, Al^{n+}, Si and Cl^- were significantly higher (ANOVA, $P < 0.05$) in acid streams compared to circumneutral streams (Table 2.3), whereas concentrations of Ca^{2+} and Mg^{2+} were significantly higher in circumneutral streams (Table 2.3). Anion, cation and metal concentrations in intermediate pH sites were generally midway between those recorded for acid and circumneutral streams (Table 2.3). Season influenced physicochemistry, with significantly higher concentrations of several cations (Na^+ and K^+), anions (Cl^-, PO_4^{3-}), non-purgeable organic carbon (NPOC) and dissolved oxygen (DO) recorded in spring. Only Si concentrations and mean water temperature were significantly higher in summer. Stream water was generally saturated or super-saturated with dissolved oxygen at all sites. No interaction between season and pH group was detected for any of the chemical variables.

2.4.2 Macroinvertebrate-environment relations

A series of ordinations were used to investigate the relationship between macroinvertebrate assemblage composition and environment. Detrended correspondence analysis (DCA) revealed that turnover in macroinvertebrate assemblage composition across streams was evident in both seasons, being more pronounced in spring (DCA axis 1 = 3.32 SD, axis 2 = 1.95 SD) than summer 2006 (DCA axis 1 = 2.13 SD, axis 2 = 1.76 SD) (Figure 2.4). Subsequent canonical correspondence analysis (CCA) of macroinvertebrate data, with manual forward selection, identified five environmental variables (pH, Al, PO_4, Cl and Mg) that significantly ($P < 0.05$) explained variation in species composition across the 30 sites in spring (Table 2.4), and four variables (Pfankuch, Mg, SO_4 and pH) that explained this variation in summer (Table 2.4). In spring, the primary gradient in the macroinvertebrate data was related to pH (see marginal effects, Table 2.4). Interset correlations of environmental variables with CCA axes indicated that variation along the first axis (15.2% of the total variation in the species data) was related primarily to pH, but also to labile aluminium concentration and chloride. Gradients along the second axis (12.6%) were most strongly correlated with dissolved phosphate concentration (Table 2.4). In summer, variation along the first axis (12.8% of the total variation in the species data) was related primarily to the Pfankuch Index of stream stability, with the second axis (9.1%) most strongly correlated with dissolved sulphate concentration (Table 2.4).

The spring and summer distribution of macroinvertebrate taxa and study sites along the environmental gradients is shown in the CCA biplots (Figures 2.5 & 2.6). In spring, the centroids for acid streams were located high on axis one (low pH and high labile aluminium) and were associated with acid-tolerant species of macroinvertebrate such as *Amphinemoura sulcicollis*, *Nemoura avicularis* and *Nemurella pictetii* (Figure 2.5). The secondary gradient of phosphate concentration separated circumneutral streams according to the level of nutrient enrichment, with enriched sites (e.g. AC16) sites supporting large populations of leaf-shredding amphipods (*Gammarus pulex*) and cased caddis (*Agapetus fuscipes*). In the summer CCA, the importance of pH was less pronounced, with axis one correlating more strongly with the Pfankuch Index of stream stability (Figure 2.6). High-scoring sites (low stability) were located mainly in the upper Wye catchment, whereas low-scoring sites (high stability) were located in the Irfon catchment. A secondary gradient of dissolved sulphate separated some of the more acid streams (high dissolved sulphate) from the rest of the group.

2.4.3 Functional composition in the absence of acidity: circumneutral streams

Eighty macroinvertebrate taxa in six functional feeding groups were collected from ten circumneutral streams across spring (mean 30.7 taxa) and summer (mean 34.5 taxa) 2006 (Figure 2.7, Table 2.5). In order of decreasing constituent taxonomic richness across both seasons, the functional groups were predators (spring 9.3, summer 11.9 taxa), grazers (8.9 spring , 10.4 summer), collector-gatherers (spring 6.4, summer 5.8), filter-feeders (spring 2.6, summer 3.1), shredders (spring 3.5, summer 3.0) and suctorial plant-feeders (spring 0, summer 0.3) (Figure 2.7, Table 2.5).

Mean density (m^{-2}) of macroinvertebrates (total and of constituent groups) was higher in summer than spring (Figure 2.7, Table 2.5). Collector-gatherers were numerically dominant, most notably in summer (spring 809 m^{-2}, summer 3736 m^{-2}) (Figure 2.7, Table 2.5). Grazers (spring 526 m^{-2}, summer 998 m^{-2}) and predators (spring 330 m^{-2}, summer 379 m^{-2}) were also important component groups in the benthos in both seasons, whereas filter feeders (spring 42 m^{-2}, summer, 528 m^{-2}), shredders (spring 133 m^{-2}, summer 486 m^{-2}) and suctorial plant-feeders (spring 0 m^{-2}, summer, 2 m^{-2}) were proportionately least abundant (Figure 2.7, Table 2.5).

In circumneutral streams, grazers present as core taxa (> 1% total numbers) in both seasons were mayflies of the Baetidae (*Baetis*) and Heptageniidae (*Rhithrogena*, *Electrogena*), and beetle larvae (*Elmis*) (Table 2.6). Additionally, nemourid stoneflies (*Amphinemoura*) occurred only in spring and ephemerellid mayflies (*Serratella*) only in summer. Shredders were dominated numerically by amphipods (*Gammarus*) and tipulid dipterans, with nemourid stoneflies (*Protonemoura*, *Nemoura*) in spring. Collector-gatherers were dominated numerically by chironomids, oligocheates and leuctrids, mainly *Leuctra inermis* in spring and *Leuctra hippopus* in summer. In spring, macroinvertebrate predators were stoneflies of the Perlidae (*Isoperla*) and Chloroperlidae (*Chloroperla*, *Siphonoperla*), empidid beetles, flatworms (*Planaria*) and mites, with the latter the most abundant predatory group in summer. Filter feeders were predominantly Simuliidae, and caddis flies (mainly Hydropsychidae), and suctorial plant-feeders were *Oxyethira* sp. (Table 2.6).

2.4.4 Effect of acidity on macroinvertebrate assemblages

Mean taxon richness decreased as acidity increased, being significantly lower in acid sites (mean spring 20.7, mean summer 24.6) compared with intermediate (mean spring 27.2, mean summer 30.8) and circumneutral sites (mean spring 30.7, mean summer 34.5), both in spring (one-way ANOVA, $df = 29$, $F = 6.231$, $P = 0.006$) and summer (one-way ANOVA, $df = 28$, $F = 7.679$, $P = 0.002$) (Figure 2.7, Table 2.5).

There was also a tendency for the total density of macroinvertebrates (mean acid 1071 m^{-2}, intermediate 1418 m^{-2}, circumneutral 1839 m^{-2} in spring; mean acid 4079 m^{-2}, intermediate 5688 m^{-2}, circumneutral 6130 m^{-2} in summer), to increase with decreasing acidity, but the relationship was not statistically significant in either season (one-way ANOVAs, $P > 0.05$; Table 2.5).

2.4.5 Effects of acidity on macroinvertebrate functional feeding groups

Mean richness, density (m^{-2}) and relative abundance (%) of macroinvertebrate taxa in each functional feeding group is displayed in Table 2.5 for each of the three pH categories (acid pH, intermediate pH and circumneutral pH). Two-way ANOVA revealed that species richness, density and relative abundance varied among functional feeding groups in both seasons (significant main effect, $P < 0.05$, Table 2.7). The main effect of stream pH group was significant for species richness in both seasons, and density in spring but not for density in summer, or relative abundance ($P > 0.05$, Table 2.7). There was however a significant interaction between functional feeding group and stream pH group for species richness and relative abundance in both seasons and for density in spring but not summer ($P < 0.05$, Table 2.7), indicating that macroinvertebrate responses to acidification differed among the functional groups. One-way ANOVA was used subsequently to examine pH effects on the taxon richness, density and relative abundance of each functional group in each season (results are shown in Table 2.5). ANOVA results for taxon richness were statistically significant for filter-feeders in spring ($df = 29$, $F = 8.538$, $P = 0.001$; acid = intermediate < circumneutral) and summer ($df = 29$, $F = 6.965$, $P = 0.004$; acid < intermediate = circumneutral), and also for grazers in both seasons (spring $df = 29$, F

= 6.061, $P = 0.007$; summer $df = 29$, $F = 5.777$, $P = 0.008$; acid < intermediate = circumneutral in both cases) (Figure 2.7, Table 2.5). Grazer density (numbers m^{-2}) differed among pH groups (spring $df = 29$, $F = 5.174$, $P = 0.013$, acid = intermediate < circumneutral; summer $df = 29$, $F = 4.023$, $P = 0.030$, acid < intermediate), but density effects on other functional groups were non-significant (Table 2.5). The relative abundance of animals in functional groups was skewed by increased acidity, with proportionally more predators in intermediate sites in spring ($df = 29$, $F = 6.342$, $P = 0.006$; intermediate > circumneutral), and more suctorial plant-feeders ($df = 29$, $F = 4.838$, $P = 0.016$; acid > intermediate = circumneutral) but fewer grazers ($df = 29$, $F = 3.595$, $P = 0.042$; acid < intermediate) in acid sites in summer (Figure 2.7, Table 2.5).

2.4.6 Trends within functional groups across a pH gradient

2.4.6.1 Collector-gatherers

In spring, the relative abundance of macroinvertebrates in each of the eight collector-gatherer taxonomic orders (Figure 2.8, panel *a*) did not differ significantly among stream pH groups ($P > 0.05$). Diptera (Chironomidae) were numerically dominant across all sites, followed by Plecoptera (*Leuctra*) and Oligochaeta (Table 2.6). Trichoptera, Coleoptera, Hemiptera, Ephemeroptera and Ostracoda constituted a very small percentage of total numbers (Figure 2.8, panel *a*). In summer, the relative abundance of Diptera (Chironomidae) was significantly higher in acid compared to intermediate pH streams ($df = 29$, $F = 6.345$, $P = 0.006$). The relative abundance of Plecoptera (*Leuctra hippopus*), on the other hand, was significantly higher in circumneutral streams ($df = 29$, $F = 5.029$, $P = 0.014$; Figure 2.8).

2.4.6.2 Filter-feeders

The filter-feeder functional feeding group consisted mainly of Diptera (Simuliidae) and Trichoptera (*Hydropsyche siltalai*) (Table 2.6). In both seasons, the relative abundance of Trichoptera was significantly higher in intermediate pH sites (spring *df* = 29, *F* = 8.411, *P* = 0.001; summer *df* = 29, *F* = 4.864, *P* = 0.016; Figure 2.8, panel *b*). Only in summer, however, was the relative abundance of Diptera significantly lower in these streams (*df* = 29, *F* = 4.778, *P* = 0.017).

2.4.6.3 Grazers

The taxonomic composition of the grazer functional group was strongly influenced by water chemistry in spring (Figure 2.8, panel *c*). In intermediate pH sites, Coleoptera (*Elmis*, *Esolus*, *Limnius* and *Oulimnius*) were numerically more abundant than Ephemeroptera (*Baetis*, *Rhithrogena* and *Electrogena*), whereas the converse was the case in the circumneutral sites. Both elmids and mayflies were scarce (Heptageniidae) or absent (Baetidae) in acid streams, whereas nemourid stoneflies (especially *Amphinemoura*) were the most abundant herbivorous group (Table 2.6). One-way ANOVA revealed that Ephemeroptera were proportionally less abundant in acid and intermediate than circumneutral streams (*df* = 29, *F* = 14.418, *P* < 0.001; Figure 2.8, panel *c*), driven mainly by baetid (mainly *Baetis rhodani*) and heptageniid (*Rhithrogena* and *Electrogena*) mayflies. The relative abundance of Coleoptera was also significantly lower in acid streams (*df* = 29, *F* = 4.789, *P* = 0.017; Figure 2.8, panel *c*), whereas Plecoptera (*Amphinemoura sulcicollis*) constituted a significantly greater proportion of macroinvertebrate individuals in acid sites (*df* = 29, *F* = 23.274, *P* < 0.001; Figure 2.8, panel *c*).

In summer, the composition of the grazers among pH groups was more uniform, with Emphemeroptera (Baetidae and Ephemerellidae) the most important order across the sites (Figure 2.8, panel *c*). Nevertheless, the percentage contribution of Ephemeroptera taxa within the grazer functional group was significantly higher in intermediate and circumneutral sites compared to acid sites (*df* = 29, *F* = 3.793, *P* = 0.035), driven by lower abundances of *Baetis* and *Seratella*. The relative abundance of Diptera was also significantly lower in acid streams (*df* = 29, *F* = 4.774, *P* = 0.017).

2.4.6.4 Predators

The relative abundance of the nine different orders contributing to the predator functional group remained constant across the three pH groups in both seasons (Figure 2.8, panel *d*). No significant differences could be found using one-way ANOVA. The taxonomic composition of the predator group, however, did vary with season (Table 2.6). In spring, Plecoptera (*Chloroperlidae*) dominated, followed by Trichoptera (*Plectrocnemia*). In summer, on the other hand, Hydracarina were the most abundant macroinvertebrate, followed by Trichoptera, Diptera (Empididae and Athericidae) and Plecoptera.

2.4.6.5 Shredders

The percentage contribution of different orders assigned to the shredder functional group did not vary significantly between pH groups (Figure 2.8, panel *e*), and remained similar between seasons. Diptera (Tipulidae) were the dominant macroinvertebrates, followed by Plecoptera (*Nemoura*) and Trichoptera (*Protonemura meyeri*). Amphipoda (*Gammarus pulex*) were only present two sites in the circumneutral pH group (Table 2.6).

2.5 DISCUSSION

Mean stream pH in 2006 varied considerably across the thirty study streams, from pH 4.4 in WY56, to pH 7.0 in LI7. Concentrations of the anion Cl^- (> 7.5 mg L^{-1}), the cation Na^+ (> 4.2 mg L^{-1}) and the metals Mn^{2+} (> 60 µg L^{-1}) and Al^{n+} (> 240 µg L^{-1}) were greatest in sites classified as acid, whereas circumneutral streams contained much higher concentrations of the micronutrients Ca^{2+} (> 2.2 mg L^{-1}) and Mg^{2+} (> 1 mg L^{-1}). Mean spring pH in eleven out of thirty study streams was less than 5.5, the critical limit for many aquatic macroinvertebrates (Sutcliffe & Carrick, 1973; Peterson & Eekhaute, 1992), and low dissolved calcium availability in 80% of sites was below the tolerance limit (2 mg L^{-1}) of the majority of aquatic gastropods and crustaceans (Sutcliffe & Carrick, 1973; Fryer, 1980; Økland, 1980a; 1980b). Furthermore, labile aluminium was higher than 0.1 mg L^{-1} in 47 and 50% of streams in spring and summer, respectively, the concentration at which aquatic organisms suffer toxic effects (Herrmann, 2001). These most recent data for upland streams in mid-Wales indicate that acid stress remains widespread, despite the reported regional trend of declining acid deposition, and are consistent with the findings of Ormerod & Durance (2009), that chemical recovery of streams in the Llyn Brianne catchment over the previous 25 years was incomplete. Lewis *et al*. (2007) reported similar findings in the upper Wye catchment, and concluded that lack of chemical recovery from acidification in low-order streams is having a continued and damaging effect on aquatic macroinvertebrate and algal populations.

All thirty study streams were oligotrophic, with mean dissolved nitrate and phosphate levels below 1 and 0.3 mg L^{-1}, respectively. This, coupled with the high level of streambed instability (mean Pfankuch Index of 55) demonstrated that upland headwater streams in Wales represent a harsh environment for aquatic organisms, regardless of acid-base status. Seasonal differences in water chemistry were observed in the study streams, with higher dissolved oxygen, and phosphate measured in spring. Due to higher water discharge rates and turbulence during winter and spring months, dissolved oxygen is often supersaturated (Hynes, 1970), particularly in highly disturbed stony streams. As temperature rises during the summer and abundance of plant and algal material increases, dissolved nutrients and oxygen are depleted (Stephenson *et al*., 1996).

The importance of water chemistry in shaping macroinvertebrate community structure was demonstrated by canonical correspondence analysis. Previous analysis of data from the River Wye and acid-sensitive catchments in Norway has revealed strong correlations between macroinvertebrate community structure and pH (Ormerod & Edwards, 1987; Larsen *et al*., 1996). Similarly in my streams, in spring at least, pH was the main factor governing differences in community composition, and a high level of species turnover existed between the most acid and circumneutral streams. Macroinvertebrate species associated with circumneutral sites included the mayflies *Baetis rhodani* and *Rhithrogena semicolorata*, which are known to be highly acid-sensitive (Smith *et al*., 1990; Guérold *et al*., 2000) and species associated with acid streams included *Nemurella pictetii*, *Nemoura avicularis* and *Amphinemoura sulcilcollis*, all of which are known to be acid-tolerant (Guérold *et al*., 1995; 2000). A secondary correlation between macroinvertebrate community composition and dissolved phosphate concentration was also revealed. The site with highest dissolved phosphate concentration, AC16, contained significant populations of *Gammarus pulex* and *Agapetus fuscipes*, both of which were scarce or absent in the other study streams.

The primary food source of both these species (aquatic plants and algae, respectively) grows best in nutrient rich waters (Hynes, 1970), and may explain why these species are limited to more enriched locations. The majority of the remaining sites contained low dissolved phosphate concentrations in comparison to AC16, emphasising their oligotrophic nature (Edwards and Brooker, 1982; Weatherley and Ormerod, 1987; Hornung *et al.*, 1990).

Macroinvertebrate taxon richness was significantly lower in acid streams in both seasons, and macroinvertebrate density was significantly lower in acid streams in spring. A similar reduction in taxon richness in acid streams compared to circumneutral streams has been reported elsewhere in the UK (Sutcliffe & Hildrew, 1989) and continental Europe (Guérold *et al.*, 2000). A total of eighty taxa were collected across all sites, which is low in comparison to the number found in similar headwater streams in France (151 taxa), Sweden (130 taxa), and Norway (127 taxa) (Larsen *et al.*, 1996; Guérold *et al.*, 2000; Petrin *et al.*, 2008a), and possibly reflects a lack of habitat or nutrient availability. Total macroinvertebrate richness was also lower in spring compared to summer. Low taxon richness in upland streams, either generally or as a result of acidification, could have important ramifications for ecosystem function and stability. The ecological consequences of biodiversity loss have been researched extensively in plant communities, and a large number of studies have demonstrated that communities with high species richness are more stable and functional relative to those with lower species richness (Tilman, 1996; Symstad *et al.*, 1998; Cardinale *et al.*, 2002). This trend is by no means universal however, with some researchers suggesting the identity of species within a community is a more accurate predictor of processing rates (Jonsson *et al.*, 2001a), and that communities experiencing non-random species losses, such as those observed as pH declines, can remain highly stable and functional (Smith & Knapp, 2003). Indeed, Chapin *et al.* (1997) suggest that differing sensitivity of organisms to a pollutant, such as acid deposition, actually results in increased community stability. However, for ecosystem stability and functioning to be maintained as species diversity declines, species loss must occur evenly across functional groups (Tilman *et al.*, 1997; Symstad *et al.*, 1998), which was not the case in the present study.

Analysis of macroinvertebrate functional composition across the pH gradient revealed a wholesale loss of grazers and filter-feeders in acid streams. Loss of filter-feeder richness in acid sites was due to the absence of several net-spinning caddis species of the genus *Philopotamus* and *Wormaldia*. Net-spinning caddis larvae are known to be highly sensitive to aluminium, a metal found in high concentrations in acid water (Herrmann, 2001). A similar reduction in filter-feeder abundance and richness has previously been reported elsewhere (Sutcliffe & Hildrew, 1989). Loss of grazer richness and density as pH declined was mainly due to the pH sensitivity of several Ephemeroptera and Coleoptera species. Acid-sensitive Ephemeroptera species included *Baetis rhodani* and *Rhithrogena semicolorata*, both of which were entirely absent from acid streams in spring. Similarly, the heptagenid mayfly *Electrogena lateralis* was absent from acid streams in summer. Elmid beetles including *Elmis aenea*, *Limnius volckmari* and *Oulimnius tuberculatus* were marginally less sensitive to low pH as they were recorded in acid streams, albeit in much lower densities. The presence of these beetles in acid streams is interesting, however, as they have previously been recorded as highly acid-sensitive and only present in circumneutral streams (Smith *et al.*, 1990). Wholesale loss of grazer density and richness at low pH is a frequently reported phenomenon (Sutcliffe & Carrick, 1973; Sutcliffe & Hildrew, 1989; Smith *et al.*, 1990; Peterson & Eekhaute, 1992; Larsen *et al.*, 1996; Friberg *et al.*, 1998; Guérold *et al.*, 2000) and is thought to be due to the combined influence of water toxicity (Sutcliffe & Hildrew, 1989) and impoverished food supply (Sutcliffe & Carrick, 1973; Collier & Winterbourn, 1987; Winterbourn *et al.*, 1992). The consequence of this loss for stream functioning, however, is unclear. Macroinvertebrate grazers play a pivotal role in algal biomass regulation and nutrient mobilisation (Wallace & Webster, 1996), so their absence in acid streams could have a profound impact on the flow of energy through the stream ecosystem. It is possible, however, that in spring at least, abundant *Amphinemoura sulcicollis* could functionally compensate for the loss of sensitive mayflies and beetles. This stonefly grazer was recorded in high densities in acid streams, and may be able to occupy the niche of *Baetis* and other acid-sensitive grazers in their absence. Further investigation is required, however, to establish the impact grazer species turnover has on grazer-periphyton interactions across an acidity gradient.

On the whole, and in disagreement with the predictions set out in my introduction, the relative abundance of predators, collector-gatherers and shredders was unaffected by changes in pH. However, despite relatively constant overall density and taxon richness of predators across sites of contrasting pH, there was some evidence to suggest that the abundance of a particular predator genus, namely *Plectrocnemia*, was higher in acid streams. Total density of *Plectrocnemia* spp. was 41.9 and 29.2 m^{-2} in acid sites, in spring and summer respectively, compared to densities of 15.6 and 22.7 m^{-2} in circumneutral streams, in spring and summer respectively, giving support to the idea that large-bodied predators benefit from a lack of fish predation in acid streams (Sutcliffe & Hildrew, 1989). The few published data available on collector-gatherer responses to acidity are contradictory. Smith and co-workers (1990) suggested that abundance of collector-gatherers is reduced at low pH, whereas Sutcliffe & Hildrew (1989) found that streams with a pH below 5.7 were dominated by them. In the present investigation, a general decrease in collector-gatherer richness and density was observed as pH declined, but this trend was not significant. It appears that the collector-gatherer group as a whole is tolerant of low stream pH, although a degree of species turnover was observed within this group as pH declined, with a higher relative abundance of Chironomidae recorded in acid streams and higher relative abundance of Plecoptera (*Leuctra hippopus*) recorded in circumneutral streams. Similarly, shredder abundance and richness remained constant across sites of contrasting pH, with the exception of *Gammarus pulex*, which has very specific water chemistry requirements (Hildrew *et al.*, 1984; Burton *et al.*, 1985; Dangles & Guérold, 2001). Case-building caddis larvae, which comprise a large proportion of the shredder group, are generally tolerant of the high aluminium levels found in acid water (Herrmann, 2001). The abundance of shredders is generally thought to be more strongly influenced by the availability of organic matter than by pH, and as such, this functional group is often dominant in acidic watercourses (Smith *et al.*, 1990). Some studies have suggested that richness (Tixier & Guérold, 2005) and abundance (Dangles & Guérold, 2000; Thomsen & Friberg, 2002) of the shredder group is reduced at low pH, but I could find no evidence for this in the present investigation.

In general, my research suggests that acidification continues to have a damaging effect on aquatic macroinvertebrate communities in upland Welsh streams.

This conclusion gives support to the recent findings of Ormerod & Durance (2009), who collated 25 years of data from the Llyn Brianne catchment, and determined that abundances of sensitive macroinvertebrate species have increased little over this time, either as a result of liming or natural improvements in stream chemistry. Over half of the acid sites examined during the present study had a minimum annual pH of less than 5.0, which is far below the tolerance limit of many freshwater invertebrates. Although detailed information regarding the effect of acidity on macroinvertebrate community structure exists, the consequences of acidity for the functional composition of macroinvertebrate communities in headwater streams has not previously been explored, and my data provide an interesting insight into the possible impact of low pH on valuable ecosystem processes. Macroinvertebrates within the grazer and filter-feeder functional groups appear most severely affected by acidification, due mainly to their intolerance of the high aluminium levels found in acid streams. The absence of grazers could have a profound impact on nutrient cycling and regulation of algal biofilms in acid streams, whereas loss of filter-feeders could have negative implications for nutrient retention and transfer of organic material to higher trophic levels. Further investigation is required to elucidate the impact losses such as these have on ecosystem processes. Within the collector-gatherer and grazer groups as least, there is evidence for species turnover between circumneutral and acid streams. It is possible, therefore, that acid-tolerant species may be able to functionally compensate for the loss of sensitive species in acid streams, and that important ecosystem functions, such as the interaction between grazers and periphyton, are maintained despite impoverished water quality. However, further investigation is required to quantify such interactions across an acidity gradient.

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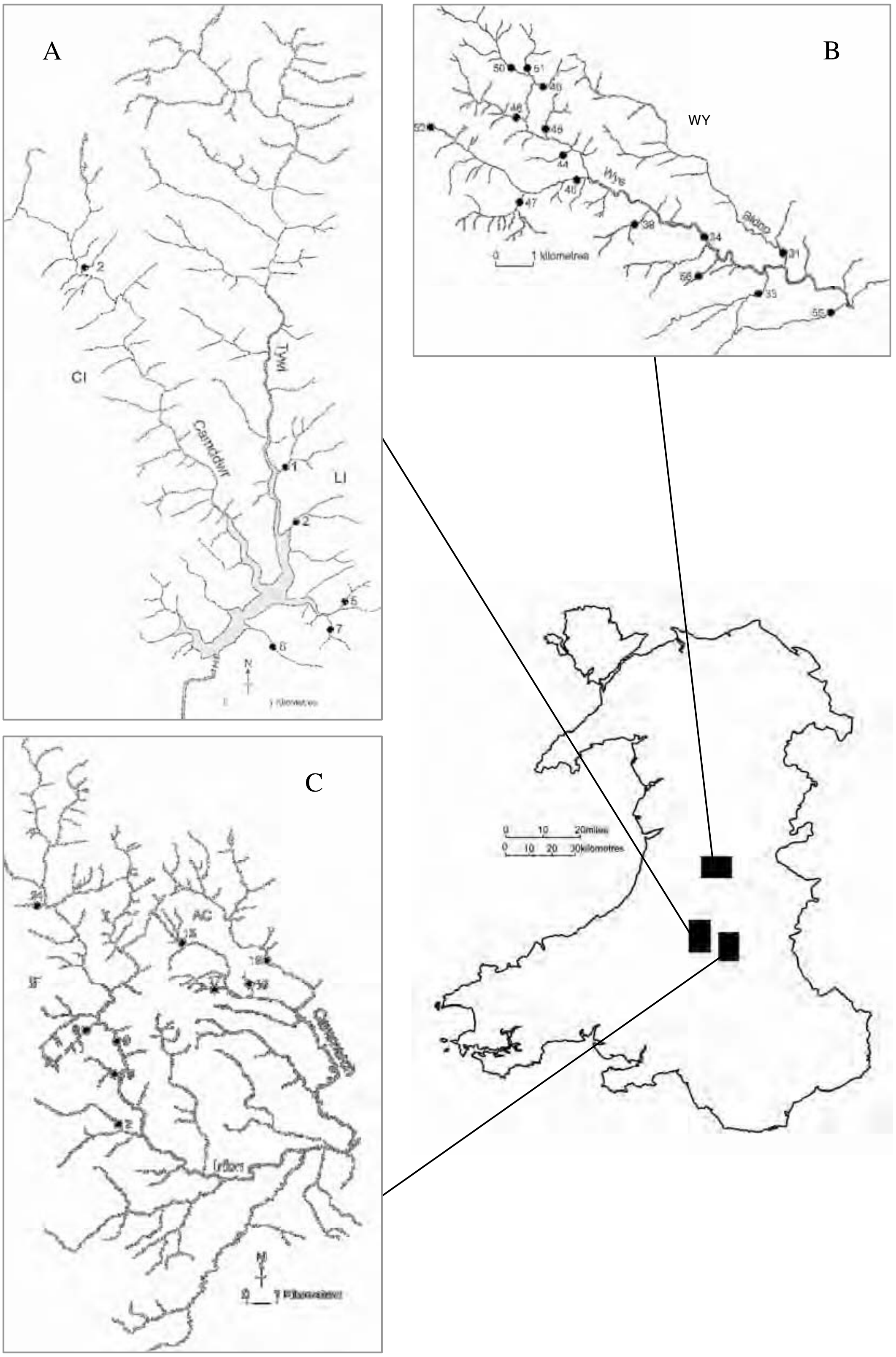


Figure 2.1 Location of 30 sampling sites in the Llyn Brianne (coded LI and CI, panel A), upper Wye (coded WY, panel B) and Irfon (coded AC and IF, panel C) catchments in mid-Wales (see Table 2.1 for geographical coordinates).

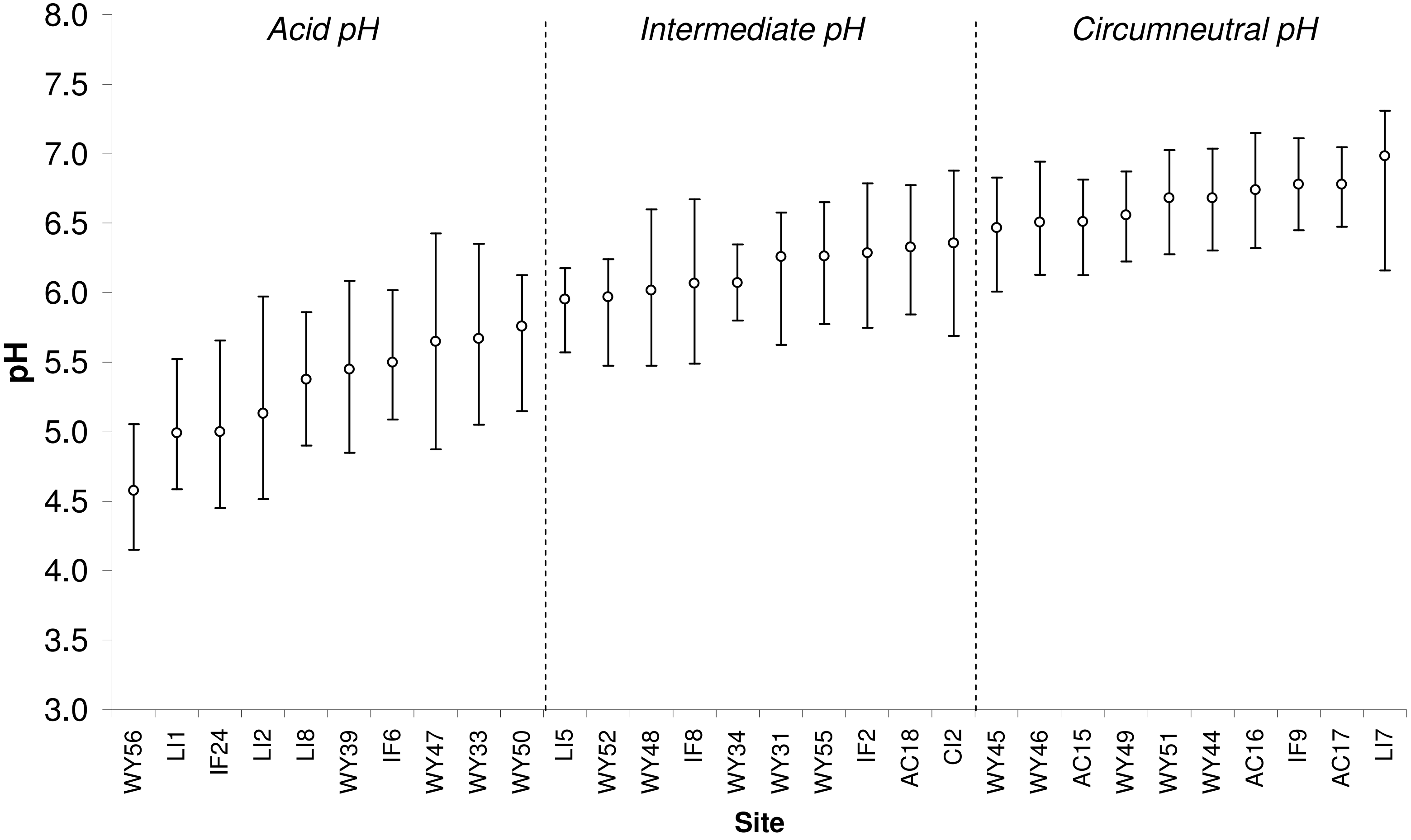


Figure 2.2 Mean (open circles), maximum and minimum (bar) pH sampled between 2003 and 2006 for thirty streams in the Llyn Brianne (n = 30), upper Wye (n = 17) and Irfon (n = 17) catchments of mid-Wales. Sites are arranged (left to right) in order of increasing mean pH, and are categorised as being of either acid, intermediate or circumneutral pH according to the classification described in Table 2.1.

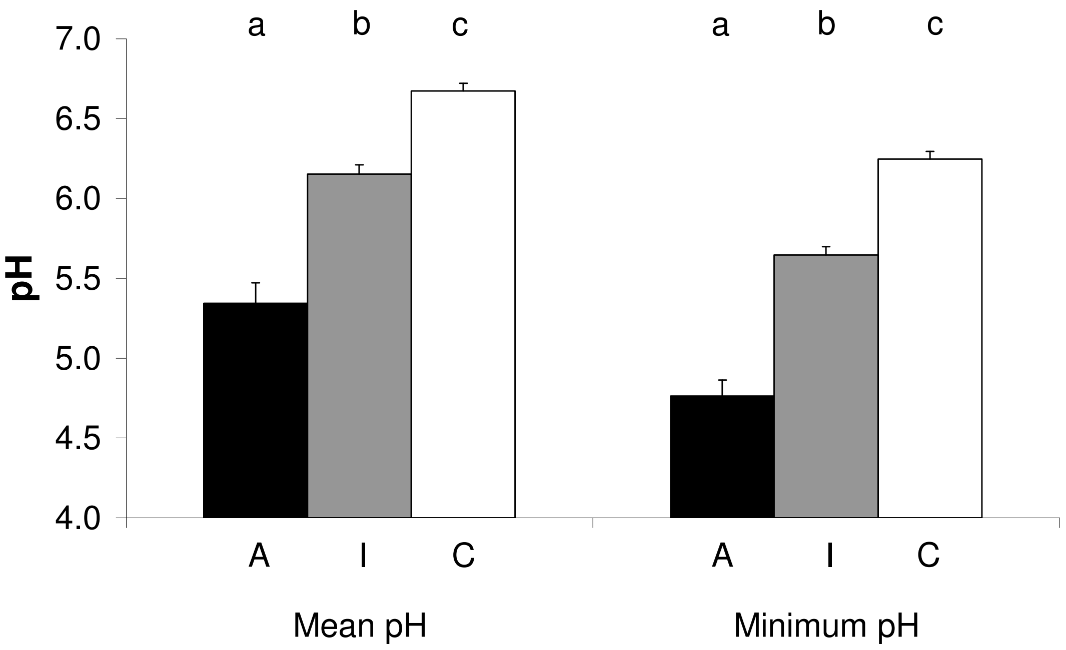


Figure 2.3 Mean and minimum (+ 1 SE) pH between 2003 and 2006 for acid pH (A, black bars), intermediate pH (I, grey bars) and circumneutral pH (C, white bars) streams. One-way ANOVA and Tukey HSD *post hoc* tests revealed differences among treatment means ($P < 0.05$), as denoted by different letters above each bar.

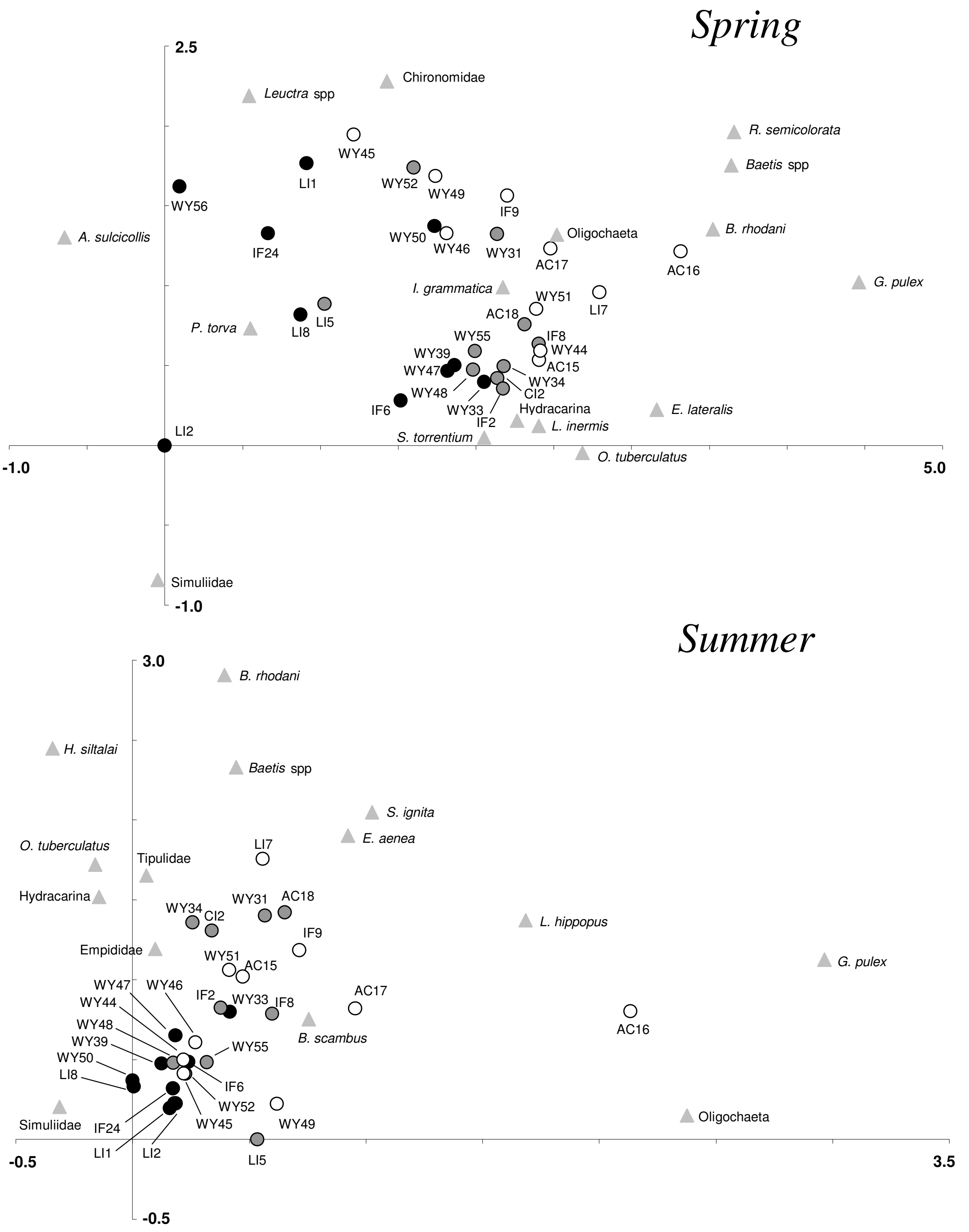


Figure 2.4 DCA ordination diagrams showing site scores (black circles, acid pH; grey circles, intermediate pH; white circles, circumneutral pH) and macroinvertebrate species scores (triangles) along the first two ordination axes, in spring and summer 2006. Eigenvalues of axes one and two are 0.484 and 0.224, respectively in spring, and 0.483 and 0.186, respectively in summer.

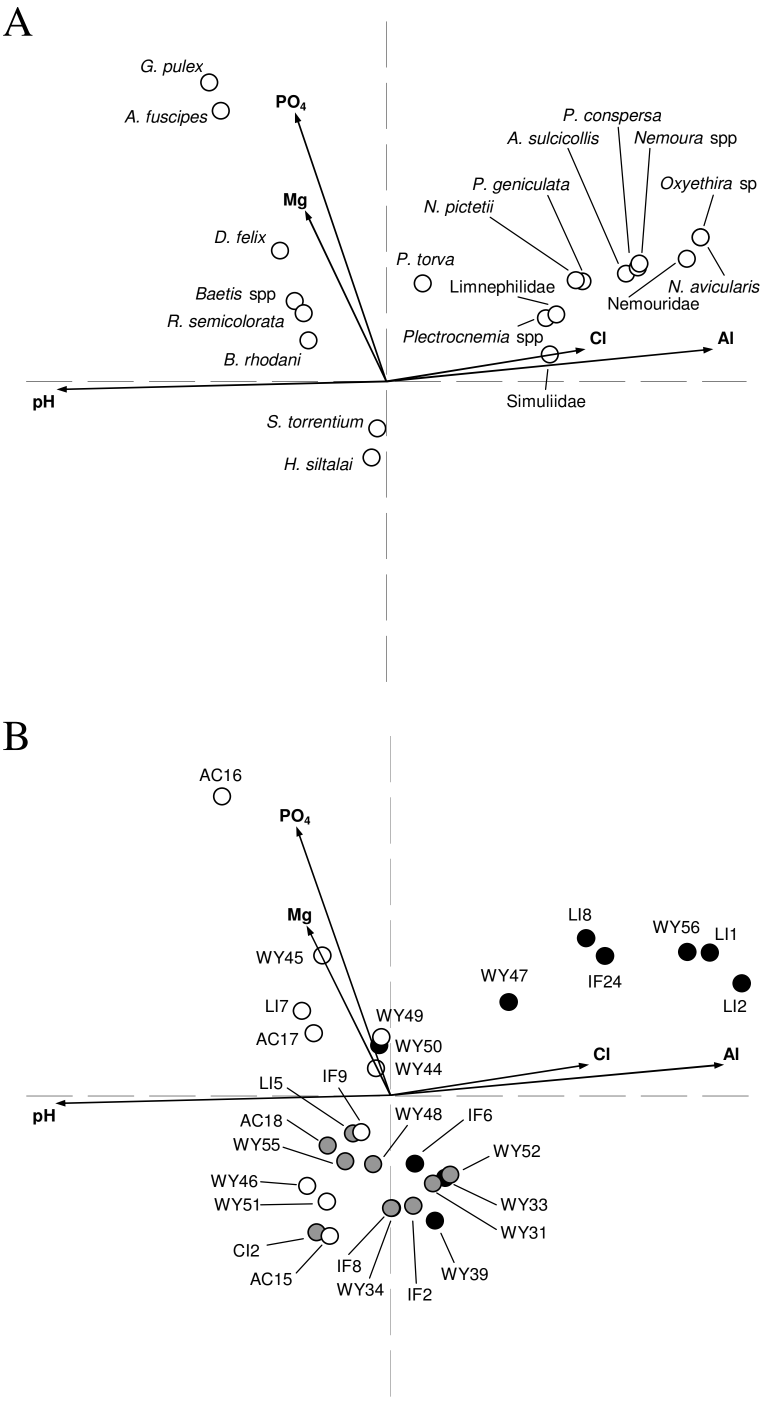


Figure 2.5 CCA ordination diagram showing the distribution of benthic invertebrates in 30 streams along a pH gradient in spring, with species scores (panel A) and site scores (panel B) shown separately (black circles, acid pH; grey circles, intermediate pH; white circles, circumneutral pH). Eigenvalues of axes one and two are 0.396 and 0.328 respectively.

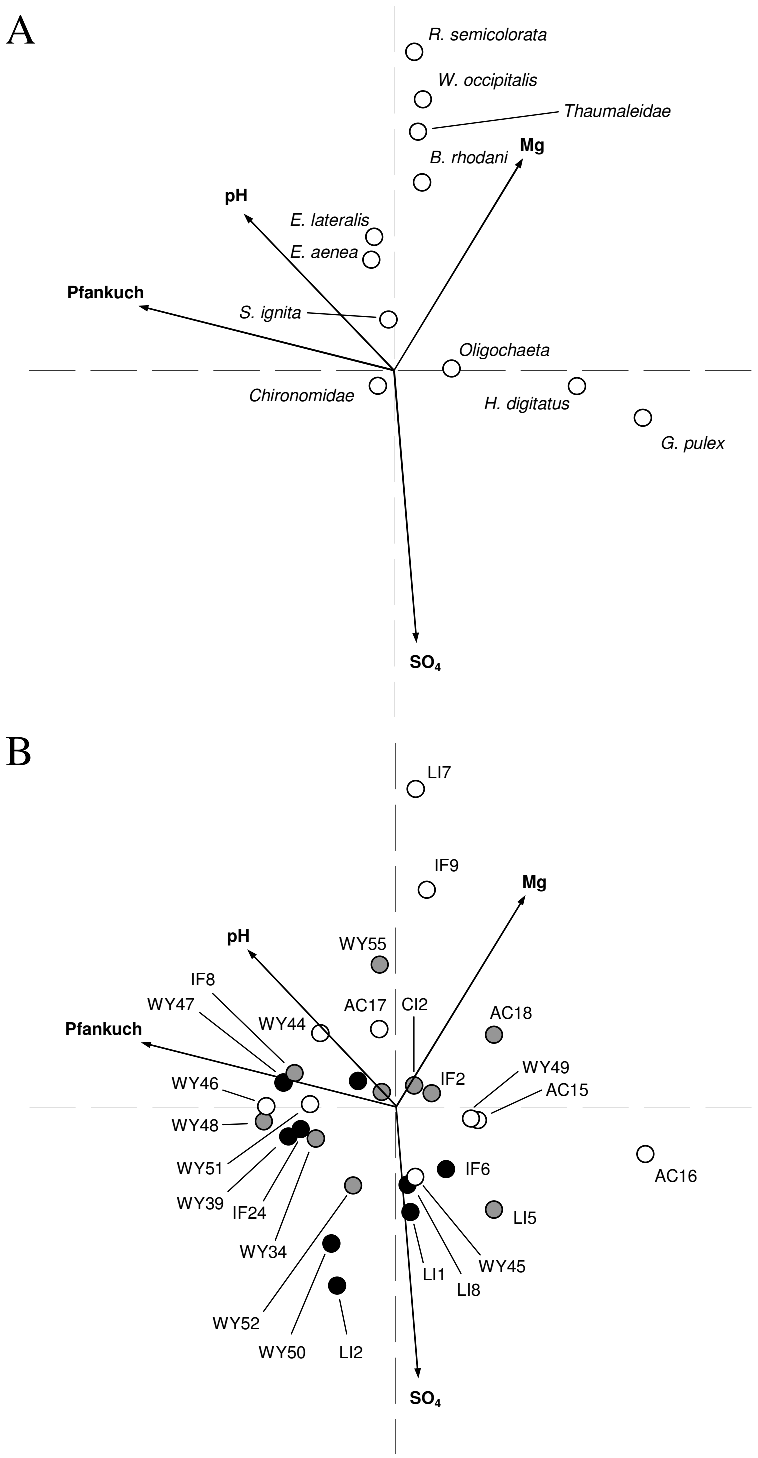


Figure 2.6 CCA ordination diagram showing the distribution of benthic invertebrates in 30 streams along a pH gradient in summer, with species scores (panel A) and site scores (panel B) shown separately (black circles, acid pH; grey circles, intermediate pH; white circles, circumneutral pH). Eigenvalues of axes one and two are 0.251 and 0.178 respectively.

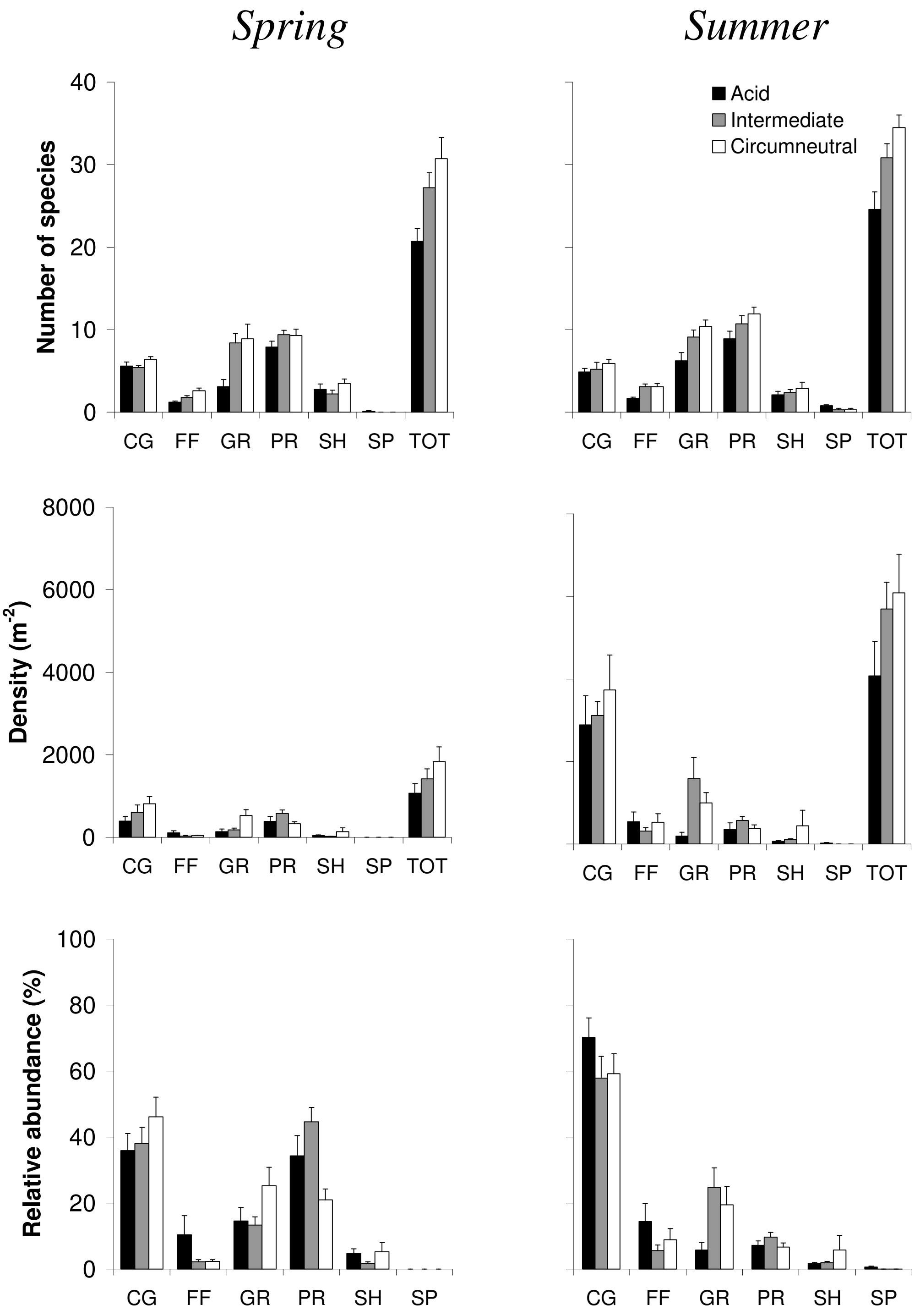


Figure 2.7 Composition of functional feeding groups (CG, collector-gatherer; FF, filter-feeder; GR, grazer; PR, predator; SH, shredder; SP, suctorial plant-feeder), in each pH category in 2006.

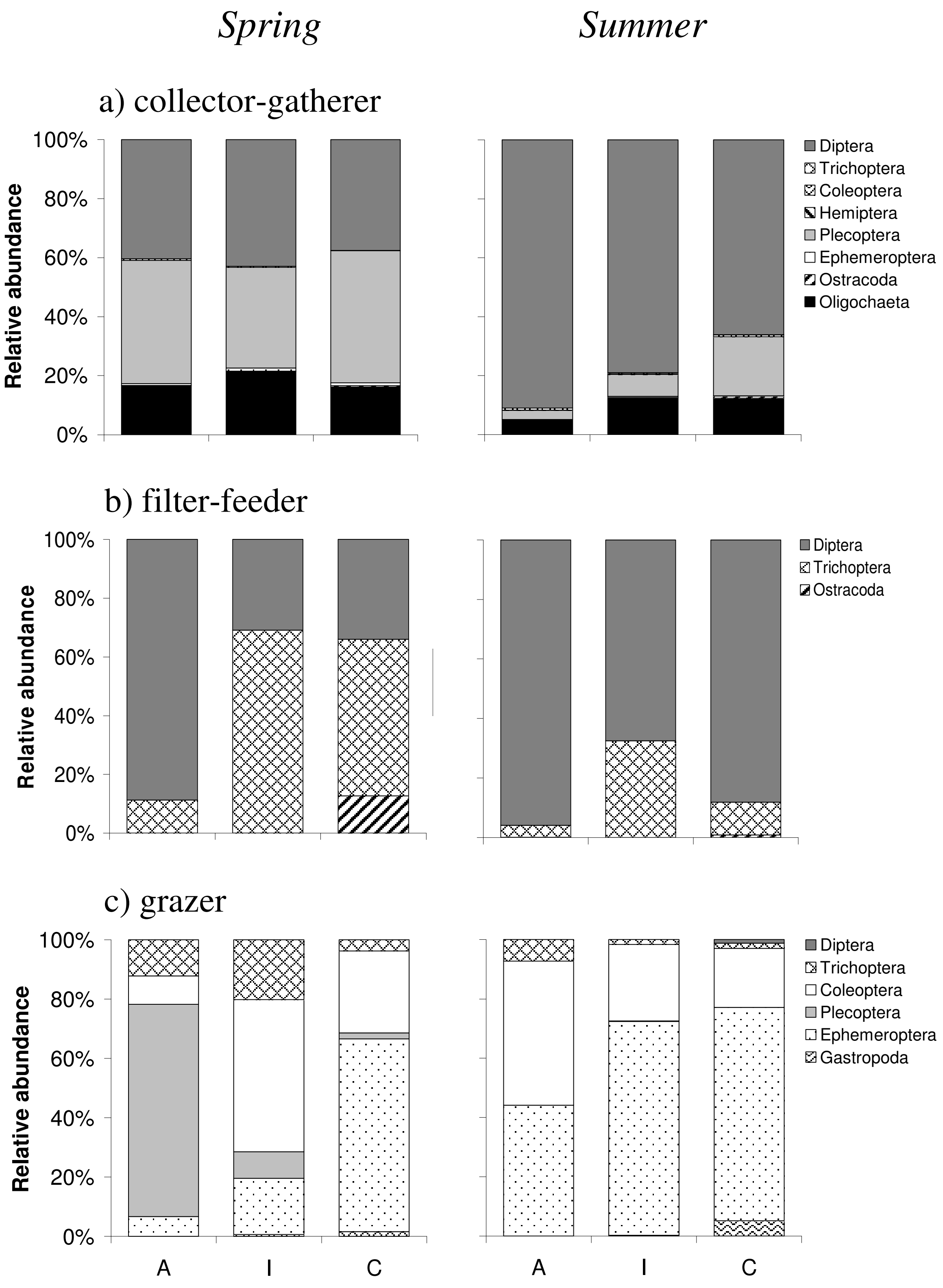
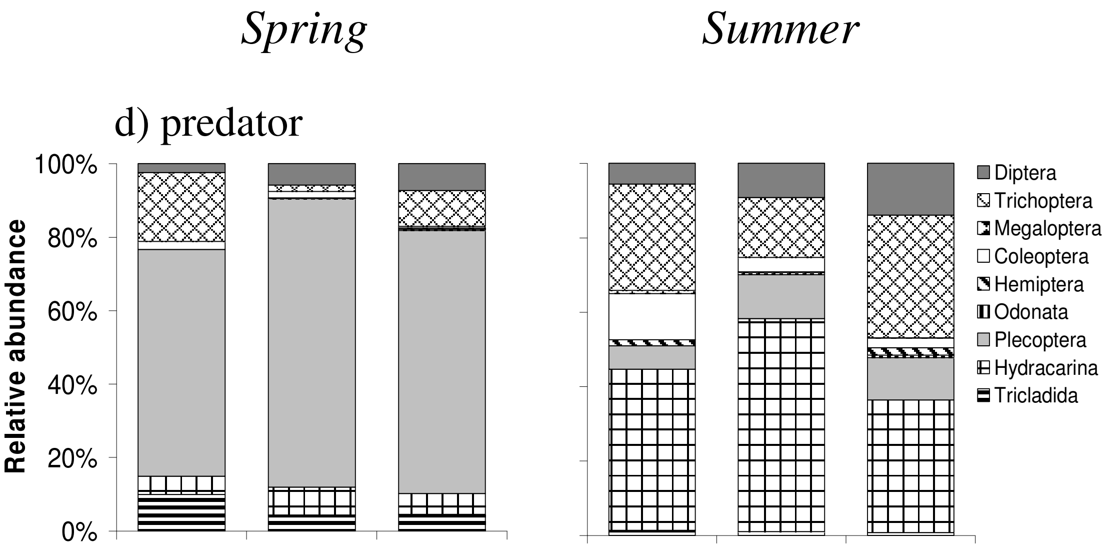


Figure 2.8 Taxonomic composition (mean relative abundance, %) of functional feeding groups across streams of contrasting pH (A, acid pH; I, intermediate pH; C, circumneutral pH) in spring and summer 2006.



e) shredder

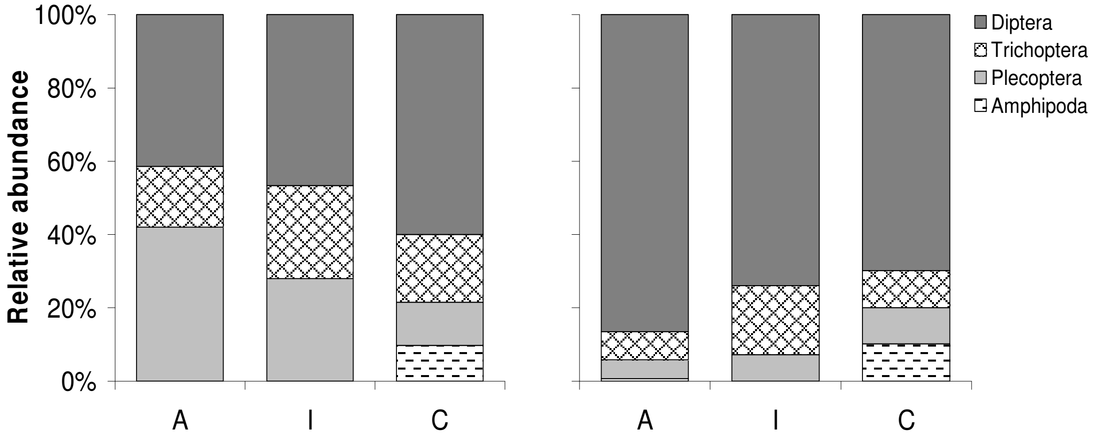


Figure 2.8 *continued.* Taxonomic composition (mean relative abundance, %) of functional feeding groups across streams of contrasting pH (A, acid pH; I, intermediate pH; C, circumneutral pH) in spring and summer 2006.

Table 2.1 Geographical location and classification (based on data collected between 2003 and 2006) of 30 study sites in the upper Wye, Irfon and Llyn Brianne catchments in mid-Wales.

Site Code	Catchment	GPS North	GPS West
Acid pH *Mean pH < 6.0; Minimum pH < 5.5*			
LI1	Llyn Brianne	52°09´71´´	3°44´52´´
LI2	Llyn Brianne	52°08´97´´	3°44´35´´
LI8	Llyn Brianne	52°07´50´´	3°44´73´´
IF6	Irfon	52°08´03´´	3°40´46´´
IF24	Irfon	52°11´21´´	3°42´61´´
WY33	Upper Wye	52°24´20´´	3°38´38´´
WY39	Upper Wye	52°25´12´´	3°41´25´´
WY47	Upper Wye	52°25´45´´	3°43´93´´
WY50	Upper Wye	52°27´23´´	3°43´94´´
WY56	Upper Wye	52°24´57´´	3°39´36´´
Intermediate pH *Mean pH 6.0 - 6.5; Minimum pH 5.5 - 6.0*			
LI5	Llyn Brianne	52°07´98´´	3°43´38´´
CI2	Llyn Brianne	52°11´95´´	3°48´51´´
IF2	Irfon	52°06´93´´	3°40´09´´
IF8	Irfon	52°08´60´´	3°40´35´´
AC18	Irfon	52°10´09´´	3°35´68´´
WY31	Upper Wye	52°24´85´´	3°37´90´´
WY34	Upper Wye	52°24´89´´	3°39´56´´
WY48	Upper Wye	52°25´74´´	3°42´42´´
WY52	Upper Wye	52°26´47´´	3°46´04´´
WY55	Upper Wye	52°23´99´´	3°36´47´´
Circumneutral pH *Mean pH > 6.5; Minimum pH > 6.0*			
LI7	Llyn Brianne	52°07´72´´	3°43´58´´
IF9	Irfon	52°08´95´´	3°41´04´´
AC15	Irfon	52°10´54´´	3°38´59´´
AC16	Irfon	52°09´64´´	3°36´46´´
AC17	Irfon	52°09´66´´	3°37´31´´
WY44	Upper Wye	52°26´11´´	3°42´86´´
WY45	Upper Wye	52°26´36´´	3°43´37´´
WY46	Upper Wye	52°26´58´´	3°43´95´´
WY49	Upper Wye	52°27´06´´	3°43´54´´
WY51	Upper Wye	52°27´24´´	3°43´80´´

Table 2.2 Physical characteristics of 30 study sites in the upper Wye, Irfon and Llyn Brianne catchments of mid-Wales. Land use is within 50 m of bank top (BL = broadleaf/mixed woodland, CP = coniferous plantation, IG = improved/semi-improved grassland, RP = rough/unimproved pasture). S.L.M is stream-link magnitude (see section 2.3.1.2 for definition).

Site	Altitude (m)	Slope (°)	Drainage Area (km^2)	Stream Order	S.L.M.	Pfankuch Index	Land Use
Acid pH							
LI1	310	5	3	3	5	58	CP
LI2	300	4	2	2	2	64	CP
LI8	316	2	1	1	1	68	CP
IF6	244	3	1	2	3	36	IG
IF24	330	7	1	1	1	71	CP
WY33	307	3	4	2	4	59	IG
WY39	325	3	2	2	3	74	CP
WY47	355	4	3	3	12	66	RP
WY50	393	3	3	3	10	54	RP
WY56	321	12	2	1	1	58	CP
Intermediate pH							
LI5	294	1	1	2	3	51	RP
CI2	368	2	1	3	8	36	RP
IF2	230	3	4	3	5	40	BL
IF8	261	3	47	4	66	69	BL
AC18	241	2	8	3	10	44	IG
WY31	298	3	12	3	17	58	IG
WY34	299	2	22	5	74	73	IG
WY48	314	2	11	4	32	71	RP
WY52	410	3	5	2	8	56	RP
WY55	290	3	5	1	1	65	IG
Circumneutral pH							
LI7	320	8	1	2	2	66	RP
IF9	261	4	2	2	3	56	IG
AC15	328	3	2	2	4	40	CP
AC16	252	7	1	1	1	20	IG
AC17	269	10	1	2	2	58	BL
WY44	357	7	1	1	1	68	IG
WY45	357	3	1	2	3	47	CP
WY46	367	3	4	3	11	58	RP
WY49	393	4	1	2	2	41	RP
WY51	392	2	2	2	3	54	RP

Table 2.3 Mean (± 1 SE) physicochemistry of sites in each pH group in spring and summer 2006. Results of ANOVA *post hoc* tests reveal significance of trends across pH groups (A, acid; I, intermediate; C, Circumneutral) and seasons (Sp, spring; Su, summer). Underlined groups were not significantly different at $P < 0.05$.

Variable	Acid pH		Intermediate pH		Circumneutral pH		*Post hoc* (Tukey)	
	Spring	Summer	Spring	Summer	Spring	Summer		
Na (mg L^{-1})	4.713 ± 0.233	4.399 ± 0.277	4.083 ± 0.144	3.532 ± 0.133	3.816 ± 0.121	3.289 ± 0.158	A > IC	Sp > Su
K (mg L^{-1})	0.179 ± 0.035	0.108 ± 0.022	0.160 ± 0.009	0.097 ± 0.012	0.207 ± 0.038	0.121 ± 0.023	AIC	Sp > Su
Ca (mg L^{-1})	1.267 ± 0.155	1.425 ± 0.260	1.355 ± 0.082	1.468 ± 0.073	2.155 ± 0.201	2.266 ± 0.213	AI < C	Sp Su
Mg (mg L^{-1})	0.789 ± 0.034	0.682 ± 0.033	0.754 ± 0.030	0.740 ± 0.045	1.017 ± 0.068	1.032 ± 0.081	AI < C	Sp Su
Mn (µg L^{-1})	76.45 ± 12.86	62.14 ± 12.74	34.32 ± 6.96	29.21 ± 6.35	20.36 ± 7.41	22.09 ± 8.24	A > IC	Sp Su
Al (µg L^{-1})	245.64 ± 36.74	240.82 ± 41.06	103.11 ± 15.73	101.41 ± 11.69	45.77 ± 5.38	55.10 ± 6.94	A > I > C	Sp Su
Fe (µg L^{-1})	88.57 ± 16.68	83.55 ± 10.15	71.92 ± 14.66	92.16 ± 22.71	48.66 ± 9.80	66.72 ± 9.76	AIC	Sp Su
Si (mg L^{-1})	1.296 ± 0.045	1.458 ± 0.090	0.995 ± 0.105	1.130 ± 0.107	1.074 ± 0.058	1.276 ± 0.177	A > IC	Sp < Su
Cl (mg L^{-1})	8.909 ± 0.473	7.794 ± 0.638	7.497 ± 0.478	5.920 ± 0.307	6.636 ± 0.191	5.400 ± 0.283	A > IC	Sp > Su
NO_3 (mg L^{-1})	0.912 ± 0.266	1.031 ± 0.214	0.506 ± 0.093	0.820 ± 0.136	1.025 ± 0.343	1.052 ± 0.244	AIC	Sp Su
SO_4 (mg L^{-1})	2.480 ± 0.414	3.672 ± 0.349	2.431 ± 0.380	2.501 ± 0.423	2.852 ± 0.439	3.186 ± 0.349	A > I	Sp Su
PO_4 (mg L^{-1})	0.040 ± 0.007	0.006 ± 0.002	0.025 ± 0.004	0.015 ± 0.006	0.033 ± 0.010	0.013 ± 0.005	AIC	Sp > Su
NPOC (mg L^{-1})	3.156 ± 0.582	2.253 ± 0.587	3.521 ± 0.215	2.676 ± 0.488	3.213 ± 0.244	1.977 ± 0.257	AIC	Sp > Su
Conductivity (µS cm^{-1})	45.12 ± 2.13	44.30 ± 2.83	39.34 ± 1.40	34.37 ± 2.12	44.14 ± 1.75	41.30 ± 1.81	AC > I	Sp Su
DO (%)	101.06 ± 0.31	100.24 ± 0.35	102.35 ± 0.71	99.53 ± 0.78	101.56 ± 0.27	99.39 ± 0.37	AIC	Sp > Su
Temperature (°C)	5.60 ± 0.16	11.23 ± 0.53	6.35 ± 0.41	12.37 ± 0.64	6.65 ± 0.38	11.74 ± 0.29	AIC	Sp < Su

Table 2.4 Importance of environmental variables entered singly (significant marginal effect) or sequentially (conditional effects) by the manual forward selection procedure during CCA of the macroinvertebrate community data set, and, the correlation coefficients describing the relationship between selected variables and the first two canonical axes (interest correlations) for the analyses. Spring and summer macroinvertebrate data were analysed separately. Analyses were considered significant at $P < 0.05$.

Marginal effects		Conditional effects				Interset correlations	
Variable	λ_1	Variable	Cum. λ_a	F	P	Axis 1	Axis 2
Spring CCA							
pH (April 2006)	0.36	pH	0.36	4.43	0.001	-0.846	-0.021
Al	0.34	Al	0.48	1.92	0.011	0.815	0.076
PO_4	0.27	PO_4	0.74	3.59	0.001	-0.244	0.723
Cl	0.23	Cl	0.88	2.17	0.003	0.535	0.089
Mg	0.18	Mg	1.05	2.38	0.002	-0.224	0.466
Summer CCA							
Pfankuch	0.20	Pfankuch	0.20	3.02	0.004	-0.619	0.153
Mg	0.16	Mg	0.35	2.40	0.010	0.342	0.547
SO_4	0.13	SO_4	0.48	2.24	0.007	0.047	-0.572
pH (July 2006)	0.12	pH	0.56	1.33	0.018	-0.369	0.395

λ_1 = eigenvalue for each single variable; cum. λ_a = cumulative total eigenvalues λ_a; P = significance level of effect, obtained by 999 Monte Carlo permutations.

Table 2.5 Mean macroinvertebrates taxon richness, density (m^{-2}) and relative abundance (%) for functional feeding groups across sites of contrasting pH in spring and summer 2006. Results of *post hoc* tests are shown only where ANOVA were significant (*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$). Abbreviations: A, acid pH; I, intermediate pH; C, circumneutral pH.

Feeding Guild	Acid pH	Interm. pH	Circum. pH	Tukey	Acid pH	Interm. pH	Circum. pH	Tukey
	Spring				Summer			
Taxon richness								
Collector-gatherer	5.6	5.4	6.4		4.9	5.2	5.8	
Filter-feeder	1.2	1.8	2.6	AI < C***	1.7	3.1	3.1	A < IC***
Grazer	3.1	8.4	8.9	A < IC**	6.2	9.1	10.4	A < IC**
Predator	7.9	9.4	9.3		8.9	10.7	11.9	
Shredder	2.8	2.2	3.5		2.1	2.4	3.0	
Suctorial plant-feeder	0.1	0.0	0.0		0.8	0.3	0.3	
Total	20.7	27.2	30.7	A < IC**	24.6	30.8	34.5	A < IC***
Density (m^{-2})								
Collector-gatherer	393.7	607.0	808.6		2887.5	3113.6	3736.3	
Filter-feeder	107.2	34.2	41.6		542.9	314.2	528.0	
Grazer	141.4	182.1	525.8	AI < C*	194.8	1589.1	998.4	A < I*
Predator	387.5	575.4	329.9		357.0	568.6	378.9	
Shredder	41.0	18.9	132.8		72.6	100.4	486.0	
Suctorial plant-feeder	0.6	0.0	0.0		24.2	2.2	2.2	
Total	1071.4	1417.6	1838.7		4079.0	5688.1	6129.8	
Relative abundance (%)								
Collector-gatherer	35.8	38.0	46.2		70.3	57.9	59.1	
Filter-feeder	10.4	2.2	2.3		14.4	5.7	8.9	
Grazer	14.7	13.4	25.2		5.8	24.7	19.5	A < I*
Predator	34.3	44.7	21.0	I > C**	7.2	9.7	6.7	
Shredder	4.7	1.7	5.3		1.7	2.0	5.8	
Suctorial plant-feeder	0.1	0.0	0.0		0.6	0.0	0.0	A > IC*
Total	100	100	100		100	100	100	

Table 2.6 Mean density (m^{-2}) and relative abundance (%, in parentheses) of core taxa (≥ 1% total numbers in at least one group of sites) in each pH group in spring (SP) and summer (SU) 2006. Functional feeding group abbreviations are CG, collector-gatherer; FF, filter-feeder; GR, grazer; PR, predator; SH, shredder (- denotes absence in group).

Feeding Group	Species	Acid pH		Intermediate pH		Circumneutral pH	
		SP	SU	SP	SU	SP	SU
CG	Oligochaeta	46.4 (4.7)	137.6 (3.4)	123.5 (7.8)	374.7 (5.9)	117.8 (6.2)	586.2 (7.0)
	Leuctra spp.	31.4 (2.2)	1.4 (0.01)	21.2 (1.3)	1 (0.02)	37.1 (1.7)	6.7 (0.1)
	Leuctra hippopus	8 (1.2)	77.9 (2.1)	2.6 (0.2)	198.7 (3.5)	16.3 (2.1)	481.3 (9.6)
	Leuctra inermis	92.5 (8.6)	-	102.1 (10)	-	241 (13.6)	-
	Leuctra nigra	16.3 (2.3)	1.4 (0.04)	-	0.6 (0.02)	11.2 (0.6)	0.6 (0.02)
	Chironomidae	191 (16.4)	2650 (64)	350 (18.2)	2501 (47)	376 (21.2)	2615 (42)
	Total	**377.6 (35)**	**2866 (70)**	**596.8 (38)**	**3074 (56)**	**738.1 (45)**	**3683 (59)**
FF	*Hydropsyche siltalai*	1 (0.1)	5.3 (0.1)	21.1 (1.3)	113.6 (2.2)	8.6 (0.6)	28.8 (0.5)
	Simuliidae	106 (10.3)	536 (14.3)	12.8 (0.9)	192 (3.4)	14.1 (0.8)	491 (8.2)
	Total	**107 (10)**	**541.3 (14)**	**33.9 (2.2)**	**305.6 (5.6)**	**22.7 (1.4)**	**519.8 (8.7)**
GR	*Baetis* spp.	-	52.3 (1.2)	8 (0.4)	859 (12.7)	148.5 (5.6)	376.3 (7.3)
	Baetis rhodani	-	4.3 (0.1)	18.9 (1.0)	55 (0.8)	138.6 (7.0)	133.4 (3.0)
	Baetis scambus	-	6.4 (0.1)	-	69.1 (1.4)	-	21.2 (0.3)
	Rhithrogena semicolorata	-	-	8 (0.4)	-	73.9 (3.2)	0.3 (0.01)
	Electrogena lateralis	4.5 (0.4)	-	16 (1.1)	7.7 (0.1)	52.8 (3.4)	31.4 (0.7)
	Seratella ignita	-	23.1 (0.4)	-	259.5 (3.9)	-	202.9 (3.7)
	Amphinemoura sulcicollis	115 (12.8)	-	9.6 (1.3)	0.6 (0.02)	9.9 (0.4)	-
	Elmis aenea	1.3 (0.1)	22 (1.1)	8 (0.6)	29.1 (0.5)	34.9 (1.7)	90.9 (2.0)
	Limnius volckmari	2.6 (0.2)	12.8 (0.5)	26.9 (2.0)	22.7 (0.4)	12.8 (0.7)	20.2 (0.3)
	Oulimnius tuberculatus	1.6 (0.1)	27.7 (1.2)	44.8 (3.2)	203.5 (3.8)	6.1 (0.5)	31.4 (0.4)
	Total	**126 (13.6)**	**156.8 (4.6)**	**149.8 (10)**	**1535 (24)**	**481 (23)**	**920.8 (18)**
PR	*Planaria torva*	21.4 (2)	2.8 (0.1)	13.8 (1.9)	3.8 (0.1)	18.2 (0.7)	1.6 (0.04)
	Hydracarina	16 (1.3)	209.1 (3.9)	54.7 (3.3)	372.2 (5.9)	17.3 (1.2)	163.8 (2.7)
	Isoperla grammatica	31.4 (2.4)	0.4 (0.01)	35.2 (2.3)	1.9 (0.03)	21.4 (1.3)	2.2 (0.05)
	Chloroperla tripunctata	2.9 (0.5)	-	8.6 (1.0)	-	19.2 (0.9)	-
	Siphonoperla torrentium	253 (22.3)	13.5 (0.3)	387 (31.2)	39 (1.0)	196 (13.0)	22.7 (0.5)
	Plectrocnemia spp.	25 (2.4)	23.5 (0.6)	3.8 (0.3)	9.6 (0.2)	8.3 (0.7)	12.2 (0.2)
	Athericidae	-	-	33.9 (1.6)	21.4 (0.3)	-	-
	Empididae	8.6 (0.8)	27 (0.4)	12.5 (0.8)	19.5 (0.4)	18.6 (1.3)	57.6 (1.0)
	Total	**358.3 (32)**	**276.3 (5.3)**	**549.5 (42)**	**467.4 (7.9)**	**299.0 (19)**	**260.1 (4.5)**
SH	*Gammarus pulex*	-	-	-	-	96 (2.8)	369.9 (4.5)
	Protonemura meyeri	10.6 (1.8)	-	-	-	2.2 (0.1)	0.3 (0.01)
	Nemoura spp.	14.7 (1.0)	0.7 (0.02)	2.9 (0.4)	-	0.3 (0.1)	-
	Tipulidae	7.7 (1.2)	59.4 (1.4)	6.7 (0.4)	74.6 (1.3)	25.3 (1.5)	45.4 (0.8)
	Total	**33 (4)**	**60.1 (1.4)**	**9.6 (0.8)**	**74.6 (1.3)**	**123.8 (4.5)**	**415.6 (5.3)**

Table 2.7 Results of two-way ANOVA testing the main effect of functional feeding group, stream pH group, and their interaction, on taxon richness, density (m^{-2}) and relative abundance (%) of benthic macroinvertebrates.

	df	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Spring		Species richness			Density (m^{-2})			Relative abundance (%)		
Functional group	5	1668.13	75.591	< 0.001	8717492	22.584	< 0.001	42067.3	60.125	< 0.001
pH group	2	85.83	9.724	< 0.001	492258	3.188	0.044	< 0.001	< 0.001	1.000
Functional × pH	10	158.83	3.599	< 0.001	1695576	2.196	0.020	4765.38	3.405	< 0.001
Error	162	715.00			12506310			22669.1		
Summer										
Functional group	5	2272.88	454.576	< 0.001	215540248	42.005	< 0.001	80230.5	118.579	< 0.001
pH group	2	84.21	42.104	< 0.001	3802298	1.853	0.160	< 0.001	< 0.001	1.000
Functional × pH	10	76.93	7.693	0.033	11318237	1.103	0.363	33.97.66	2.511	0.008
Error	162	611.88	3.777		166254207			21921.8		

CHAPTER THREE

IMPACT OF ACIDITY ON ALGAL-GRAZER INTERACTIONS IN UPLAND HEADWATER STREAMS

3.1 SUMMARY

1. Acidification experiments often report an increase in algal abundance in response to acid treatments, and it has been suggested that algal proliferation is driven by the loss of acid-sensitive macroinvertebrate grazers which typically suppress algal growth. This 'grazer release' hypothesis is yet to be tested in natural stream environments however, and it remains to be tested whether algal biomass in upland headwater streams is regulated primarily by abiotic or biotic factors.
2. Here I report research which investigated the effect of acidification on algal-herbivore interactions in a suite of upland streams in mid-Wales. I examined trends in the abundance of benthic algae and macroinvertebrate grazers, and measured the strength of herbivory, across streams ranging in pH from profoundly acid to circumneutral, in order to establish the extent to which algal abundance is regulated by abiotic (e.g. water quality) or biotic (grazing) factors in acid-sensitive river catchments.
3. Estimates of algal abundance and grazing pressure were obtained using artificial substrata (5×2 pairs of unglazed clay tiles) placed in 30 streams of contrasting pH during spring and summer 2006 (56 d experimental period). Quantitative estimates of grazer community structure (species identity, taxon

richness and density) and water quality were also made during the experimental period in order to elucidate the primary influence on algal biomass.

4. Algal abundance did not correlate with any water chemistry variables (pH, Al^{n+}, NO_3^-, PO_4^{3-}, Si) in either season. However, algal abundance was generally lower in acid streams in summer, and I speculate that nutrients may be limiting to algal growth in these sites.
5. Herbivore impact on algal abundance in spring was highest in circumneutral streams with abundant grazer populations. However, grazing pressure was undetectable in acid sites and there was no evidence for algal proliferation at low pH. In summer, grazing pressure was weak or absent across the sites, possibly due to the small size of mayfly larvae during this period. Overall, these findings are inconsistent with the ‘grazer release’ hypothesis.
6. I conclude that high levels of hydrological and chemical disturbance reduce the strength of algal-grazer interactions in upland headwater streams, and that there is little evidence for ‘top-down’ control of algal biomass in the majority of upland sites.

3.2 INTRODUCTION

Macroinvertebrate grazers are an important component of benthic communities in running waters, and form an essential link in the food web between benthic algae and predators (Biggs, 1996; Stevenson, 1996). Over the past three decades, a substantial body of literature has developed describing the nature of algal-grazer interactions in streams (e.g. see reviews by Hildrew, 1992; Reynolds, 1992; Steinman, 1996). Many controlled experiments have revealed strong top-down effects of macroinvertebrate grazers on the presence and abundance of benthic algae (Gregory, 1983; Lamberti & Moore, 1984; Feminella *et al.*, 1989; Lamberti *et al.*, 1989). In their review, Feminella & Hawkins (1995) reported that 70% of herbivory studies showed a significant decrease in periphyton abundance in the presence grazers. For example, Hill & Knight (1987) found that periphyton biomass halved in the presence of the grazer *Ameletus validus* (Ephemeroptera). Similarly, Feminella *et al.* (1989) demonstrated that algal standing crop was significantly reduced in the presence of caddisfly larvae and snails. The strength of algal-grazer interactions is rarely static however, but varies with season (Biggs, 1996). During summer months, algal production is generally greater due to increased day length, solar energy input and, in turn, water temperature (Lund, 1965). The level of grazing pressure, on the other hand, tends to reflect the abundance of algal food available to grazers, and is synchronised with their seasonal abundance and life-cycle (Reynolds, 1992). Despite the profusion of studies relating to herbivory in streams, most have been conducted in physically stable, productive systems or in controlled laboratory or field enclosures, often with a limited range of taxa, notably snails, caddis and mayflies (Hildrew, 1992). By contrast, relatively little is known about the strength of interactions in nutrient poor upland ecosystems, or about the effects of environmental pollution on this important trophic pathway.

Macroinvertebrate grazer-scrapers are a functionally important component of most pristine stream benthic communities, but they are sensitive to a variety of environmental stressors, most notably inputs of acid anions. Acid streams are frequently characterised by lower abundance and diversity of the grazer functional group (Sutcliffe & Carrick, 1973; Sutcliffe & Hildrew, 1989; Smith *et al*., 1990; Peterson & Eekhaute, 1992; Larsen *et al*., 1996; Friberg *et al*., 1998; Guérold *et al*., 2000), and sensitive species are generally absent in streams with a mean pH < 5.5 (Sutcliffe & Carrick, 1973; Økland, 1980b; Økland, 1980a; Haines, 1981; Stoner *et al*., 1984). Elimination of sensitive species in acid streams occurs primarily as a result of osmotic stress and impaired ion regulation caused by the presence of high dissolved hydrogen and aluminium concentrations (Sutcliffe & Hildrew, 1989; Herrmann, 2001).

Whilst effects of low pH on macroinvertebrate grazer density and richness have been well described, there is no consensus among the few studies undertaken to date as to the responses of benthic algal assemblages to decreasing pH, and specifically, to the corresponding depletion of the consumer population. The majority of research in this area has focused on lentic algal communities (e.g. Schindler *et al*., 1985; Edberg *et al*., 2001; Vinebrooke *et al*., 2003), and there is a basic lack of understanding as to how stream benthic algae respond to acidification. Where the impact of acidity on stream algae has been examined, these studies have been focused on changes to algal community structure (Moss, 1973; Mulholland *et al*., 1986; Marker & Willoughby, 1988; Planas *et al*., 1989; Winterbourn *et al*., 1992, Kinross *et* *al*., 1993; Hirst *et al*., 2004; Ledger & Hildrew, 2005), with fewer studies focusing on algal abundance. Acidification experiments in artificial stream mesocosms have revealed a positive relationship between acidity and algal biomass (Hendrey, 1976; Hall *et al*., 1980; Allard & Moreau, 1985; Parent *et al*., 1986; Planas *et al*., 1989), with up to five-fold increases in algal abundance found in acidified channels relative to circumneutral controls (Allard & Moreau, 1985), whereas studies conducted in more natural stream environments have found no such trend (Maurice *et al*., 1987; Marker & Willoughby, 1988; Collier & Winterbourn, 1990; Winterbourn *et al*., 1992; Kinross *et al*., 1993).

In cases where algae have proliferated under acidic conditions the 'grazer release' hypothesis has been put forward in explanation (Hall *et al*., 1980; Hendrey, 1976; Stokes, 1986; Planas *et al*., 1989). Experimental studies using manipulated channels have shown an inverse relationship between grazer abundance and algal biomass (Lamberti & Resh, 1983; Yasuno *et al*., 1985; Feminella *et al*., 1989; Winterbourn, 1990), suggesting a 'top-down' influence on algal standing crop. Where acidification reduces grazer density (Sutcliffe & Carrick, 1973; Townsend *et al*., 1983; Mulholland *et al*., 1986; Økland & Økland, 1986; Rosemond *et al*., 1992), easing grazing pressure, periphyton may proliferate. Experiments in circumneutral streams involving direct manipulation of grazer density lend support to this theory. For example, Hill and Knight (1987) found that periphyton biomass declined with increasing density of the mayfly *Ameletus validus,* and Feminella *et al.* (1989) found more algae on tiles raised above the substratum to exclude crawling grazers (including caddisfly larvae and snails) than on control tiles placed directly on the stream bed. However, few studies have examined the extent of these trophic dynamics in acid streams (but see Sutcliffe & Carrick, 1973; Townsend *et al*., 1983; Mulholland *et al*., 1986; Økland & Økland, 1986; Rosemond *et al*., 1992), and it is still unclear whether algal biomass in acid-sensitive upland river catchments is regulated primarily by biotic or abiotic factors.

The research described in this chapter examines the effect of acidification on algal-grazer interactions in 30 streams distributed across the upper Wye, Irfon and Llyn Brianne catchments. Trends in the abundance of epilithic algae and macroinvertebrate grazers were traced across streams of contrasting pH, and in-stream experimental substrata were used to determine the strength of the interaction between grazers and algae across the pH gradient. Consistent with the 'grazer release' hypothesis, I predicted that the influence of grazers on benthic algae would decrease with increasing acidity, in response to the declining strength of herbivory. Furthermore, algal abundance in the presence and absence of grazers was compared to determine whether biofilms in upland headwater streams are regulated primarily by abiotic or biotic factors.

3.3 METHODS

3.3.1 Sampling design

Estimates of algal abundance and macroinvertebrate grazing were obtained using artificial substrata (unglazed clay tiles, surface area 64 cm^2) placed in 30 headwater streams during spring (mid-March to mid-May) and summer (mid-June to mid-August) 2006. The use of tiles as artificial substrates has been shown to reduce algal sampling variability and minimise disturbance to the stream habitat (Lamberti & Resh, 1985). At each site, two tiles were placed pairwise in five 'run' habitats as substrata for algal colonization. Each pair of tiles was attached to a clay house brick for stability and anchored to the stream bed using a metal stake. Grazing macroinvertebrates were excluded from one tile in each pair by coating the edge of the tile with petroleum jelly (McAuliffe, 1984a; Jacoby, 1985; Dudley & D'Antonio, 1991). The second tile in the pair served as an untreated control.

Tiles were left *in situ* for a period of 56 days in both spring and summer. At the end of each sampling period, tiles were removed from the stream bed, and any invertebrates present on the surface of the tiles collected. Algal biofilm was removed from the upper surface of each tile using a toothbrush, and washed into a 24 ml polypropylene bottle containing stream water. Care was taken not to incorporate any petroleum jelly into the sample. Biofilm samples were placed immediately in a cool box (0°C) and frozen within five hours of collection. Aliquots were subsequently drawn from these samples for algal abundance estimates (ash-free dry mass and chlorophyll *a*).

Aquatic macroinvertebrates were collected at random from riffles in each of the 30 streams at the mid-point of each tile incubation period (~ 4 weeks) using a standard Surber sampler (mesh aperture 250 µm, 0.0625 m^2 area), and preserved in 4% formaldehyde. At each site, a contemporaneous water sample was filtered immediately using cellulose-nitrate filter membranes (Whatman, 0.45 µm, 47 mm diameter), transferred to sterile plastic bottles and refrigerated (< 48 h). Samples for metal (Ca^{2+} and Al^{n+}) analysis were acidified after collection to pH 2.0 using 70% Aristar nitric acid. Dissolved oxygen concentration (YSI 550A DO Meter), total dissolved solids and conductivity (Myron Utrameter II 6P) were measured directly in the field. Stream water pH was measured monthly between March and August 2006 using a handheld pH meter (HANNA HI9024C). The physical stability of the stream bed at each site was assessed using the Pfankuch Index (Pfankuch, 1975) (see Chapter 2).

3.3.2 Laboratory protocols

Concentrations of anions ($NO_3^-$and PO_4^{3-}) in water samples were determined by ion chromatography (Dionex, ICS-2000) using the gradient method. Metal (Ca^{2+} and Al^{n+}) and metalloid (Si) concentrations were analysed using an Agilent 7500ce ICP-MS. Non-purgeable organic carbon (NPOC) was measured using a TOC analyser (Shimadzu TOC-V CSH).

Biofilm samples were defrosted and diluted to 24 ml were necessary using deionised water. Chlorophyll pigments were extracted from 5-10 ml aliquots of biofilm slurry using 10 ml of 90% acetone (extracted overnight at 5°C, in the dark), following freeze-drying. The concentration of chlorophyll *a* was calculated after measuring absorbance of the extraction at wavelengths of 664 nm, 647 nm and 630 nm (WPA Lightwave S2000 UV/Visible Spectrophotometer, and 1 cm quartz cuvettes) and correcting for turbidity by subtracting absorbance at 750 nm (Jeffrey & Humphrey, 1975; Sterman, 1988). A further 5-10 ml aliquot of biofilm slurry was placed in a crucible for ashing (500°C for 4 h). Ash-free dry mass (AFDM) was estimated as the difference in mass (mg) between oven dried (80°C) and ashed crucibles.

Surber samples were rinsed to remove fixative, and all macroinvertebrates then sorted from debris and identified to the lowest practicable taxonomic unit (generally species level, excepting Diptera and Oligochaeta) using a binocular (Zeiss Stemi 2000), and where necessary, compound microscope (Nikon Optiphot-2).

3.3.3 Data analysis

Due to the high level of similarity between chlorophyll *a* and ash-free dry mass trends only chlorophyll *a* results are presented here. Chlorophyll *a* abundance ($\mu g\ cm^{-2}$) on 'grazed' and 'ungrazed' tiles provides an estimation of algal abundance in the presence and absence of grazers. The level of herbivory (herbivore impact) at each site was estimated as the percentage difference in algal abundance (chlorophyll *a*) between grazed and ungrazed tiles, and was calculated as follows:

$$\text{Herbivore impact (\% change)} = \frac{\text{Ungrazed} - \text{Grazed}}{\text{Ungrazed}} \times 100$$

Negative values indicated resource depression by grazers, whereas positive values reflected increased algal abundance under grazing. Zero values indicated that macroinvertebrate grazers had no impact on algal abundance.

Pearson's product-moment correlation was used 1) to relate algal abundance on ungrazed tiles to water chemistry (NO_3^-, PO_4^{3-}, Ca^{2+}, Al^{n+} and Si) and physical variables (stream order, slope, Pfankuch Index), and 2) to relate stream pH to algal abundance ($\mu g\ cm^{-2}$), herbivore impact (%) and the richness and density of grazers (m^{-2}).

3.4 RESULTS

3.4.1 Macroinvertebrate grazers

Grazer taxon richness ranged between 1 and 18 in spring, and 2 and 14 in summer across the 30 study sites (Figure 3.1). In both spring and summer, grazer taxon richness correlated strongly with mean stream pH (spring $r = 0.539$, $P = 0.002$; summer $r = 0.657$, $P < 0.001$; Figures 3.1a & 3.1b). In spring, the grazer functional group in circumneutral streams consisted mainly of Ephemeroptera (*Baetis*, *Rhithrogena* and *Electrogena*) and Coleoptera (*Elmis*, *Limnius* and *Oulimnius*) whereas in acid sites, grazers were mainly nemourid stoneflies (*Amphinemoura sulcicollis*) and elmid beetles (Chapter 2, Table 2.6). In summer, the grazer functional group was compositionally more similar in circumneutral and acid streams, consisting mainly of Ephemeroptera (*Baetis*, *Rhithrogena*, *Electrogena* and *Seratella*) and Coleoptera (*Elmis*, *Limnius*, *Oulimnius* and *Esolus*) (Chapter 2, Table 2.6).

Grazer density (across all sites) ranged between 3 and 1203 individuals per m^2 in spring and between 13 and 5216 individuals per m^2 in summer (Figures 3.1c and 3.1d). In spring, the density of Ephemeroptera declined with decreasing pH and the density of Plecoptera increased (Figure 3.1e), resulting in no net difference in grazer density across the pH gradient (Pearson's product moment correlation, $P > 0.05$; Figure 3.1c). In summer, by contrast, grazer density decreased with increasing acidity ($r = 0.342$, $P < 0.1$; Figure 3.1d).

3.4.2 Algal biomass

Mean algal biomass (as chlorophyll *a*) on 'ungrazed' tiles varied considerably across the sites in both spring (range 0.02 – 3.64 µg chlorophyll *a* cm^{-2}) and summer (range

0.15 – 9.44 µg chlorophyll *a* cm^{-2}), but this inter-site variation in algal biomass did not correlate with any of the water chemistry (NO_3^-, PO_4^{3-}, Ca^{2+}, Al^{n+} and Si) or physical variables (stream slope, stream order) in either season ($P > 0.05$), including stream pH (spring $r = 0.021$, $P = 0.914$; summer $r = 0.289$, $P = 0.121$; Figures 3.2a & 3.2b). Algal biomass on ungrazed tiles did however correlate negatively with the Pfankuch Index, but only in summer ($r = -0.514$, $P = 0.004$).

3.4.3 Herbivory

Mean algal biomass on grazed tiles followed a similar trend to that of ungrazed tiles (Figures 3.2c & 3.2d), and showed no correlation with stream pH (Pearson's correlation; spring $r = -0.029$, $P = 0.879$; summer $r = 0.324$, $P = 0.080$). Herbivore impact (% change in chlorophyll *a*) ranged from weakly negative (resource depression) to weakly positive (resource growth) in both spring (range -59% to 42%, Figure 3.2e) and summer (range -30% to 46%, Figure 3.2f). The relationship between herbivore impact and pH was weakly significant in spring ($r = -0.369$, $P = 0.054$; Figure 3.2e) and non-significant in summer ($P > 0.05$; Figure 3.2f).

3.5 DISCUSSION

Acidified streams are harsh environments for aquatic organisms, although much debate still surrounds the influence of acid stress on periphyton growth and biomass (e.g. Planas *et al.*, 1989 vs. Winterbourn *et al.*, 1992). Experiments that attempt to mimic acidification in artificial channels have shown that algal biomass increases as pH declines in response to the loss of acid-sensitive grazers (Hendrey, 1976; Hall *et al.*, 1980; Allard & Moreau, 1985; Parent *et al.*, 1986; Planas *et al.*, 1989). The results of the present study, however, do not support these findings, but do corroborate existing empirical research in natural systems that report no effect of acidity on algal standing crop (Marker & Willoughby, 1988; Collier & Winterbourn, 1990; Winterbourn *et al.*, 1992; Kinross *et al.*, 1993, Ledger & Hildrew, 2005). The disparity in results from these two approaches (natural vs. artificial acidification) can be explained by the fact that artificial acidification experiments assess only the immediate impacts of acid toxicity on community structure, notably the disappearance of herbivores and corresponding increases in algal biomass. In natural systems, however, acidification acts over a much longer timescales, allowing further shifts in the presence and abundance of acid-tolerant species to occur, some of which may deliver compensatory dynamics that counteract the immediate effects of acidity observed experimentally.

Although algal biomass did not correlate directly with stream pH in this study, the algal layers were impoverished in the majority of acid streams and I speculate that nutrient limitation may constrain algal growth in acid sites. While my analysis found no relationship between algal growth and any physicochemical variables, the study streams described here are known to be hydrochemically dynamic (Kowalik &

Ormerod, 2006; Ormerod & Durance, 2009). It is already known that availability of essential cations such as calcium, sodium and potassium is reduced in acid streams (Mason, 2002), but whether nutrients are also limited remains unclear (Winterbourn *et al.*, 1992; Planas, 1996). All thirty study streams were oligotrophic, with dissolved nitrate and phosphate concentrations below 1 and 0.3 mg L^{-1}, respectively, which is emphasised by the relatively low algal biomass found even in circumneutral streams. Algal biomass in the absence of grazers averaged 0.81 and 2.02 µg cm^{-2} (chlorophyll *a*) in spring and summer respectively, which is similar to the maximum algal abundance found in other oligotrophic streams in the UK (Winterbourn *et al.*, 1992; Kinross *et al.*, 1993), but much lower than quantities found in more enriched streams (5 to 15 µg cm^{-2}) (Lamberti & Resh, 1983; Rosemond *et al.*, 1993). It may be the case that low nutrient availability in acid headwater streams offsets the impact of reduced consumption by grazers, and prevents algal proliferation (or 'grazer release') in their absence.

Seasonal differences in algal biomass existed in the present study, and were most apparent in circumneutral streams, where mean chlorophyll *a* was approximately five times greater in algae samples collected in summer compared to spring. These findings contrast with those of Kinross *et al.* (1993), who reported that season did not have an impact on the standing crop of algae. These contrasting results may be due to differing abiotic regimes in the two stream types. In physically disturbed streams such as those in mid-Wales, periphyton standing crop in winter and spring months is likely to be regulated by rapid increases in water flow, which dislodge algal layers (Biggs, 1996). Indeed, measurement of the Pfankuch Index (Pfankuch, 1975) of stream stability at the study sites (mean 55, minimum 20, maximum 74) revealed similar values to those recorded for glacial Alaskan and Alpine streams (Maiolini & Lencioni, 2001; Milner *et al*., 2006). Sites with such high Pfankuch Index values are unlikely to provide suitable conditions to allow the proliferation of algae.

A primary aim of this chapter was to investigate the influence of invertebrate grazers on periphyton biomass in upland headwater streams. Literature on algal-herbivore interactions is well developed, and a number of meta-analyses have investigated the strength of these linkages in running waters. For example, Feminella & Hawkins (1995) collated data from 89 research papers on herbivore-periphyton interactions published between 1975 and 1993, and calculated that 70% reported a significant reduction in algal biomass by grazers (at ambient density). A large proportion of the remaining 30% of studies reported a change in algal community composition in the presence of grazers. The majority of studies included in the analysis, however, were conducted in artificial channels (56%) or in several sections of the same stream (29%) and so it is difficult to relate these findings to natural streams that have been acidified as a result of the deposition of anthropogenic pollutants. My data, from experiments conducted in natural systems, failed to find a consistent impact of grazers on periphyton biomass. Only in spring, in a handful of sites, could herbivore impact be detected. This resource depression was significantly related to pH, and corresponded with a higher abundance of mayfly larvae in circumneutral streams. In most other cases, particularly in summer months, herbivore impact on algal biomass was either weak or absent, despite higher overall densities of grazing invertebrates between June and August. This may in part be due to the fact that nymphs of the dominant grazer, *Baetis* spp., were first instars and hence unable to exert significant effects on algal resources, despite their large numbers.

Furthermore, I found no evidence for 'grazer release' in my acid streams; that is, algae did not proliferate in the absence of grazers. The observed pattern may be explained by a degree of turnover in the species complement across the pH gradient, whereby declines in the abundances of mayflies with increasing acidity were offset by increased abundances of stoneflies. My findings contrast with those of Feminella & Hawkins (1995), who suggested that algal communities in streams are regulated primarily by biotic factors, notably grazing by macroinvertebrates. This may be the case for physically benign lowland streams with stable flow and chemistry. However, for upland streams similar to the ones studied here, algal-grazer interactions are likely to be weakened by frequent episodes of hydrological and chemical disturbance. This idea is consistent with the predictions of the harsh-benign hypothesis of Peckarsky *et al*. (1990), which suggests that biotic interactions are relatively feeble in streams subjected to harsh abiotic regimes. Grazer-periphyton interactions may be further weakened by the inability of the primary grazers in this study, the mayfly larvae (including *Ephemerella*, *Baetis*, *Heptagenia* and *Paraleptophlebia*) to make much impact on algal abundance (McAuliffe, 1984b; Colletti *et al*., 1987; Karouna & Fuller, 1992).

In conclusion, the results of this investigation show that algal abundance in upland streams is regulated primarily by hydrological conditions rather than pH. Furthermore, the impact of macroinvertebrate grazers on algae is not influenced by stream acid-base status, and there is currently no evidence for 'grazer release' of algal resources in acidified headwater streams. The oligotrophic nature of upland catchments, combined with harsh abiotic conditions they endure, preclude any influence of pH by weakening algal-grazer interactions.

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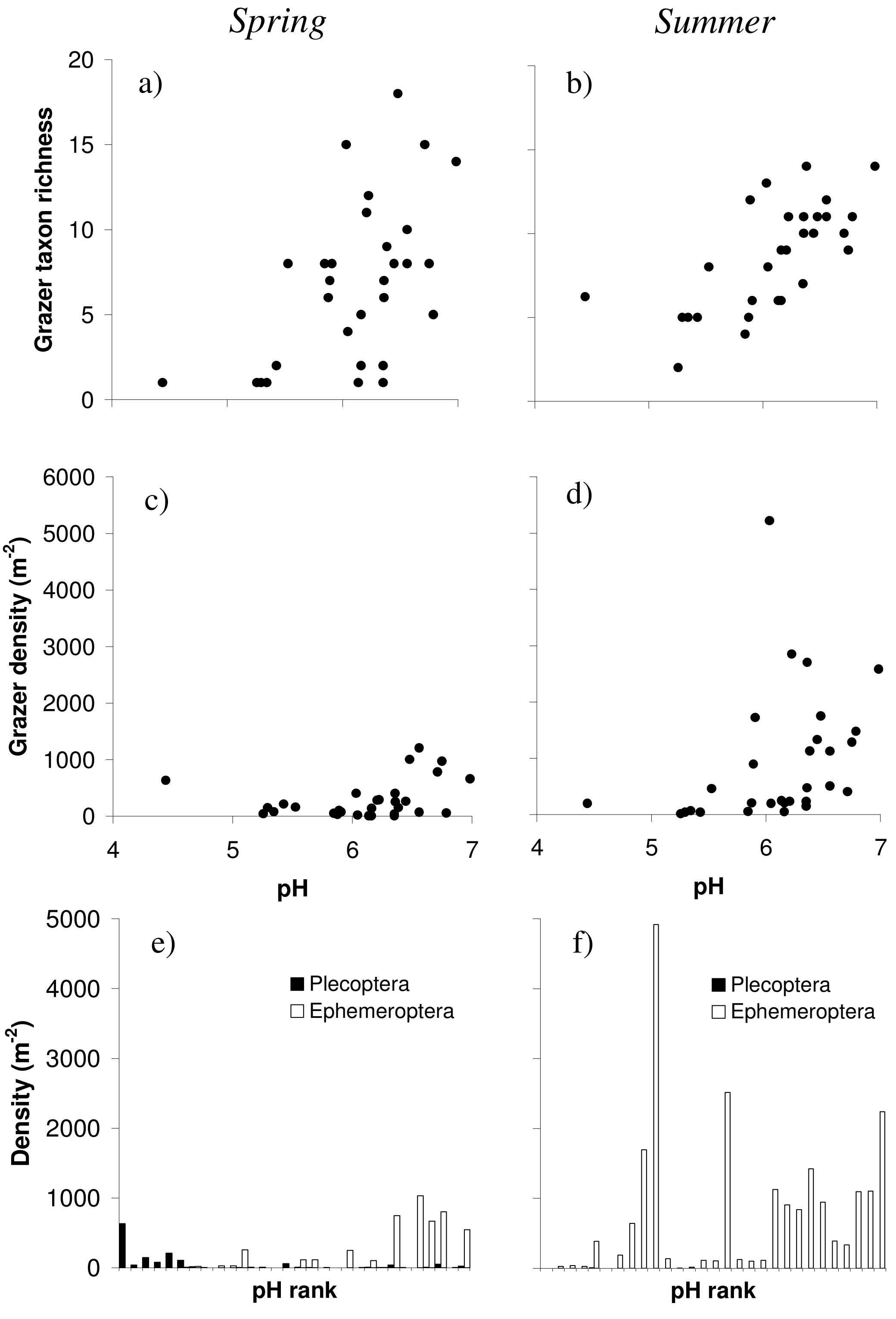

Figure 3.1 Mean taxon richness (a, b) and density (m^{-2}; c, d) of macroinvertebrate grazers in thirty streams of contrasting pH, and density (m^{-2}; e, f) of two dominant grazer orders in sites ordered (left to right) by increasing pH, in spring and summer 2006.

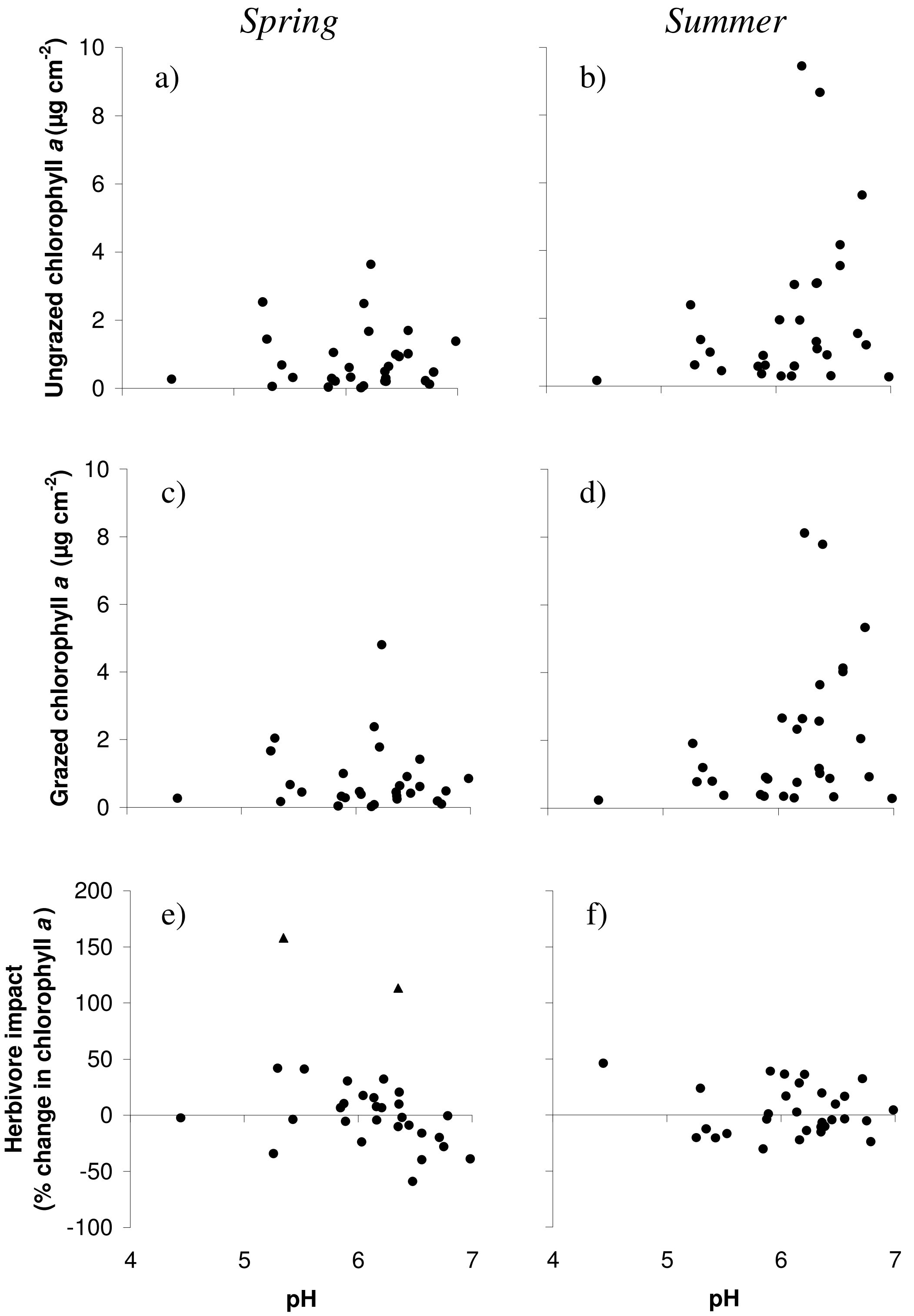

Figure 3.2 Mean chlorophyll *a* (µg cm^{-2}) on 'ungrazed' (a, b) and 'grazed' tiles (c, d), and herbivore impact (% change in chlorophyll *a*; e, f) in thirty streams of contrasting pH, in spring and summer 2006. Triangles denote statistical outliers not included in analysis.

CHAPTER FOUR

LEAF LITTER BREAKDOWN AS A MEASURE OF ECOSYSTEM INTEGRITY IN STREAMS OF CONTRASTING pH

4.1 SUMMARY

1. Leaf litter decomposition is used increasingly as a measurable indicator of environmental stress and stream ecosystem health. The research presented in this chapter investigates the effect of surface water acidification on decomposition processes in upland streams in mid-Wales. Breakdown rates of oak leaves (*Quercus robur* L.) were determined across thirty headwater streams of contrasting pH in both spring and summer 2006. Comparisons of macroinvertebrate shredder assemblage structure and the relative contribution of microbes and macroinvertebrates to the breakdown process were made in order to elucidate the mechanisms underlying leaf breakdown.
2. Total breakdown rates ranged between 0.0017 and 0.0206 k d^{-1} across the sites in spring, and from 0.0034 to 0.0428 k d^{-1} in summer, and were positively correlated with stream pH in both seasons. Microbes made the greatest contribution to the overall breakdown process in both seasons, but this contribution declined with increasing acidity and total Al, and with decreasing Ca^{2+} concentrations. Macroinvertebrate contributions to decomposition were positively correlated with the abundance and richness of macroinvertebrate shredders in leaf bags, but were not related to the abundance and richness of shredders in the benthos.

3. Richness of leaf-shredding invertebrates in the benthos and in leaf bags was not significantly related to stream acidity, whereas shredder abundance in leaf bags was lower in acid streams. In general, shredder species richness was low (< 3 taxa average) and taxonomic composition of the shredder community similar across sites. However, breakdown rates were particularly high (0.010 to 0.021 k d^{-1} in spring, 0.011 to 0.043 k d^{-1} in summer) in two circumneutral streams (AC16 and AC17) with large populations of *Gammarus pulex* (Amphipoda).
4. Research findings indicate that acidification of upland headwater streams results in impaired ecosystem function, primarily though negative effects on microbial assemblages. Invertebrate-controlled breakdown was low in most sites, regardless of pH, reflecting the oligotrophic nature of the streams and the paucity of macroinvertebrate shredders in the stream benthos.

4.2 INTRODUCTION

The effects of acidification on benthic macroinvertebrate community structure have been studied for several decades, and are well known (e.g. Sutcliffe & Carrick, 1973; Sutcliffe & Hildrew, 1989; Peterson & Eeckhaute, 1992; Tixier & Guérold, 2005). More recently, attention has turned to the relationship between these structural shifts and key ecosystem processes, including leaf litter decomposition (Hildrew *et al*., 1984; McKie *et al*., 2009), since functional responses to environmental stress are increasingly regarded as valuable indicators of ecosystem health (Simon *et al*., 2009). Assays of the litter breakdown pathway are a particularly efficient means of evaluating the effects of pollutants, including acid deposits, on aquatic systems (Gessner & Chauvet, 2002).

Research over several decades has advanced knowledge of the processing of leaf litter by aquatic organisms in running waters (e.g. Petersen & Cummins, 1974; Webster & Benfield, 1986; Gessner, 1999; Tiegs *et al*., 2009). Allochthonous inputs are the primary source of energy in forested catchments (e.g. Webster & Benfield, 1986; Wallace *et al*., 1997), and the key agents of leaf litter breakdown can be estimated empirically (e.g. Dangles & Guérold, 2001; Dangles *et al*., 2004b; Boyero *et al*., 2006). Both microbes, principally bacteria and hypomycete fungi, and macroinvertebrates, act as agents of leaf litter decomposition in streams (Bärlocher & Kendrick, 1974; Suberkropp & Klug, 1980; Gessner & Chauvet, 1994; Baldy *et al*., 1995; Sampaio *et al*., 2001; Pascoal *et a*l., 2005; Bergfur *et al*., 2007). Microbial breakdown rates are often controlled by water chemistry, including key nutrient concentrations (Pascoal *et al*., 2005; Baldy *et al*., 2007; Bergfur *et al*., 2007) and dissolved metals (Carlisle & Clements, 2005), and water temperature (Webster &

Benfield, 1986), which all influence microbial activity and biomass. However, in many upland streams water pH is regarded as a key variable controlling microbially-mediated litter decomposition (Webster & Benfield, 1986). Reduced breakdown rates in acid streams (e.g. Hildrew *et al.*, 1984; Dangles *et al.*, 2004a; Petrin *et al.*, 2008) may reflect constraints on microbial activity and respiration (e.g. Mulholland *et al.*, 1987; Groom & Hildrew, 1989; Dangles *et al.*, 2004a) that result from elevated aluminium concentrations (Mulholland *et al.*, 1987; Dangles *et al.*, 2004a; Baudoin *et al.*, 2008), and low dissolved calcium (Dangles *et al.*, 2004a).

Research suggests that macroinvertebrate shredders are less sensitive to acid stress than microbial assemblages. Nevertheless, taxonomic richness (Tixier & Guérold, 2005), abundance (Dangles & Guérold, 2000; Thomsen & Friberg, 2002) and overall biomass (Griffith & Perry, 1993; Dangles & Guérold, 2001) of shredder assemblages is often lower in acid streams compared to circumneutral equivalents. For example, the leaf shredding amphipod *Gammarus* is severely affected by freshwater acidification, and is rarely found below pH 5.8 – 6.0 (Hildrew *et al.*, 1984; Burton *et al.*, 1985; Dangles & Guérold, 2000; 2001). Plecopteran shredders, by contrast, are generally considered tolerant of acidic conditions (e.g. Sutcliffe & Carrick, 1973; Townsend *et al.*, 1983; Tierney *et al.*, 1998), although this has recently been challenged (Tixier & Guérold, 2005). Reduced shredder abundance at low pH may also be related to reduced food quality (Hildrew *et al.*, 1984), as both the feeding activity (Mackay & Kersey, 1985; Dangles & Guérold, 2001; Dangles *et al.*, 2004a), and growth (Groom & Hildrew, 1989; Thomsen & Friberg, 2002) of shredders fed acid-conditioned leaves is often impaired. There is compelling evidence to suggest that many shredders prefer leaves that have been preconditioned by microbes

(Bärlocher & Kendrick, 1974; Cummins & Klug, 1979; Sutcliffe *et al*., 1981), which may be damaged at low pH (e.g. Suberkropp & Klug, 1980; Groom & Hildrew, 1989; Dangles *et al*., 2004a). Biomass and density of certain shredder species, including *Gammarus pulex*, is also positively related to nutrient availability (Baldy *et al*., 2007; Bergfur *et al*., 2007), which is generally lower in acid streams (Grahn *et al*., 1974; Dickson, 1978). The importance of macroinvertebrate shredders to the breakdown process is unequivocal however. In general, sites with high shredder richness (Jonsson & Malmqvist, 2000; Jonsson *et al*., 2001; Huryn *et al*., 2002; Boyero *et al*., 2007), abundance (Mulholland *et al*., 1987; Jonsson *et al*., 2001) and biomass (Huryn *et al*., 2002) support faster litter breakdown rates, although species identity is also an important determinant of decomposition (Boyero *et al*., 2006; 2007). For example, breakdown rates are reportedly faster in sites supporting abundant populations of the amphipod *Gammarus* than those dominated by stoneflies or caddis (Dangles & Malmqvist, 2004). Despite corroboratory studies supporting the idea that leaf breakdown is slower in acid streams (e.g. Hildrew *et al*., 1984; Mulholland *et al*., 1987; Dangles & Guérold 2000; Petrin *et al*., 2008), the pattern is not ubiquitous (Mason, 2002; Dangles *et al*., 2004b). It is therefore essential that the chemical parameters influencing macroinvertebrate shredder assemblages are fully explored, so that the diversity and functional integrity of shredder communities can be restored in streams that have been affected by pollution.

The research presented in this chapter aimed to determine the extent to which acidification affects decomposition rates (leaf breakdown) across streams falling along an acidification gradient in mid-Wales. Whilst the historical relationship between macroinvertebrate assemblage structure and pH is well known across these sites (Stoner *et al*., 1984; Weatherley & Ormerod, 1987; Weatherley *et al*., 1989; Ormerod & Durance, 2009), recent shifts in water chemistry imply recovery could be underway (Bradley & Ormerod, 2002; Ormerod & Durance, 2009), and the current status of the structural and functional integrity of these apparently perturbed ecosystems is worthy of further analysis. A key objective was to determine how microbial and macroinvertebrate breakdown responds across the pH gradient. Consistent with the notion that acidification damages organisms at every trophic level, I predicted that decomposition by both microbes and macroinvertebrates would be impaired at low pH, and that inter-site variation in macroinvertebrate breakdown was related to the presence and abundance of macroinvertebrate shredders across the pH gradient.

4.3 METHODS

4.3.1 Sampling design

Decomposition rates were examined in 30 study streams across three river catchments in spring and summer 2006 (see Chapter 2 for site descriptions). Sites were divided into three pH groups (acid, intermediate and circumneutral) on the basis of mean and minimum pH between 2003 and 2006. Post-abscission leaves of English Oak (*Quercus robur*) were collected in the autumn of 2005, air-dried in the laboratory and stored in a cool, dry environment. Previous research has suggested that leaves can be stored for up to a year prior to use with no significant loss of mass over this period (Burton *et al.*, 1985). English Oak was chosen for this experiment as it is one of the only species common to all three study catchments. Two types of leaf bag were constructed (tetrahedral shape, approximately 10 × 15 × 5 cm) following a standard design (Mulholland *et al.*, 1987; Jonsson *et al.*, 2001; Dangles *et al.*, 2004a; Boyero *et al.*, 2006). The first was made from coarse plastic mesh with an aperture large enough to allow most invertebrates through (4 mm). The second was designed to exclude aquatic macroinvertebrates, and was made from precision polyamide netting (0.3 mm aperture). Leaf bags containing 5 g air-dried leaves were tied in pairs (one coarse mesh and one fine mesh, 20 cm twine length) to the bricks (Burton *et al.*, 1985) used for algal sampling (Chapter 3), and secured to the stream bed using metal stakes. A total of ten bags (five of each type) were placed in five run habitats. Extra leaf bags were transported to and from the study sites, but not placed into streams, in order to estimate handling losses.

The experiment was repeated in spring and summer 2006, commencing in mid-March and mid-June, respectively. Leaf bags were left *in situ* for a period of 56 days in both spring and summer, after which they were collected using a hand net (250 µm mesh) to capture any emigrating invertebrates, and immediately frozen to arrest decomposition. Any invertebrates and leaf fragments small enough to pass through the 4 mm mesh bags were placed in labelled plastic vials and preserved in 70% Industrial Methylated Spirit (IMS).

Five aquatic macroinvertebrate samples were collected from riffles in each of the 30 sites in April and July 2006 (Chapter 2) using a Surber sampler (0.0625 m^2, 250 µm mesh aperture) and preserved in 4% formaldehyde solution. Water samples were collected in polyethylene bottles and filtered immediately though cellulose-nitrate filter membranes (Whatman, 0.45 µm pore size, 47 mm diameter). Filtered samples were then transferred to sterile bottles, refrigerated and analysed within two days of collection. Water samples for metal analysis (Al^{n+}, Ca^{2+}) were acidified to pH 2.0 using 70% Aristar nitric acid. Stream pH was measured *in-situ*, monthly between March and August 2006, using a handheld pH meter (HANNA HI9024C). Dissolved oxygen concentration (YSI 550A DO Meter), total dissolved solids and conductivity (Myron Ultrameter II 6P) were measured contemporaneously. A suite of physical variables (slope, width class, riparian land use, Pfankuch Index of stream stability) were also recorded at each site (Chapter 2).

4.3.2 Laboratory protocols

Defrosted oak leaves were gently washed with deionised water to remove silt and sand, and any macroinvertebrates found in the leaf bags were removed and stored in 70% IMS. Any leaf fragments larger than 0.25 cm^2 were also cleaned in this way (Jonsson *et al.*, 2001). Leaves were air-dried to constant weight and conversion factors between AFDM (ignition for 4 h at 550°C) and air-dried mass were calculated to estimate AFDM in the bags (Mulholland *et al*., 1987; Rowe *et al*., 1996; Dangles *et al*., 2004a), after correcting for handling loss (Mackay & Kersey, 1985; Rowe *et al*., 1996) and initial leaching (Dangles & Malmqvist, 2004). In addition, a small number of oak leaves that had been stored in the laboratory and not used in experiments were oven dried (40°C for 24 h) and ground to powder. Between 1-2 mg of sample was combusted at 950°C (Carlo Erba EA1110 Flash Elemental Analyser) to measure percentage nitrogen and carbon content. Elemental analysis revealed that oak leaves (prior to submergence) contained between 0.9 and 1.3% nitrogen, and between 47.6 and 48.5% carbon, giving an average carbon to nitrogen ratio of 44:1.

Macroinvertebrates collected in the Surber samples and leaf bags were sorted from debris and transferred to vials of 70% IMS. All macroinvertebrates were identified to the lowest practicable taxonomic unit (generally species), excepting Diptera and Oligochaeta, using a binocular (Zeiss Stemi 2000), and where necessary, compound (Nikon Optiphot-2) microscope. Water samples were analysed for anions (NO_3^-, PO_4^{3-}) with a Dionex ICS-2000 ion chromatograph, and cations and metals (Al^{n+}, Ca^{2+}) using an Agilent 7500ce ICP-MS (Chapter 2). Non-purgeable organic carbon (NPOC) was measured using a TOC analyser (Shimadzu TOC-V CSH).

4.3.3 Data analysis

Total leaf decomposition (microbial and invertebrate breakdown) was estimated from the loss of leaf dry mass (g) from coarse mesh bags during the 56-day incubation period whereas microbial-only decomposition was the loss of leaf mass from fine mesh bags (e.g. Dangles & Guérold, 2001; Dangles *et al*., 2004b; Boyero *et al*., 2006). Breakdown rates in fine mesh and coarse mesh bags in each of the sites were estimated by fitting data to the negative exponential decay model described by Petersen & Cummins (1974):

$$W_t = W_0.\mathrm{e}^{-kt}$$

where W_t is the AFDM remaining at time t in days, W_0 is the initial AFDM at the beginning of the experiment, and $-k$ is the daily breakdown rate (e.g. Gessner & Chuavet, 1994; Dangles *et al*., 2004a; Boyero *et al*., 2006). For the purpose of clarity, $-k$ is reported in all text, figures and tables as a positive value, k (e.g. Huryn *et al*., 2002; Dangles *et al*., 2004a; Boyero *et al*., 2006). For each pair of leaf litter bags (fine and coarse), leaf breakdown rate (k d^{-1}) for the fine-mesh bag (microbial breakdown) was subtracted from the value for the coarse-mesh bag (total breakdown) to give breakdown attributable to macroinvertebrates (invertebrate breakdown). These data were also expressed as the ratio of microbial to invertebrate breakdown.

Counts of macroinvertebrates found in Surber samples were used to calculate the average density (m^{-2}) of each shredder taxon. Macroinvertebrate data used for analyses were expressed as shredder taxon richness and shredder density per m^2 for Surber data, or as numbers of individuals or taxa per leaf bag. Abundance/density data were also expressed as relative abundance where Surber and leaf bag data were to be compared.

Two-way ANOVA was used to determine whether breakdown rates (total, microbial, invertebrate and ratio of microbial to invertebrate breakdown) and shredder community descriptors (abundance and taxon richness in Surber samples and leaf bags) differed significantly among the three pH groups (acid, intermediate, circumneutral pH) and between seasons (spring, summer). Breakdown rate data were not normally distributed, and thus double square root-transformed to equalise variances. *Post hoc* pairwise comparison of means (pH groups and seasons) was carried out using *t*-tests or Tukey's HSD test.

Spearman's rank-order correlation was used on untransformed data to determine whether breakdown rates (total, microbial, invertebrate and ratio of microbial to invertebrate breakdown), shredder abundance and shredder richness (in Surber samples and leaf bags) correlated with chemical (pH, Al^{n+}, Ca^{2+} NO_3^-, PO_4^{3-}) or habitat (Pfankuch Index, slope, stream width class) variables. Due to the exceptionally high breakdown rate and shredder abundance recorded at one site (AC16), correlations were only considered significant if they produced *P* values of less than 0.05 in analyses where AC16 was both included and excluded.

Detrended Correspondence Analysis (DCA, with detrending by segments, and without transformation or downweighting of rare species) was performed on shredder abundance data (from Surber samples) using CANOCO 4.5 (ter Braak & Šmilauer, 2002) to explore variations in the shredder community across the thirty study streams.

Finally, in order to determine whether the taxonomic composition of shredder assemblages in the stream and leaf bags was similar, a comparison of richness and relative abundance of each shredder taxon in the benthos and leaf bags was made using Spearman's rank-order correlation analysis. Furthermore, two-tailed *t*-tests (P = 0.05) were used to compare the relative abundances (%) of the most abundant functional feeding groups (grazers, shredders and predators) in the benthos versus leaf bags.

4.4 RESULTS

4.4.1 Leaf litter breakdown

Across the 30 sites, total leaf decomposition ranged between 0.0017 and 0.0206 k d^{-1}, in spring and 0.0034 and 0.0428 k d^{-1} in summer (pH group means are shown in Figure 4.1; Table 4.1). The contribution of microbes to total decomposition was generally greater (1.7× in spring and 5.1× in summer) than that attributable to macroinvertebrate shredders (Figure 4.2). Breakdown by microbes was slower in spring than summer (Figure 4.1; Table 4.1). Thus, across the sites, decomposition by microbes ranged between 0.0009 and 0.0069 k d^{-1} in spring, and 0.0027 and 0.0072 k d^{-1} in summer, whereas invertebrate breakdown ranged from less than 0.0001 k d^{-1} to 0.0136 k d^{-1} in spring and 0.0356 k d^{-1} in summer (pH group means are shown in Table 4.1).

In both seasons, mean breakdown rates (total and fractions) tended to increase with decreasing acidity (Figure 4.1). Two-way ANOVA revealed that both total and microbial breakdown rates were significantly slower in the more acid sites (Table 4.2), whereas differences in invertebrate decomposition, and in the ratio of microbial: invertebrate breakdown among streams of contrasting pH were not statistically significant ($P > 0.05$, Table 4.2). Seasonal effects were statistically significant for total and microbial breakdown (spring < summer, $P < 0.05$), but not for invertebrate or the ratio of microbial to invertebrate breakdown (Table 4.2).

In spring, Spearman's rank-order correlation indicated that the rate of microbial leaf decomposition increased with decreasing aluminium concentrations, and increasing pH (Figure 4.3) and dissolved calcium concentration ($r_s = 0.418$, $P = 0.021$). Invertebrate breakdown rates, however, were positively correlated with shredder abundance and taxon richness in the leaf bags (Figure 4.3). Factors significantly correlated with total breakdown rate in spring were (in order of decreasing significance level) dissolved aluminium (Spearman's rank correlation coefficient $r_s = -0.526$, $P = 0.003$), spring pH ($r_s = 0.510$, $P = 0.004$), leaf bag shredder abundance ($r_s = 0.462$, $P = 0.010$), dissolved phosphate ($r_s = -0.410$, $P = 0.024$) and leaf bag shredder richness ($r_s = 0.370$, $P = 0.044$).

In summer, invertebrate breakdown rates were again positively correlated with the abundance and richness of shredders in the leaf bags, whereas microbial breakdown was correlated significantly with calcium concentration ($r_s = 0.457$, $P = 0.013$) but not dissolved aluminium concentration or pH ($P > 0.05$, Figure 4.4).

4.4.2 Shredders in benthic assemblages

A total of 17 shredder taxa were identified in benthic Surber samples across streams and seasons (Table 4.3). The most taxonomically diverse groups were the Trichoptera (10 taxa) and Plecoptera (4 taxa). Mean taxon richness for shredders was similarly low (range 2.1-3.5 taxa, Table 4.1) across streams of contrasting pH (two-way ANOVA $P > 0.05$, Table 4.2), and across seasons (two-way ANOVA $P > 0.05$, Table 4.2). Although the overall mean density (m^{-2}) of shredders was higher in circumneutral streams (Table 4.1), differences among groups were not statistically significant (two-way ANOVA, $P > 0.05$, Table 4.2). Two-way ANOVA and *post hoc* tests also showed that shredder density (m^{-2}) was significantly lower in spring than summer (Table 4.2).

Shredder assemblages in acid streams were characterised by nemourid stoneflies in spring and cased caddis in summer (Table 4.3). However, larval

Tipulidae dominated the shredder assemblage in both seasons. Tipulids were also the dominant shredders in intermediate pH sites, whereas amphipods (*Gammarus pulex*), tipulids and a variety of cased caddis larvae were important to varying degrees in circumneutral benthic assemblages (Table 4.3). *Gammarus pulex* was locally abundant but restricted to only two circumneutral (≥ 6.4) streams (AC16 & AC17) with relatively high dissolved phosphate concentration (≥ 0.03 mg L^{-1}).

Detrended correspondence analysis (DCA) of shredder abundance data revealed high assemblage turnover along the first axis of the ordination, in both spring (axis 1 = 5.1 SD, axis 2 = 3.2 SD) and summer (axis 1 = 3.7 SD, axis 2 = 1.4 SD) (Figure 4.5). Eigenvalues of axes one and two were 0.901 and 0.478, respectively in spring, and 0.942 and 0.218, respectively in summer. Three groups of sites were differentiated on the DCA biplot; those with communities characterised by either an abundance of nemourid stoneflies (e.g. *Nemoura avicularis* and *Protonemura meyeri*), or *Gammarus pulex*, or a diversity of large cased caddis (e.g. *Halesus* spp., *Sericostoma personatum* and *Chaetopteryx villosa*). In general however, the majority of sites exhibited a great deal of overlap in assemblage composition, with a number of sites generating similar scores along axis 1 and 2 of the DCA (Figure 4.5).

4.4.3 Functional composition of leaf bag macroinvertebrates

Overall, shredders were not overrepresented in leaf bags in either season (Figure 4.6), in contrast to collector-gatherers (mainly chironomids), which were significantly more abundant in leaf bags. The relative abundance of predators was similar in the leaf bags and substrate in spring, but was significantly lower in the leaf bags in summer.

Shredder abundance in leaf bags was lowest in acid streams compared to intermediate and circumneutral streams but did not vary between seasons, whereas shredder richness was greater in spring compared to summer but similar among streams of contrasting pH (Table 4.2). The structure of macroinvertebrate assemblages in leaf bags was broadly dissimilar to that in the benthos as revealed by Surber sampling (Table 4.3). Whilst shredder taxon richness in leaf bags correlated strongly with that in the benthos (spring $r_s = 0.750$, $P < 0.001$; summer $r_s = 0.565$, $P = 0.001$), shredder density did not ($P > 0.05$ in both seasons). Spearman's rank-order correlation of mean relative abundances of shredders in benthos versus leaf bags were only statistically significant for circumneutral pH streams in spring and intermediate pH streams in summer (Table 4.4). In all other circumstances, lack of correlation was due to the aggregation species such as *Chaetopteryx villosa, Potamophylax* spp. and *Sericostoma personatum* in the leaf bags (Table 4.3).

4.5 DISCUSSION

The total breakdown of oak leaves ranged from 0.0017 to 0.0206 k d^{-1} in spring and from 0.0034 to 0.0428 k d^{-1} in summer, but was generally lowest in acid streams. Petersen & Cummins (1974) compared breakdown rates of several different leaf species, and suggested that rates less than 0.005 k d^{-1} should be considered 'slow', and rates of between 0.005 and 0.010 k d^{-1} considered 'moderate'. According to this classification, in spring, 87% of streams used in the present study had slow processing rates, and 7% of sites had moderate breakdown rates, whereas in the summer, these values changed to 57% and 37%, respectively. In two sites (AC16 and AC17) however, mean total breakdown rates varied between 0.010 and 0.021 k d^{-1} in spring and 0.011 and 0.043 k d^{-1} in summer, which would be considered 'fast' to exceptionally 'fast' (c.f. Petersen & Cummins, 1974).

In the majority of the study streams (> 75%) microbes made the greatest contribution to the overall leaf breakdown process. In spring, the average ratio of microbial to invertebrate breakdown (across all streams) was 1.7 to 1. In summer, when water temperature was higher, this increased to 5.1 to 1. The essential contribution made by microbes to the decomposition process has been reported elsewhere (Bärlocher & Kendrick, 1974; Baldy *et al*., 1995; Dangles, 2002), although the majority of studies relating to factors controlling litter breakdown do not compare the relative contribution of microbes and invertebrates to the decomposition process (e.g. Gessner & Chauvet, 1994; Dangles & Guérold, 2000), as has been done in the present investigation. Stream pH and dissolved aluminium concentration were the most important factors controlling microbe-mediated breakdown rates, but only in spring when water chemistry was at its most variable and the acid streams exhibited lower pH. A number of other studies have reported that the rate of colonisation (Chamier, 1987) and overall activity of microbes (Suberkropp & Klug, 1980; Burton *et al*., 1985; Mackay & Kersey, 1985; Mulholland *et al*., 1987; Groom & Hildrew, 1989; Dangles *et al*., 2004a; Merrix *et al*., 2006) was reduced in acid streams, possibly as a result of hydrogen and aluminium toxicity (Mulholland *et al*., 1987; Dangles *et al*., 2004a; Baudoin *et al*., 2008).

The contribution of macroinvertebrates to the total breakdown rate (i.e. invertebrate breakdown rate) was directly related to the abundance and taxon richness of shredders in the leaf bags, but was not related to the richness or density of shredders in the substrate, or any physicochemical variables, including pH. Several other studies have found a strong correlation between shredder communities in coarse-mesh leaf bags and breakdown rates (Mulholland *et al*., 1987; Jonsson *et al*., 2001; Huryn *et al*., 2002), but these studies did not correct for the microbial contribution to breakdown, nor did they examine the shredder communities in the stream benthos.

Shredder communities in the study streams were similar to those recorded in oligotrophic streams in France (Dangles *et al*., 2001; Dangles, 2002; Dangles *et al*., 2004a) and Scandinavia (Jonsson & Malmqvist, 2000; McKie *et al*., 2006), and consisted mainly of Plecoptera (*Nemoura* and *Protonemura*), Trichoptera (*Halesus*, *Potamophylax* and *Sericostoma personatum*) and Amphipoda (*Gammarus pulex*). Despite slight shifts in the shredder community composition as pH decreased (from *Gammarus* and caddis larvae to stonefly larvae), the number of shredder species in both substrates (benthos and leaf bags) remained fairly constant, averaging less than three taxa per sample. In the Garonne River in France, a comparable number of shredder taxa were recorded (Chauvet *et al*., 1993). In the Touyre, however, up to seven taxa were documented (Chauvet *et al*., 1993), and up to 11 and 23 have been recorded in headwater streams in Sweden and North America, respectively (Jonsson *et al*., 2001; Huryn *et al*., 2002). This discrepancy may in part be due to variations in the way that researchers assign macroinvertebrate species to functional feeding groups. For example, some studies relying on the Merritt & Cummins (1984) system classify *Leuctra* as shredders, whereas in this study *Leuctra* have been classified as collector-gatherers (after Moog, 1995). Discrepancies notwithstanding, it would still appear that shredder communities in upland Welsh streams are impoverished.

Rapid breakdown in a handful of streams (AC16 and AC17) was attributed to the presence of the amphipod *Gammarus pulex*. Shredder identity is important in determining the speed of litter breakdown (Boyero *et al*., 2006; 2007), and it is often the case that sites containing powerful shredders, such as *Gammarus,* experience faster breakdown rates than sites that contain mainly weak shredders, such as Plecoptera and Trichoptera (Griffith & Perry, 1993; Dangles & Guérold, 2000; Dangles *et al*., 2004a; Dangles & Malmqvist, 2004). *Gammarus*, however, has specific water chemistry requirements, and is only usually found in streams of a relatively high trophic status, where the pH is greater than 5.8 (Hildrew *et al*., 1984; Burton *et al*., 1985; Dangles & Guérold, 2001) and dissolved calcium concentration is greater than 4.5 µg L^{-1} (Dangles & Guérold, 2001). In this study, *G. pulex* was only present in streams where mean pH and calcium levels were greater than 6.4 and 2.8 mg L^{-1}, respectively, and where phosphate concentrations were ≥ 0.03 mg L^{-1}, adding support to the idea this species can only survive in neutral, nutrient and calcium-rich streams (Hildrew *et al*., 1984; Burton *et al*., 1985; Dangles & Guérold, 2001). With the exception of *G. pulex*, and in contrast to the microbial community, pH had little impact on the diversity of the shredder community and its ability to break down leaf litter, as the oligotrophic nature of these upland catchments results in a widespread suppression of shredder richness. Only Plecopteran and Trichopteran shredders are able to survive in the majority of these headwater streams, resulting in universally slow breakdown rates.

A further explanation for the low diversity of shredders in these headwater streams may be the poor quality of available food. Oak leaves, the substrate used in this experiment, are an important component of deciduous leaf litter in upland Welsh river catchments (Edwards & Brooker, 1982), but are a nutritionally poor food source for aquatic macroinvertebrates (Sampaio *et al.*, 2001, Loranger-Merciris *et al.*, 2008). The initial carbon to nitrogen ratio of dried oak leaves in this experiment was 44:1, which is comparable to a ratio of 35:1 recorded elsewhere (Kaushik & Hynes, 1968; Bjelke & Herrmann, 2005). This is in comparison to a ratio of 19:1 and 25:1 recorded for alder and willow, respectively, in other similar studies (Chauvet *et al.*, 1993; Bjelke & Herrmann, 2005). Research involving detailed examination of the chemical composition of oak leaves has shown that they contain a low percentage of nitrogen, high levels of lignin, phenol and tannins, and have a high protein precipitation capacity (Sampaio *et al.*, 2001; Bjelke & Herrmann, 2005). High phenol and tannin levels are capable of deactivating fungal enzymes (Suberkropp & Klug, 1980; Sampaio *et al.*, 2001), and therefore cause a reduction in microbial conditioning and breakdown. When fed to shredders, oak leaves also cause lower macroinvertebrate growth rates and higher levels of mortality (Bjelke & Herrmann, 2005). It is unsurprising, therefore, that oak leaves often decompose more slowly than other species. In their review of published breakdown rates for different plant species, Webster & Benfield (1986) found that out of 15 species of woody plant, only needles of heather and pine decomposed slower than leaves from oak trees. In the current investigation, the total breakdown rate for oak in acid pH, intermediate pH and circumneutral streams was 0.0027, 0.0033 and 0.0057 k d^{-1}, respectively, in spring, and 0.0048, 0.0051 and 0.0095 k d^{-1}, respectively, in summer. This is in comparison to rates of greater than 0.25 k d^{-1} for maple, ash, birch and cherry leaves in streams in the Hubbard Brook in North America, and rates of greater than 0.027 k d^{-1} for alder and willow leaves in streams in Germany (Webster & Benfield, 1986).

In this study, breakdown rates were measured by submerging mesh bags containing leaf material, forming resource-rich patches in which shredders accumulated. *Chaetopteryx villosa* (Limnephilidae), for example, was found in much higher numbers in the leaf bags compared to the benthos, possibly because this species constructs its case from leaf fragments, in addition to using leaves as its main source of food (Hynes, 1993). Several researchers have suggested that certain invertebrates use leaf bags as shelter rather than as a food source (Dobson *et al.*, 1995; Cortes *et al.*, 1997; Dangles *et al.*, 2001, Sampaio *et al.*, 2001), and that this is more likely to happen when slow decaying species are used in decomposition experiments (Dangles *et al.*, 2001). Shredders may also aggregate in the leaf bags when the natural supply of leaves in the stream is impoverished (Cortes *et al.*, 1997). In this investigation, collector-gatherers accounted for around 40% and 80% of individuals found in leaf bags in spring and summer, respectively, which is similar to values reported elsewhere (Li *et al.*, 2009). In such cases, the collector-gatherer species may be taking advantage of an abundant supply of coarse particular organic matter.

Despite these findings however, the use of artificial leaf bags remains the best way to measure leaf decomposition, which in turn is one of the most successful methods of assessing the functional integrity of aquatic environments (Gessner & Chauvet, 2002; Simon *et al*., 2009).

The main aim of this chapter was to determine the extent to which acidification affects leaf breakdown rates, as a measure of ecosystem function and health, in upland headwater streams. Damage to every trophic level in aquatic ecosystems as a result of acidification has been widely reported (Chapter 1), and the assumption that this damage would translate to reduced microbe and invertebrate-mediated breakdown was largely correct. The ability of microbes to break down leaf litter is severely reduced at low pH, possibly as a result of hydrogen and aluminium toxicity. The impact of acidity on macroinvertebrate shredder communities and invertebrate-controlled breakdown was less obvious, however. Shredder communities were impoverished in the majority of my study streams, regardless of pH, possibly due the oligotrophic nature of upland catchments, the high levels of physical disturbance they experience and their inability to retain leaf litter (Dobson *et al*., 1995; Lepori *et al.,* 2005). Nevertheless, the presence of a highly acid-sensitive species, *Gammarus pulex*, in two physically stable, circumneutral streams, enhanced breakdown rates. The combination of damage to microbe and shredder communities at low pH resulted in significantly lower total breakdown rates in acid streams. These findings demonstrate that freshwater acidification damages ecosystem function in upland headwater streams primarily though effects on the microbial community. Measuring litter breakdown is a highly integrative approach to assessing the impact of pollution on ecosystem function, as it allows the simultaneous evaluation of damage to microbe and invertebrate communities. These findings emphasise the importance of such assessment in tracking recovery from surface water acidification, and other catchment-scale pollution problems.

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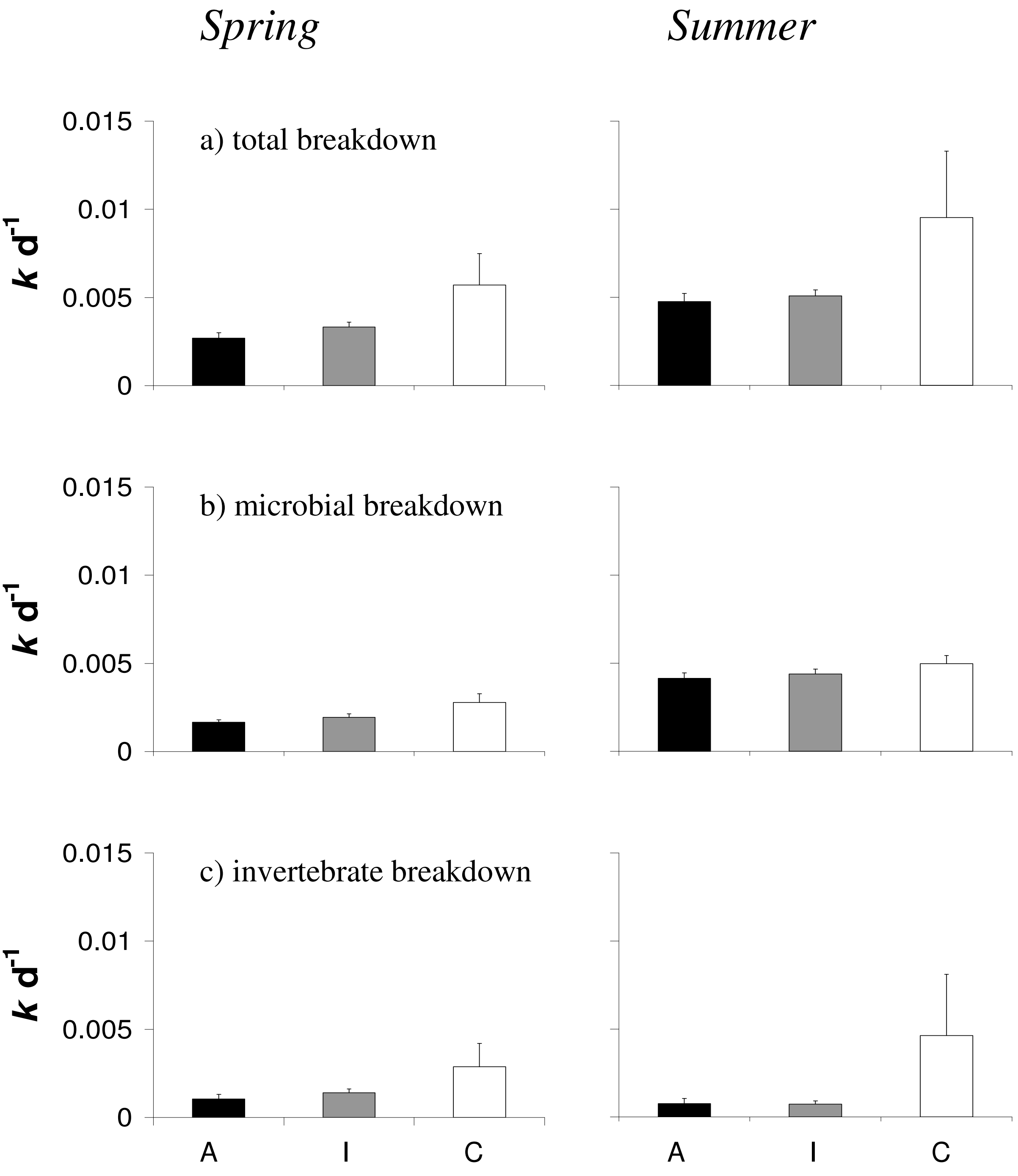

Figure 4.1 Mean breakdown rates (k d^{-1}, + 1 SE) in each of three pH groups (A, acid pH; I, intermediate pH; C, circumneutral pH), in spring and summer 2006 (see Table 4.2 for results of statistical analysis).

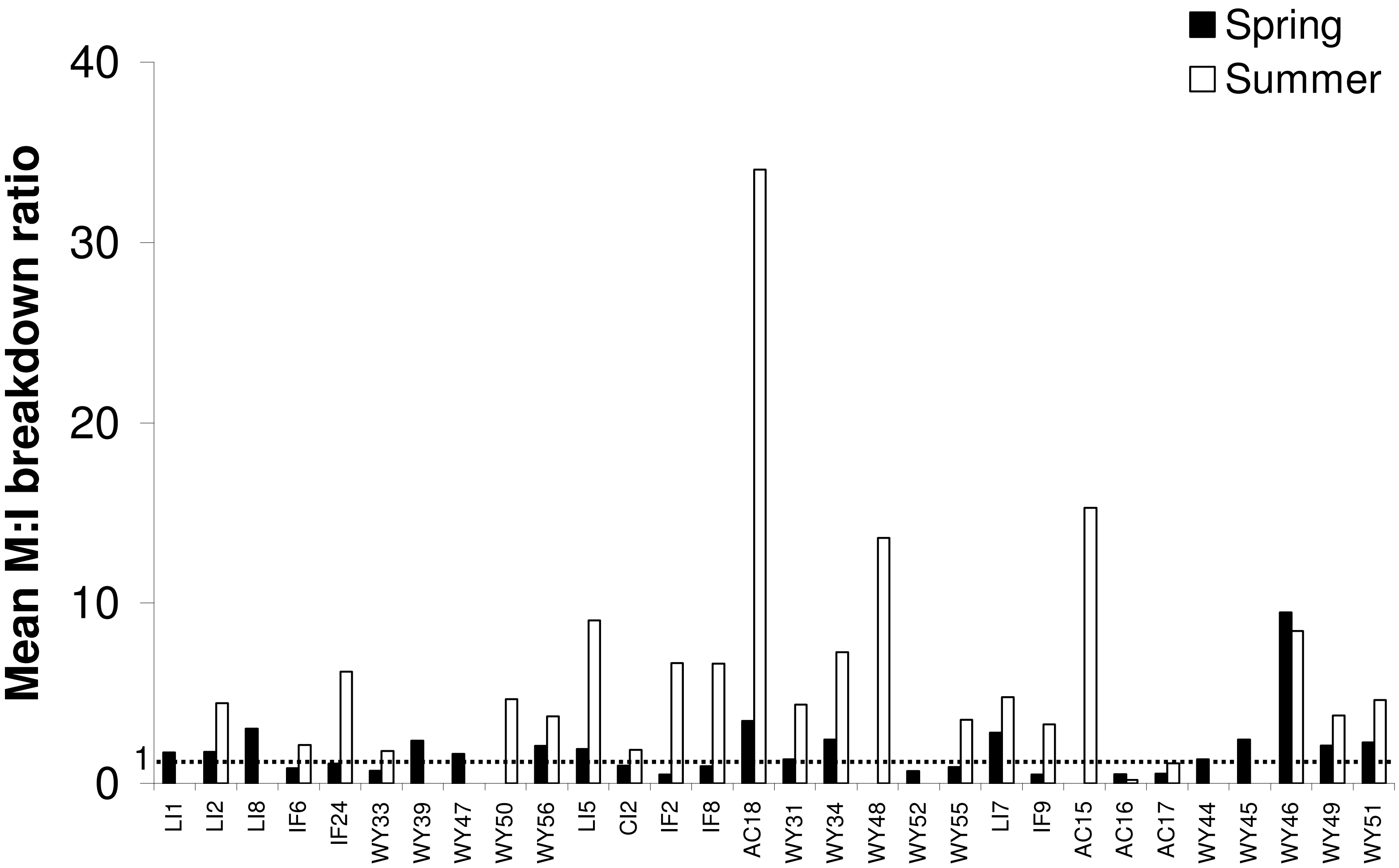

Figure 4.2 Ratio of microbial to invertebrate breakdown in 30 streams of contrasting pH in spring and summer 2006. Sites are in order (from left to right) of increasing mean pH (2006). Dashed line marks ratio of 1 (equal microbial and invertebrate breakdown).

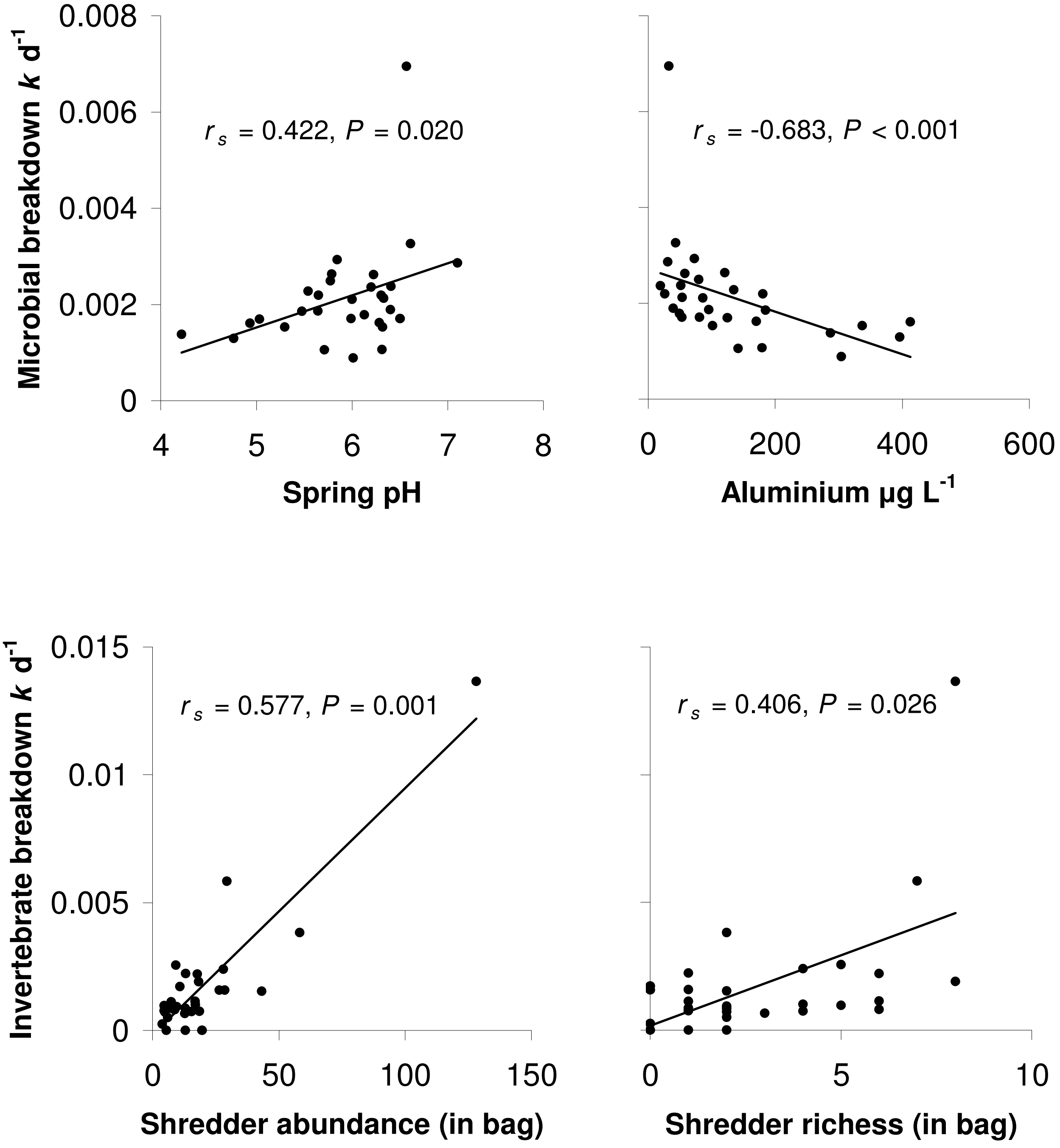

Figure 4.3 Main factors influencing breakdown rates in spring 2006. Trend lines are shown on data where Spearman's rank-order correlation was significant ($P < 0.05$).

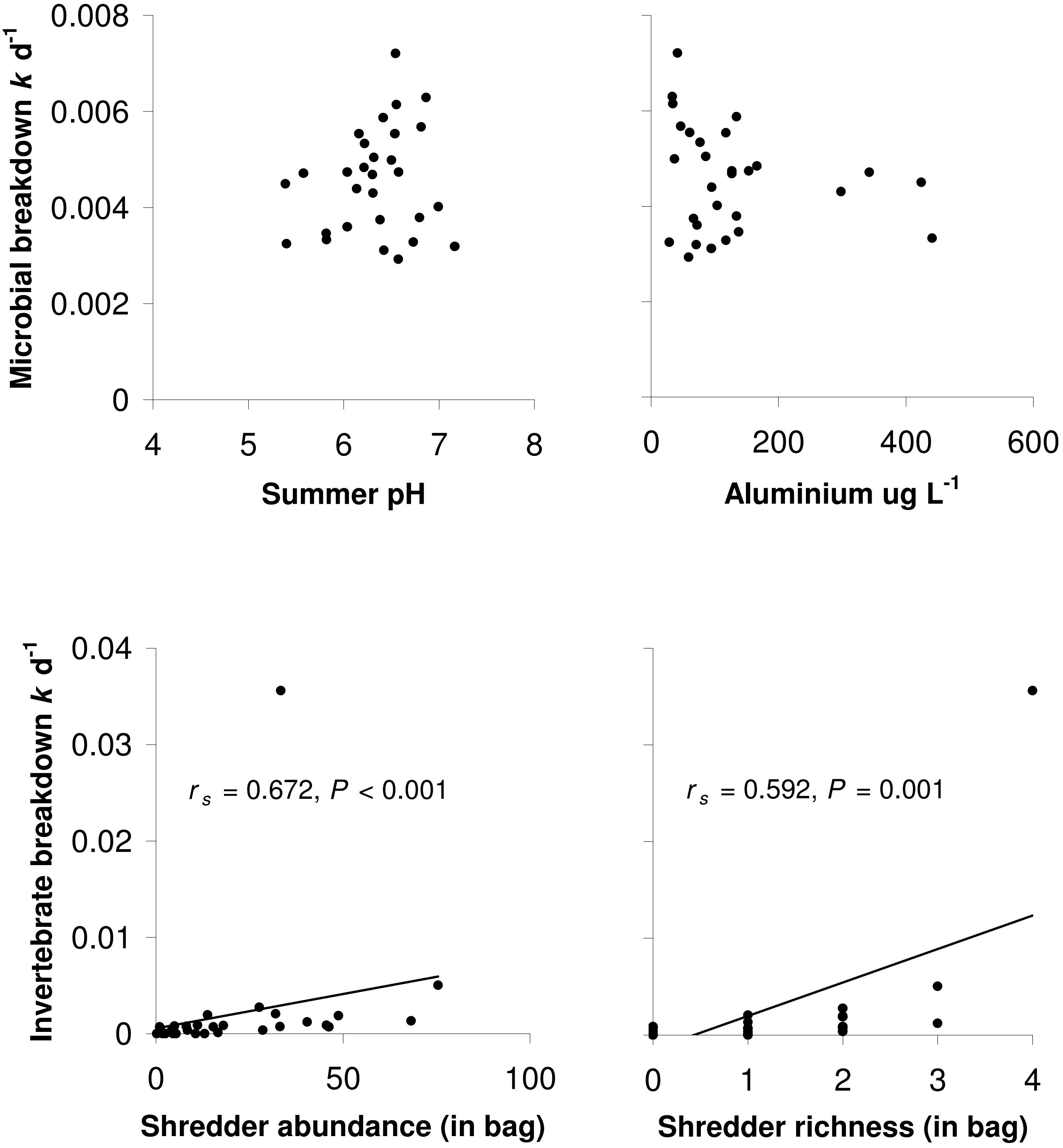

Figure 4.4 Main factors influencing breakdown rates in summer 2006. Trend lines are shown on data where Spearman's rank-order correlation was significant ($P < 0.05$).

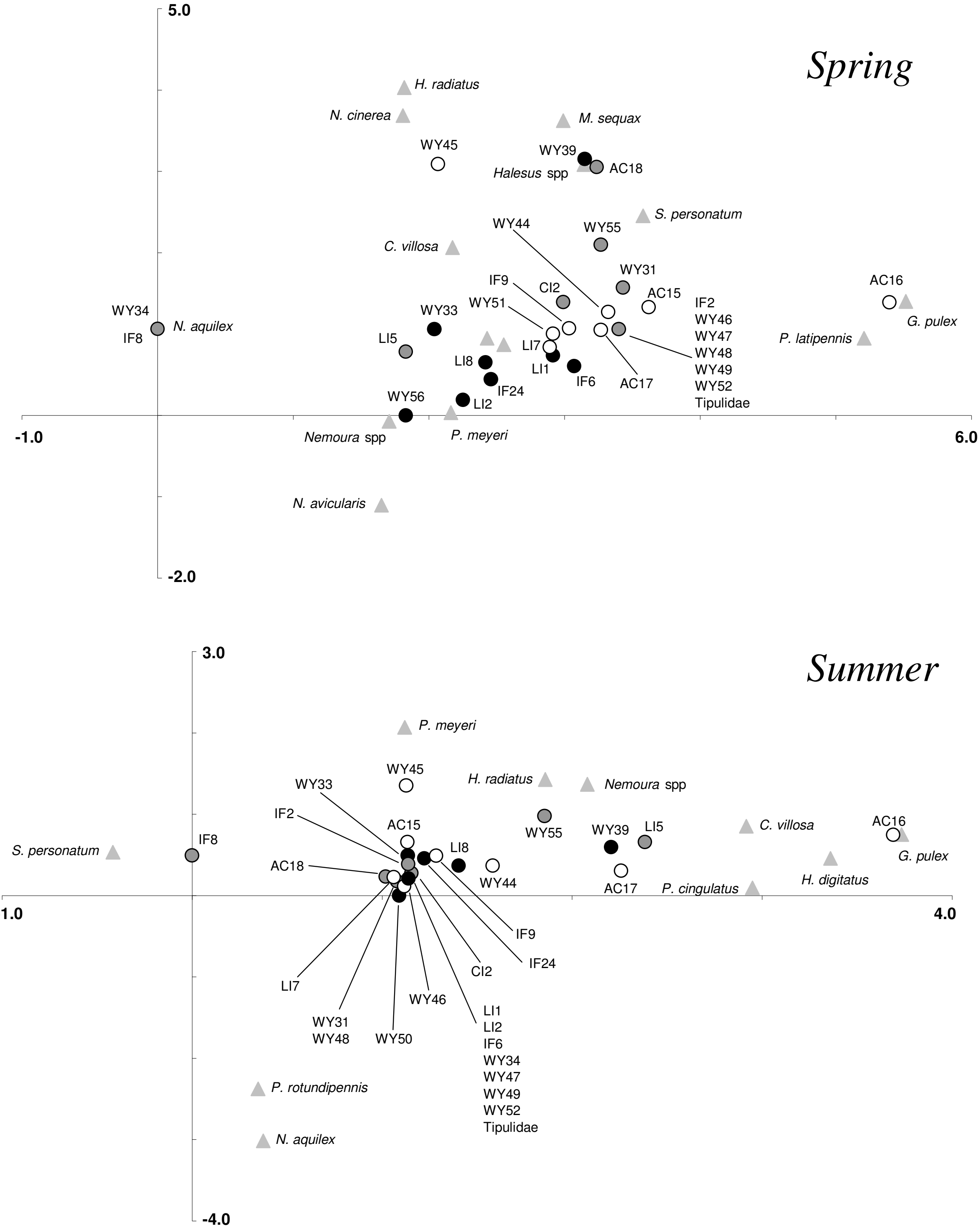

Figure 4.5 DCA ordination diagrams describing the distribution of benthic shredders in 30 streams of contrasting pH in spring and summer 2006. Species scores (triangles) and sites scores (circles: black circles, acid pH; grey circles, intermediate pH; white circles, circumneutral pH) are plotted on the same diagram.

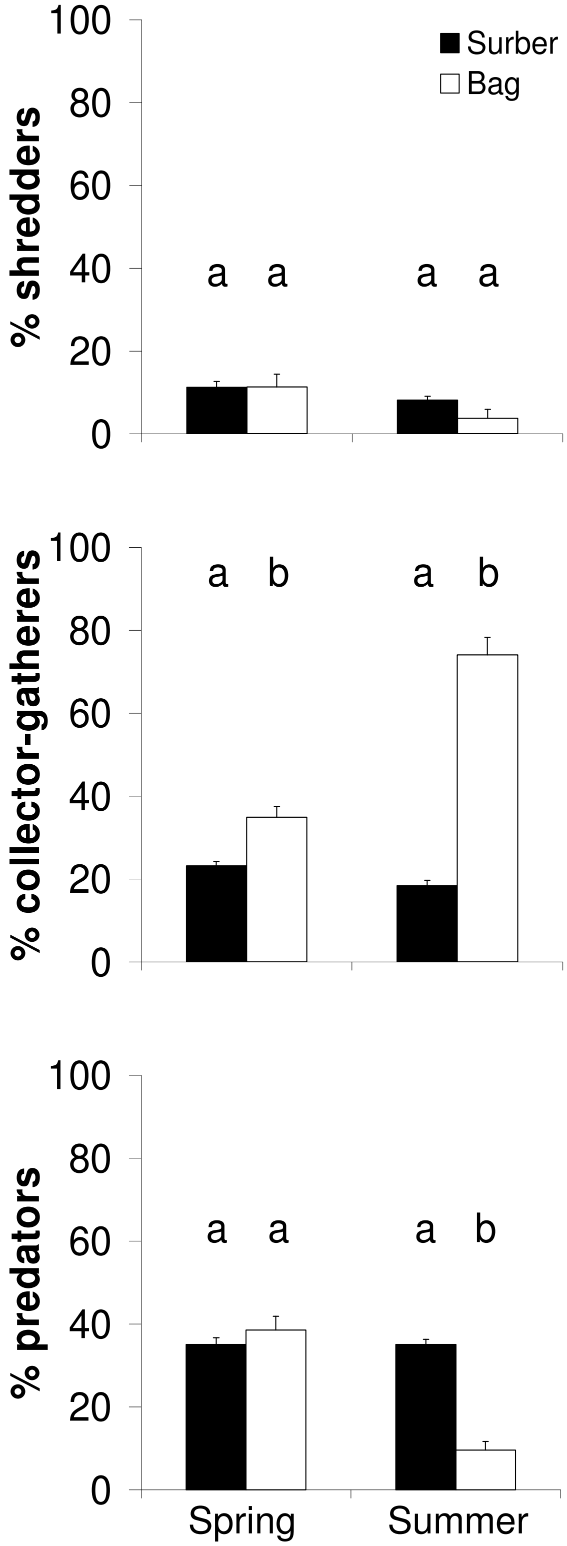

Figure 4.6 Mean relative abundance (% of total density) of the three dominant functional feeding groups (+ 1 SE) in Surber samples and litter bags in spring and summer 2006. Groups with different lowercase letters above bars have significantly different means ($P < 0.05$).

Table 4.1 Leaf litter breakdown rates and shredder community descriptors (mean ± 1 SE) for each of the three pH groups, in spring and summer 2006.

	Acid pH	Intermediate pH	Circumneutral pH
Spring			
Total breakdown (k d^{-1})	0.0027 ± 0.0003	0.0033 ± 0.0003	0.0057 ± 0.0018
Microbial breakdown (k d^{-1})	0.0017 ± 0.0001	0.0019 ± 0.0002	0.0028 ± 0.0005
Invertebrate breakdown (k d^{-1})	0.0010 ± 0.0003	0.0014 ± 0.0002	0.0029 ± 0.0013
Ratio M to I breakdown	1.53 ± 0.28	1.34 ± 0.33	2.20 ± 0.87
Shredder density (m^{-2})	41.0 ± 18.7	18.9 ± 5.3	132.8 ± 99.0
Shredder richness	2.8 ± 0.6	2.2 ± 0.5	3.5 ± 0.5
Summer			
Total breakdown (k d^{-1})	0.0048 ± 0.0005	0.0051 ± 0.0003	0.0095 ± 0.0038
Microbial breakdown (k d^{-1})	0.0041 ± 0.0003	0.0044 ± 0.0003	0.0050 ± 0.0005
Invertebrate breakdown (k d^{-1})	0.0008 ± 0.0003	0.0007 ± 0.0002	0.0046 ± 0.0035
Ratio M to I breakdown	2.30 ± 0.74	8.71 ± 3.06	4.15 ± 1.50
Shredder density (m^{-2})	72.6 ± 20.4	100.4 ± 19.9	486.0 ± 421.5
Shredder richness	2.1 ± 0.4	2.4 ± 0.3	3.0 ± 0.8

Table 4.2 Results of two-way ANOVA testing the effect of stream pH and season on leaf litter breakdown and shredder community descriptors. Results of pairwise comparisons (Tukey HSD) are also shown (abbreviations: A = acid pH, I = intermediate pH, C = circumneutral pH; Sp = spring, Su = summer). Means of underlined groups were not significantly different (*P* > 0.05).

	df	SS	*F*	*P*	Tukey
Total breakdown rate (k d^{-1})					
Season	1	0.014	11.277	0.001	Sp < Su
pH group	1	0.011	4.387	0.017	AI < C
Season × pH group	2	< 0.001	0.072	0.930	
Error	54	0.069			
Microbial breakdown rate (k d^{-1})					
Season	1	0.032	99.799	<0.001	Sp < Su
pH group	2	0.003	5.392	0.007	AI < C
Season × pH group	2	0.001	0.869	0.415	
Error	54	0.017			
Invertebrate breakdown rate (k d^{-1})					
Season	1	0.017	2.265	0.138	Sp Su
pH group	2	0.020	1.332	0.272	AIC
Season × pH group	2	0.003	0.210	0.811	
Error	54	0.404			
Ratio of microbial to invertebrate breakdown					
Season	1	0.479	2.118	0.151	Sp Su
pH group	2	0.714	1.577	0.216	AIC
Season × pH group	2	1.072	2.368	0.103	
Error	54	12.221			
Shredder density m^{-2} (benthos)					
Season	1	8.011	6.805	0.012	Sp < Su
pH group	1	2.534	1.076	0.348	AIC
Season × pH group	2	1.436	0.610	0.547	
Error	54	63.566			
Shredder richness (benthos)					
Season	1	0.017	0.189	0.666	Sp Su
pH group	2	0.065	0.362	0.698	AIC
Season × pH group	2	0.138	0.744	0.466	
Error	54	4.823			
Shredder abundance (leaf bag)					
Season	1	0.059	0.247	0.621	Sp Su
pH group	2	2.127	4.424	0.017	A < IC
Season × pH group	2	0.410	0.852	0.432	
Error	54	12.982			
Shredder richness (leaf bag)					
Season	1	2.983	9.886	0.003	Sp > Su
pH group	2	<0.001	<0.001	1.000	AIC
Season × pH group	2	0.285	0.488	0.616	
Error	54	16.292			

Table 4.3 Mean relative abundance (% of total density) and mean percentage occurrence (%, in brackets) of shredder taxa in both the benthos and in litter bags, in streams from three pH groups. Samples were collected in spring and summer 2006.

Species	Acid pH		Intermediate pH		Circumneutral pH	
	Benthos	Bag	Benthos	Bag	Benthos	Bag
Spring						
Gammarus pulex	-	-	-	-	9.8 (20)	11.3 (20)
Niphargus aquilex	4.4 (10)	-	20.0 (20)	-	-	-
Protonemura meyeri	23.8 (50)	9.8 (20)	-	-	4.5 (30)	9.3 (20)
Nemoura spp.	17.1 (40)	24.9 (40)	6.7 (10)	10.8 (20)	0.9 (10)	5.3 (20)
Nemoura avicularis	0.7 (10)	-	-	-	-	-
Nemoura cinerea	0.6 (20)	9.3 (20)	1.3 (10)	2.5 (10)	6.4 (10)	3.2 (10)
Chaetopteryx villosa	-	6.4 (20)	5.8 (30)	30.1 (50)	1.1 (10)	33.9 (70)
Halesus spp.	-	3.3 (20)	3.3 (10)	10.7 (20)	2.0 (20)	0.8 (20)
Halesus radiatus	-	3.6 (20)	-	3.9 (20)	0.9 (10)	8.3 (40)
Micropterna sequax	-	-	3.3 (10)	-	-	-
Potamophylax spp.	-	2.2 (10)	-	18.4 (50)	-	0.4 (20)
Potamophylax cingulatus	3.6 (20)	20.3 (50)	0.7 (10)	5.2 (20)	8.6 (50)	8.0 (20)
Potamophylax latipennis	2.2 (10)	-	-	0.7 (10)	1.5 (20)	0.02 (10)
Potamophylax rotundipennis	12.5 (20)	-	2.9 (20)	-	1.5 (30)	0.02 (10)
Beraea sp.	-	-	-	-	1.7 (10)	-
Sericostoma personatum	-	-	9.3 (30)	1.8 (20)	1.4 (30)	-
Tipulidae	34.9 (80)	20.1 (50)	46.7 (60)	15.9 (40)	60.0 (100)	19.5 (60)
Summer						
Gammarus pulex	-	-	-	-	11.0 (20)	18.3 (10)
Niphargus aquilex	0.7 (11)	-	-	-	0.3 (10)	-
Protonemura meyeri	2.9 (33)	-	1.1 (20)	-	10.6 (40)	-
Nemoura spp.	2.2 (22)	16.7 (10)	6.1 (10)	7.1 (10)	0.4 (20)	30.0 (20)
Chaetopteryx villosa	6.9 (22)	7.4 (20)	7.9 (30)	47.6 (40)	5.8 (40)	13.8 (30)
Halesus digitatus	-	-	-	-	0.7 (20)	-
Halesus radiatus	-	-	0.6 (10)	14.3 (20)	-	3.2 (20)
Potamophylax cingululatus	0.8 (11)	-	0.6 (10)	-	3.9 (20)	-
Potamophylax rotundipennis	-	-	0.2 (10)	4.8 (10)	-	24.7 (30)
Sericostoma personsonatum	-	11.1 (10)	9.7 (50)	4.8 (10)	0.9 (20)	10.0 (10)
Tipulidae	86.5 (100)	64.8 (40)	73.9 (100)	21.4 (20)	66.4 (90)	-

Table 4.4 Results of Spearman's rank correlation of shredder relative abundances in Surber samples and litter bags, in spring and summer 2006 (r_s = correlation coefficient, P = significance level. Test is significant (*) at $P < 0.05$).

	Spring		Summer	
	r_s	P	r_s	P
Acid pH	0.415	0.097	0.530	0.093
Intermediate pH	0.420	0.093	0.706	0.015*
Circumneutral pH	0.507	0.038*	-0.258	0.444

CHAPTER FIVE

COMPETITION AS A BARRIER TO THE RECOLONISATION OF AN ACID-SENSITIVE GRAZER

5.1 SUMMARY

1. Declining acid deposition, together with restoration efforts, has led the amelioration of acidity in upland streams, but the recovery of biota in these ecosystems has been disappointing. The biotic resistance hypothesis proposes that acidification-induced shifts in biodiversity and species interactions could render damaged communities closed to reinvasion when water chemistry is restored. Here, I investigate the possibility that the performance of acid-sensitive macroinvertebrate recolonists in chemically restored streams is negatively impacted by strong competitive interactions with resident species.
2. A competition experiment using in-stream enclosures was undertaken in a limed stream in mid-Wales. Specifically, I tested for the competitive effect of an abundant resident stonefly, *Leuctra inermis* (Kempny), on the performance (growth rate) of a focal colonist species, *Baetis rhodani* (Pictet). An additive experimental design was employed in which *B. rhodani* was exposed to several (0, 0.25, 0.5 and 1 times ambient) densities of its putative competitor, *Leuctra*. I found that the growth rate of *B. rhodani* (2.29% dry mass d^{-1}) was not impacted by the presence of *L. inermis* (0.85% dry mass d^{-1}) at any of the densities tested. Algal biomass in experimental treatments did not differ significantly from ungrazed controls, indicating that neither taxon depressed resources.

3. Dietary overlap among the two species was examined for nymphs collected from the stream and experimental enclosures, using gut contents analysis. Results showed that both *B. rhodani* and *L. inermis* consumed diatoms, filamentous green algae, coccoid green algae, detritus and inorganic material in varying proportions, suggesting that the potential for competition over food resources in natural populations of these species exists.
4. The results of this study do not support the hypothesis that resident species constrain colonist populations by affecting growth rates through competition for limited resources or interference. Further work is required to assess the potential for independent and interactive effects of other common residents on colonists, acting through competition or predation.

5.2 INTRODUCTION

Declining acid deposition across the northern hemisphere over recent decades has led to widespread improvements in the water quality of formerly acidified surface waters (Reynolds *et al.*, 1999; Fowler *et al.*, 2005). In some areas, the process of chemical recovery has been accelerated through the application of powdered limestone to catchments (e.g. Fjellheim & Raddum, 1992; Bradley & Ormerod, 2002; Raddum & Fjellheim, 2003). However, despite apparent improvements to the acid-base status of many river catchments, evidence for corresponding biological recovery is limited (Alewell *et al.*, 2000; Driscoll *et al.*, 2001; Moerke & Lamberti, 2003; Pretty *et al.*, 2003; Yan *et al.*, 2003; Monteith *et al.*, 2005). One of the most extensive liming programmes in the UK was established in mid-Wales during the 1980s (Ormerod & Durance, 2009). In the upper Tywi catchment, lime applications increased stream base flow pH (> 6.0) and decreased aluminium concentrations (Bradley & Ormerod, 2002), whereas the response of biota did not followed model predictions (Weatheley & Ormerod, 1992; Rundle *et al.*, 1995) and there was a lack of persistence of many macroinvertebrate species, including mayfly larvae (Bradley & Ormerod, 2002). A similarly disappointing response to liming has been observed in the nearby upper Wye catchment (Lewis *et al.*, 2007), with scant recolonisation of acid-sensitive macroinvertebrate and diatom species. Research in mid-Wales is consistent with the findings of work undertaken elsewhere in providing little convincing evidence that biological recovery is underway where water quality has improved (Soulsby *et al.*, 1997; Gee, 2001). Several hypotheses have been proposed to explain the failure of acid-sensitive macroinvertebrate taxa to recolonise watercourses that are apparently recovering chemically (see Bradley & Ormerod, 2002; Holt & Yan, 2003; Ledger &

Hildrew, 2005). These hypotheses, which are not mutually exclusive, include (1) dispersal limitation, (2) resource limitation, (3) abiotic resistance, and (4) biotic resistance.

Freshwater acidification often affects entire river catchments, resulting in the geographical fragmentation of acid-sensitive macroinvertebrate populations, and it has been suggested that biological recovery could be constrained by the poor dispersal abilities of sensitive species (Snucins *et al.*, 2001). *Dispersal limitation* could be an important consideration for less mobile aquatic organisms, such as gastropods, but may also be a significant factor for mobile winged insects where recovering sites are distant from colonist sources. However, recent research indicates that dispersal constraints are unlikely to be a major factor controlling biological recovery of streams in mid-Wales (Bradley & Ormerod, 2002). Several studies have shown that adult aquatic insects travel considerable distances (up to 1.1 km) and disperse readily among catchments (Petersen *et al.*, 1999; Briers *et al.*, 2004). Similarly, recent surveys in mid-Wales found the adults of eight acid-sensitive species in the riparian vegetation of acid streams, even though these species had not been recorded as larvae in these streams for over twenty years (Masters *et al.*, 2007). Elsewhere, Snucins (2003) revealed slow (~8 years) recolonisation of an acidic lake accessible to recolonists (< 5 km), and argued that other factors must place limits on recovery.

Acidification may also act indirectly through *resource limitation* by changing food quality and quantity available to aquatic macroinvertebrates (Sutcliffe & Carrick, 1973; Thomsen & Friberg, 2002). There is unequivocal evidence that taxonomic composition of diatom and macrophyte assemblages differ significantly between circumneutral and acid streams (Ormerod *et al.*, 1987; Ledger & Hildrew, 2001; Hirst *et al.*, 2004), and several authors have suggested that the absence of grazer-scrapers in acid streams may be due to reduced food quality (Sutcliffe & Carrick, 1973; Townsend *et al.*, 1983; Mackay & Kersey, 1985; Dangles & Guérold, 2000). Winterbourn *et al.* (1985) found that stream invertebrates produced faeces with low organic content when offered epilithon from acid streams and reasoned that this was because the microbially-impoverished epilithic layers from acid streams were a poor food source. Whether algal communities in limed or naturally recovering streams are of poorer quality than those in circumneutral streams has yet to be tested experimentally however, and I hope to explore this hypothesis in Chapter 6.

Recent research has shown that acid episodes may still occur in sites regarded as being recovered chemically from acidification (Bradley & Ormerod, 2002; Kowalik & Ormerod, 2006). Acid-sensitive recolonists to such systems may face *abiotic resistance* whereby persistence is governed by acid episodicity caused by heavy rainfall or snowmelt (Bradley & Ormerod, 2002; Lepori *et al.*, 2003, Kowalik & Ormerod, 2006). However, in many instances, pH fluctuations in stream networks are becoming less severe (Davies *et al.*, 2005), and cannot explain observed delays in the biological recovery of chemically restored sites (Monteith *et al.*, 2005).

Acidification leads to profound shifts in the presence and abundance of species across the food web (Chapter 1). Changes in assemblage structure and the resulting strength and shape of species interactions may impart a degree of *biotic resistance* to the reestablishment of acid-sensitive populations once water quality is restored. Community closure (*sensu* Lundberg *et al.*, 2000) may arise where species loss occurs unevenly across functional groups, altering trophic structure (Hildrew *et al.*, 1984; Planas, 1996). For example, in circumneutral streams, grazers interact strongly with benthic algae, controlling algal biomass and altering species composition (Hill & Knight, 1987; Vinebrooke, 1996). In acidified streams, by contrast, grazers are extirpated, potentially leaving an empty niche that can be invaded by acid-tolerant species. Research has shown that stoneflies of the Nemouridae, often characterised as shredders, can in fact feed and grow on a diet of algae (Malmqvist *et al.,* 1991; Friberg & Jacobsen, 1994; Ledger & Hildrew, 2000a; 2001), and regulate algal biomass by grazing in acid streams (Ledger & Hildrew, 2000b). In this case study, acid-tolerant stoneflies occupy the niche of acid-sensitive herbivores and to some degree functionally compensate for their absence. Since the same study showed that nemourids compete with one another for the impoverished layers of benthic algal food, it could be that recolonising species also face competition with residents for limited algal resources in previously acid streams and that this may ultimately contribute to a lack of persistence under improved chemical conditions. Support for the idea of 'competitive resistance' has been found in acid lake communities (Keller & Yan, 1998; Shurin, 2000; Holt & Yan, 2003; Vinebrooke *et al.*, 2003; Frost *et al.*, 2006), where delayed recovery of algal and zooplankton communities following chemical restoration has been attributed to competition with persistent acid-tolerant species.

Research presented in this chapter addressed the potential for *biotic resistance* within a chemically restored stream of the upper Wye in mid-Wales. Specifically, I assessed the degree of resource overlap between the stonefly *Leuctra inermis*, an abundant resident of acid streams (Stoner *et al.*, 1984; Smith *et al.*, 1990; Lancaster *et al.*, 1996; Friberg *et al.*, 1998; Dangles & Guérold, 2000), and the mayfly *Baetis rhodani*, an acid-sensitive grazer that lacks persistence in limed tributaries (Bradley &

Ormerod, 2002). *Leuctra* has been shown to exploit heavily algal resources in acid streams (Ledger & Hildrew, 2005) and it could be that the collective effect of the population is to deplete the availability of algae to invading species. Using a growth experiment, I tested the hypothesis that the growth rate of a recolonist *Baetis*, could be affected by *Leuctra,* its putative competitor. I predicted that the growth of *Baetis* would be higher in the absence of *Leuctra*, and that the growth of the mayfly would be negatively related to the abundance of the stonefly.

5.3 METHODS

5.3.1 Site description

The experiment was conducted in a stretch of the upper Wye River (WY34, 52° 24´89´´N, 3° 39´56´´W) over 23 days in August 2007. The river at this point is approximately 10 m wide, has a stream order of 5 (Strahler, 1957) and a stream link magnitude of 74 (Shreve, 1966). The river bed consists of small boulders, cobbles and gravels, and despite its shallow gradient (2°), is highly unstable at the reach level (Pfankuch Index of 73; Pfankuch, 1975). Land use adjacent to the experimental stretch of the River Wye is predominantly improved grassland used for sheep grazing, although ~ 13% of the surrounding land has been afforested with conifers (Forestry Commission, 2002). In July 2003, The Wye and Usk Foundation began liming hydrological source areas of the Wye in an attempt to improve water quality for fish. Although the main river is not limed directly, it is subject to the cumulative influence of lime-treated tributaries, and is characterised by stable circumneutral pH during summer months (5.7 to 6.1 during the experimental period). Mean annual dissolved calcium, nitrate and phosphate levels (2002-2006) in the river are approximately 2, 0.4 and 0.012 mg L^{-1}, respectively. The algal biofilm in the upper River Wye is dominated by the diatoms *Achnanthidium minutissimum*, *Fragilaria* cf. *capucina* var. *gracilis* and *Eunotia exigua* (I. Jüttner, *pers. comm.*). Macroinvertebrate communities are generally impoverished (22 taxa recorded in both spring and summer 2006) but contain a number of acid-sensitive species, including *Baetis rhodani* and *Rhithrogena semicolorata*.

5.3.2 Experimental design

An additive experimental design (Inouye, 2001) tested the effect of interspecific competition from resident *Leuctra inermis* on the performance (growth rate) of a focal colonist species, *Baetis rhodani*, using in-stream enclosures (plastic containers, 15 cm length × 10 cm width × 5 cm height). Seven treatments were established, representing various density combinations of *Baetis* and *Leuctra*. In four treatments, the number of *B. rhodani* individuals was held constant at low density (5 individuals) and exposed to varying densities (0, 0.25, 0.5 and 1 times ambient) of its putative competitor, *L. inermis* (Table 5.1). In two further treatments, each species was held in the absence of the other (at equivalent densities, 20 individuals) to compare growth rates and assess the impact of each species on algal resources. Finally, in an ungrazed control, algal resources were enclosed without animals. The mean ambient density of each species was determined from Surber samples (Surber net, 0.0625 m^2, 250 µm mesh; Surber, 1970) collected 24 hours before the experiment began.

Enclosures were placed longitudinally to the flow in blocks of seven in well-lit stony riffles for a period of 23 d. Blocks were replicated seven times (i.e. a total of 49 enclosures) resulting in a 7 × 7 randomised block design. The side walls of the enclosures contained six circular apertures (12.6 cm^2 each) of 300 µm mesh. The mesh size allowed water flow through enclosures but prevented departure or arrival of macroinvertebrates. The mesh walls were cleaned at regular intervals during the experiment to reduce clogging. Enclosures were placed in areas of moderately high water velocity, thereby offsetting flow reductions caused by the mesh, and secured to the stream bed using metal stakes. Two rinsed, biofilm-coated cobbles from the same stretch of river were placed into each cage. Individuals of *Baetis rhodani* and *Leuctra* *inermis* were collected from riffles upstream of the experimental area using a standard FBA pond net (250 µm mesh, 230 mm × 255 mm frame, 300 mm bag depth), and were placed in the enclosures on day zero (the quantity depended on the treatment, see Table 5.1). For each species, individuals of approximately equal size (mean body length *Baetis* 4.8 mm, *Leuctra* 7.4 mm) were selected for use in the experiment. Macroinvertebrates for gut contents analysis and growth determination were sampled at the beginning (day 0, 100 *Baetis*, 100 *Leuctra*) and end of the experiment, and preserved in 70% IMS.

5.3.3 Estimating the quantity of algae

Algal biofilm was sampled from the surface of twenty pebbles outside the enclosures (ambient), and from both pebbles inside each of the enclosures. Biofilm was removed from the upper surface of each pebble using a toothbrush, and washed into separate scintillation vials with stream water. These vials were frozen immediately and placed in the dark. The scrubbed surface was carefully traced onto an acetate sheet and the area (cm^2) subsequently determined from the mass of the tracings. The biofilm samples were later defrosted in the laboratory and made up to 24 ml with water. Estimates of ash-free dry mass (AFDM) were made by placing aliquots of biofilm slurry (5-10 ml) in crucibles for ashing (500°C for 4 h). AFDM was estimated as the difference between oven-dried (80ºC) and ashed crucibles. Further 5-10 ml aliquots of biofilm slurry were freeze-dried using an Edwards Modulyo Freeze Dryer attached to an Edwards two-stage vacuum pump. The freeze-dried algae samples were then left overnight at 5ºC to extract in 90% acetone solution for estimation of chlorophyll. Absorption of light was measured at wavelengths of 664 nm, 647 nm and 630 nm using a WPA Lightwave S2000 UV/Visible Spectrophotometer, and corrected for turbidity by subtracting absorbance at 750 nm (Sterman, 1988). From these data it was possible to calculate the concentration of chlorophyll *a* using the equations of Jeffrey and Humphrey (1975). Algal biomass was expressed as µg of chlorophyll *a* and mg ash-free dry mass per unit area.

5.3.4 Gut contents analysis

Twelve individuals of *B. rhodani* and *L. inermis*, from the benthos and enclosures (48 individuals in total), were rinsed in deionised water to remove preservative. The head of each individual was removed, and the contents of the fore-gut 'teased' onto a glass slide under a stereoscopic microscope (Zenith STZ-4500) as described by Cummins (1973). The contents were then dispersed into a drop of deionised water using forceps. Prepared slides were examined under a compound microscope (Nikon Alphaphot YS2-T), and particles present in the gut contents were divided into five categories; diatoms, filamentous green algae, coccoid green algae, detritus and inorganic debris. A qualitative ranking of the percentage of each particle type in the total particle area was estimated (Cummins, 1973). Particle areas were calculated by multiplying the number of particles of each type in the field of view by the mean area of each particle type, calculated from 50 randomly selected particles (Ledger & Hildrew, 2001).

5.3.5 Measuring macroinvertebrate growth

In the laboratory, the identity of macroinvertebrate species was confirmed and the length of individuals collected at the beginning and end of the experiment was measured using a dissecting microscope and eye-piece graticule. The length of individuals (BL, mm) was converted to dry mass (DM, mg) using length-dry mass regression equations previously determined for *B. rhodani* and *L. inermis* (Burgherr & Meyer, 1997).

For *B. rhodani*:

$$\text{Ln DM} = -5.55 + 2.66 \,.\, \text{Ln BL}$$

For *L. inermis*:

$$\text{Ln DM} = -6.12 + 2.66 \,.\, \text{Ln BL}$$

The mean instantaneous growth rates (Gw) for each species in each enclosure was then calculated from the initial (W_0) and final dry mass (W_t) of individuals by:

$$\text{Gw } \% \text{ d}^{-1} = [\text{Ln} \,.\, W_t - \text{Ln} \,.\, W_0/t] \,.\, 100$$

where t is the 23 d of the experiment.

5.3.6 Data analysis

Dietary overlap of *Baetis rhodani* and *Leuctra inermis* was investigated using detrended correspondence analysis (DCA) in CANOCO 4.5 (ter Braak & Šmilauer, 2002). Samples of individuals collected from both the benthos (prior to the start of the experiment) and from the experimental enclosures (at the end of the experimental period) were included. Percentage contribution of each food particle type (diatoms, filamentous green algae, coccoid green algae, detritus and inorganic debris) in gut contents of each species was treated as 'species' data in the analysis.

One-way ANOVA was used to examine the effect of *L. inermis* density on the growth rate of *B. rhodani* (treatments 1-4, Table 5.1) and compare mean algal biomass (as chlorophyll *a* and ash-free dry mass per cm^2) across the seven experimental treatments. Data were tested for equal variances prior to analysis (Levene's test). A *t*-test was used to compare mean growth rates of *B. rhodani* individuals held at low (5 individuals per enclosure) and high (20 individuals per cage) density. All tests were considered significant at $P < 0.05$.

5.4 RESULTS

Gut content analysis revealed substantial overlap in the diet of *Baetis rhodani* and *Leuctra inermis* (Figure 5.1). Individuals of both species, collected from the benthos and experimental enclosures, consumed diatoms, filamentous green algae, coccoid green algae, detritus and inorganic debris of varying proportions (Figure 5.1). Benthic *B. rhodani* consumed proportionally more diatoms and coccoid green algae, and less detritus, than *L. inermis* (Figure 5.1). Detrended correspondence analysis (DCA) of gut contents revealed that inter-individual variation in the proportions of different food types consumed was greater for *B. rhodani* than *L. inermis* (Figure 5.2), and generally greater for caged than benthic nymphs of both species (Figure 5.2).

During the 23 d enclosure experiment, individuals of bivoltine *B. rhodani* grew an average of 2.29% dry mass day^{-1}, compared to a rate of 0.85% dry mass day^{-1} for univoltine *L. inermis*. One-way ANOVA revealed no significant effect of *L. inermis* density on *B. rhodani* growth rate ($df = 18$, $F = 0.378$, $P = 0.770$; Figure 5.3). Furthermore, a t-test revealed that *B. rhodani* growth rates at low and high density were similar ($df = 9$, $t = 0.319$, $P = 0.757$).

One-way ANOVA revealed no significant difference in algal biomass (as chlorophyll *a* and AFDM per cm^2) among treatments (chlorophyll *a* $df = 48$, $F = 0.095$, $P = 0.996$; AFDM $df = 48$, $F = 0.253$, $P = 0.955$; Figure 5.4). Thus, neither *B. rhodani* nor *L. inermis* significantly reduced algal abundance in enclosures.

5.5 DISCUSSION

Several hypotheses have been advanced to explain the absence of biological recovery in surface waters where acidity is ameliorating (Alewell *et al*., 2000; Driscoll *et al*., 2001; Moerke & Lamberti, 2003; Pretty *et al*., 2003; Yan *et al*., 2003; Monteith *et al*., 2005). Among these, the biotic resistance hypothesis has perhaps received the least attention, although mathematical models predict that changes in food web structure in polluted sites result in conditions that render assemblages resistant to invasion (Lundberg *et al*., 2000). Failure of acid-sensitive algal species to recolonise lakes following chemical improvements has been attributed to competitive exclusion by persistent acid-tolerant species (Vinebrooke *et al*., 2003), but to my knowledge, the biotic resistance hypothesis has not been tested in the context of stream acidification.

In the present investigation, the diet of both *Baetis rhodani* and *Leuctra inermis* was similar, consisting of diatoms, filamentous and coccoid green algae, detritus and inorganic material. The ability of these two species to exploit the same resources confirms that there is potential for interspecific competition to occur in these upland streams. Similarity of diet between individuals collected from the benthos and enclosures to some degree justifies the use of cages as venues for experiments, since interactions between *B. rhodani* and *L. inermis* are predicated on realistic resource exploitation. Variation in the proportions of each food type eaten by individuals of *B. rhodani* was greater than for *L. inermis*, both in the benthos and experimental enclosures, demonstrating the differing feeding strategies of these two species. In natural systems, *Baetis* is the more mobile of the two and moves between a greater number of food patches in a given time period, resulting in greater variation in the proportions of food types consumed by individuals. *Leuctra*, on the other hand, is more sedentary, which is reflected in the consistency of proportions of different food types collected among individuals of this species. Interestingly, the results presented here challenge the idea that grazers, such as *B. rhodani*, avoid feeding on filamentous algae due to its unpalatable nature (Reynolds, 1992; Junger & Planas, 1993), as this form of algae was a component of the diet of individuals both from the enclosures and the benthos. Indeed, filamentous algae may in fact constitute an important part of the diet of *B. rhodani*, particularly in summer months when this form of algae is most abundant in streams (Brown, 1961).

Despite a high level of dietary overlap in *B. rhodani* and *L. inermis*, I could find no direct evidence for competition between these two species, as *B. rhodani* growth was unaffected by the presence of its competitor. It is known that some stoneflies, including nemourids (Ledger & Hildrew, 2001) and leuctrids (Hynes, 1993) have similar trophic requirements to mayflies, and may functionally compensate for their loss under acidic conditions (Ledger & Hildrew, 2000b), such that competition from generalist stoneflies may hinder the recolonisation of acid-sensitive mayflies once water chemistry has been restored. The results of my in-stream mesocosm experiment, however, showed no support for the idea that strong biotic interactions, namely competition, render chemically restored streams closed to reinvasion by acid-sensitive species (Lundberg *et al.*, 2000). I could also find no evidence that intraspecific competition between *Baetis* individuals reduces their success (Kohler, 1992), as growth rates at low and high densities were similar. I cannot however refute the hypothesis that strong competitive interactions prevent recolonisation of *Baetis* to chemically restored streams completely, as the interactions between *B. rhodani* and *L. inermis,* and *Baetis* conspecifics reported here are only a small subset of the possible competitive interactions between species that may occur in my study stream, and represent an even smaller subset of the interactions that may occur globally in acid-sensitive catchments. Furthermore, the competition hypothesis is a subset of the ‘biotic resistance’ hypothesis, and does not take into account the possible impact of other biotic interactions on the success of *B. rhodani* in chemically restored streams. For example, the presence of predators has been found to have a negative impact on *Baetis* populations, both directly through their consumption, and indirectly through sub-lethal effects of the predator’s presence (Kratz, 1996; McPeek & Peckarsky, 1998).

Algal biomass in experimental enclosures did not differ from that found in the ungrazed control treatment, which may further explain why the presence of *L. inermis* did not affect the growth of *B. rhodani*. The competition hypothesis proposes that strong competitive interactions reduce the success of recolonists where focal resources are limiting (Ledger & Hildrew, 2000b). The lack of significant grazing pressure in experimental enclosures suggests however that *Leuctra* is not capable of regulating the resources available to *Baetis* such that they become limiting to this species. Impoverished food quality resulting from nutrient limitation, on the other hand, may represent a more immediate threat to the success of recolonising grazers than competition for limited resources (Townsend *et al.*, 1983; Winterbourn *et al.*, 1985; Sutcliffe & Hildrew, 1989; Winterbourn *et al.*, 1992). Indeed, in the present investigation the growth rate of *B. rhodani* was 2.29% dry mass day^{-1}, which is lower than the value reported elsewhere (4.51% DM d^{-1}) for a similar species, *Baetis alpinus*, in Alpine streams (Breitenmoserwursten & Sartori, 1995). As the *quantity* of algae in my enclosures was probably not limiting to *Baetis*, the discrepancy in growth rates between the two studies may indicate that this grazer species is limited by the nutritional *quality* of algae in limed streams such as the Wye. The growth rate of *L. inermis* in this experiment, on the other hand, was much greater than the rate recorded (0.37% DM d^{-1}) in an acid stream in the Peak District (Pretty *et al*., 2005), indicating that this species is limited neither by the quantity nor the quality of food in limed streams.

The strength of biotic interactions in upland headwater streams, including competition, may be reduced by high levels of physical and hydrological disturbance (Peckarsky *et al*., 1990). Evidence to support the competition hypothesis has so far only been found in lentic systems (Keller & Yan, 1998; Shurin, 2000; Holt & Yan, 2003; Vinebrooke *et al*., 2003; Frost *et al*., 2006), which are more hydrologically and chemically stable than stream environments. The harsh abiotic conditions found in streams may reduce population numbers and weaken biotic interactions. Moreover, periods of episodic acidity during high flow may represent a more immediate threat to the survival and fitness of acid-sensitive recolonists (Lepori *et al*., 2003).

It is clear that the concept of 'biotic resistance' in macroinvertebrate communities recovering from acidification is a controversial one. It is however a potentially important factor in explaining the delay in biological recovery of streams and rivers. Despite recovery of water chemistry in many affected catchments, it has yet to be determined if the changes to aquatic communities are also reversible. In the present study, I found no direct evidence to support the theory that macroinvertebrate communities damaged by acidification are closed to reinvasion once water chemistry has improved. It may be that biotic interactions (i.e. competition) in highly disturbed systems such as the upper Wye are not strong enough to reduce the success of reinvasion by acid-sensitive grazers. However, a great deal more research is required in order to completely discount the ‘biotic resistance’ hypothesis. The impact of predation pressure on acid-sensitive recolonist species requires particular attention.

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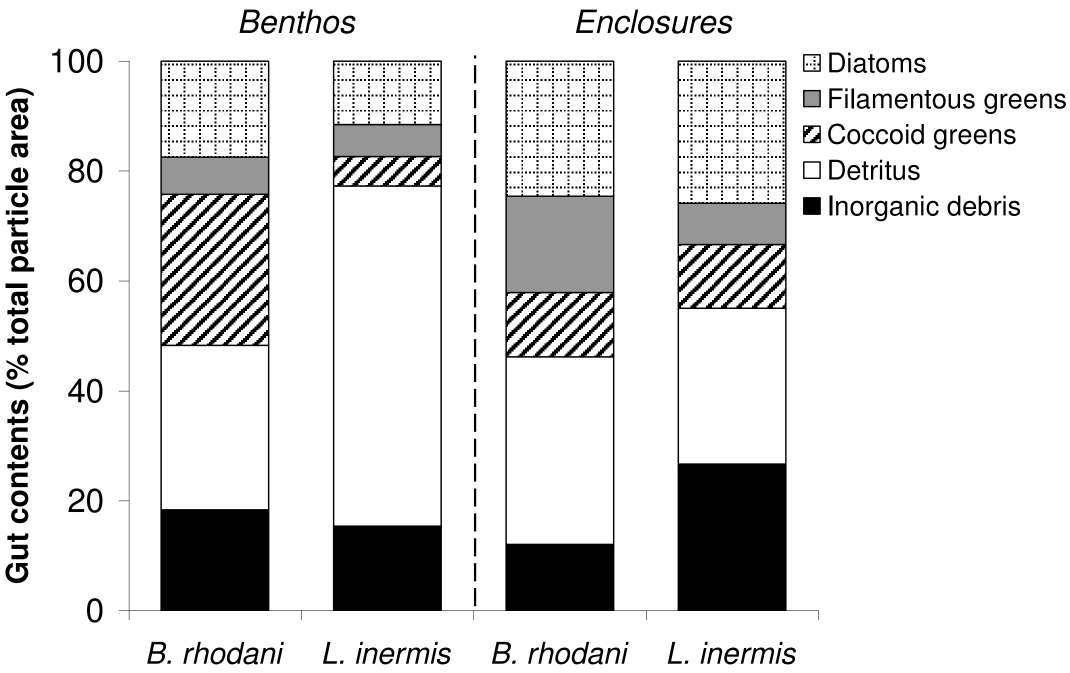

Figure 5.1 Mean percentage composition (food category area as percentage of total particle area) of gut contents collected from nymphs of *Baetis rhodani* and *Leuctra inermis* sampled from the benthos prior to the start of the experiment, and from the experimental enclosures at the end of the experiment.

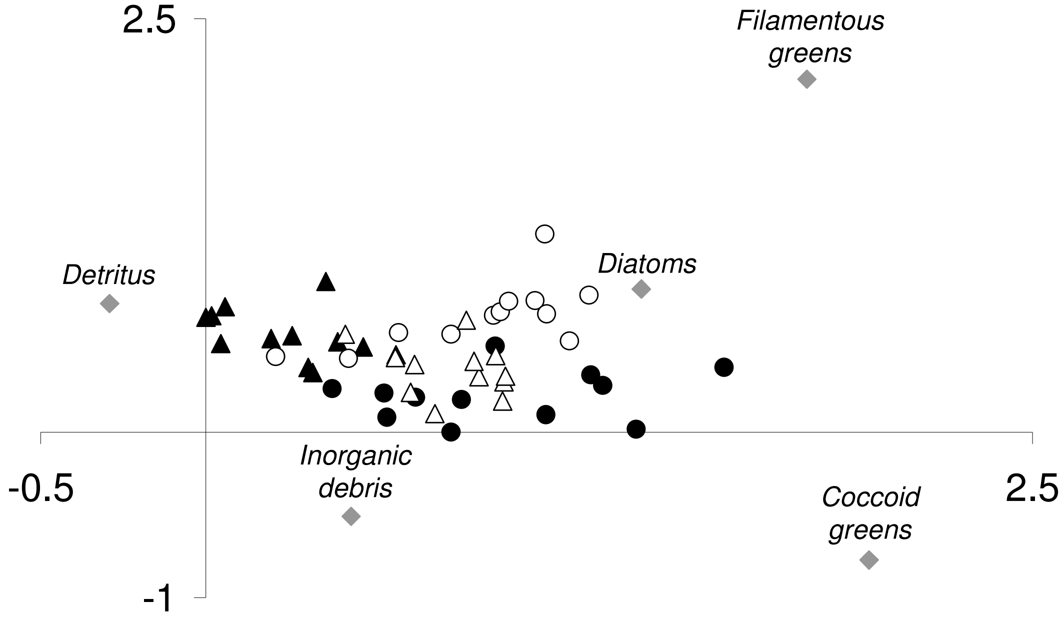

Figure 5.2 DCA ordination diagram of diet for *Baetis rhodani* (circles) and *Leuctra inermis* (triangles) from benthic samples (open symbols) and enclosures (closed symbols). Centroids for each food particle type (grey diamonds) are also shown. Axis one has a length of 1.568 SD and an eigenvalue of 0.154, whereas axis two has a length of 1.197 SD and an eigenvalue of 0.062.

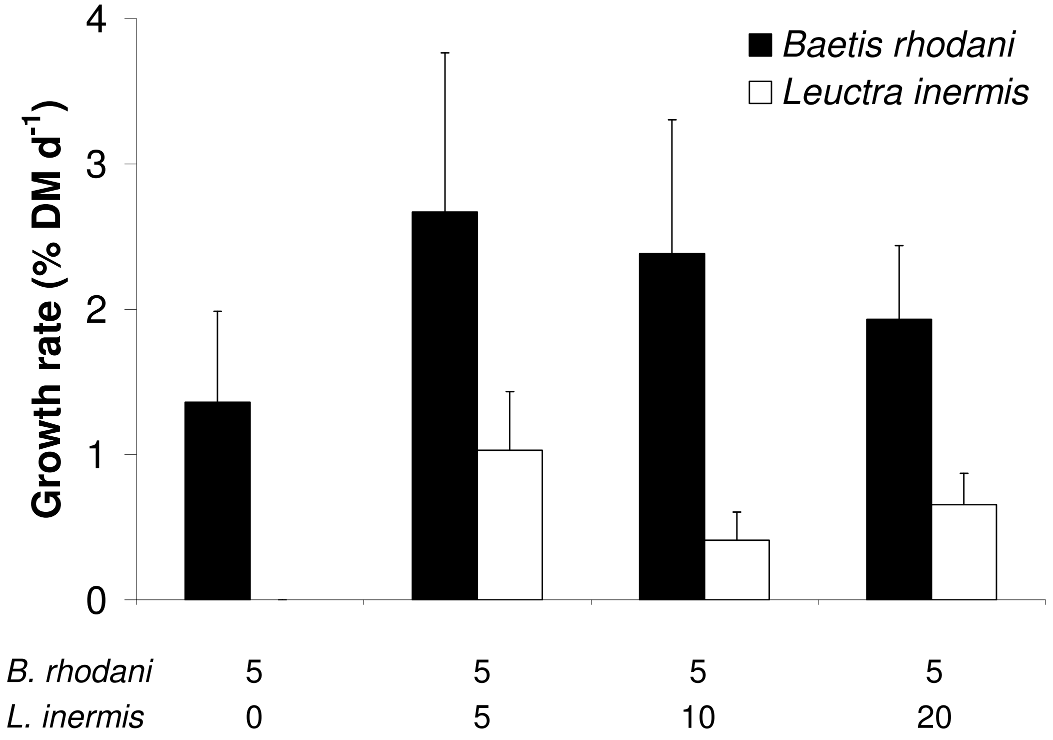

Figure 5.3 Mean (+ 1 SE) instantaneous growth rate (% increase in dry mass per day) of *Baetis rhodani* and *Leuctra inermis* in each treatment. Each of the four experimental treatments exposes *B. rhodani* (at low density of 5 individuals per cage) to different densities of its competitor, *L. inermis*.

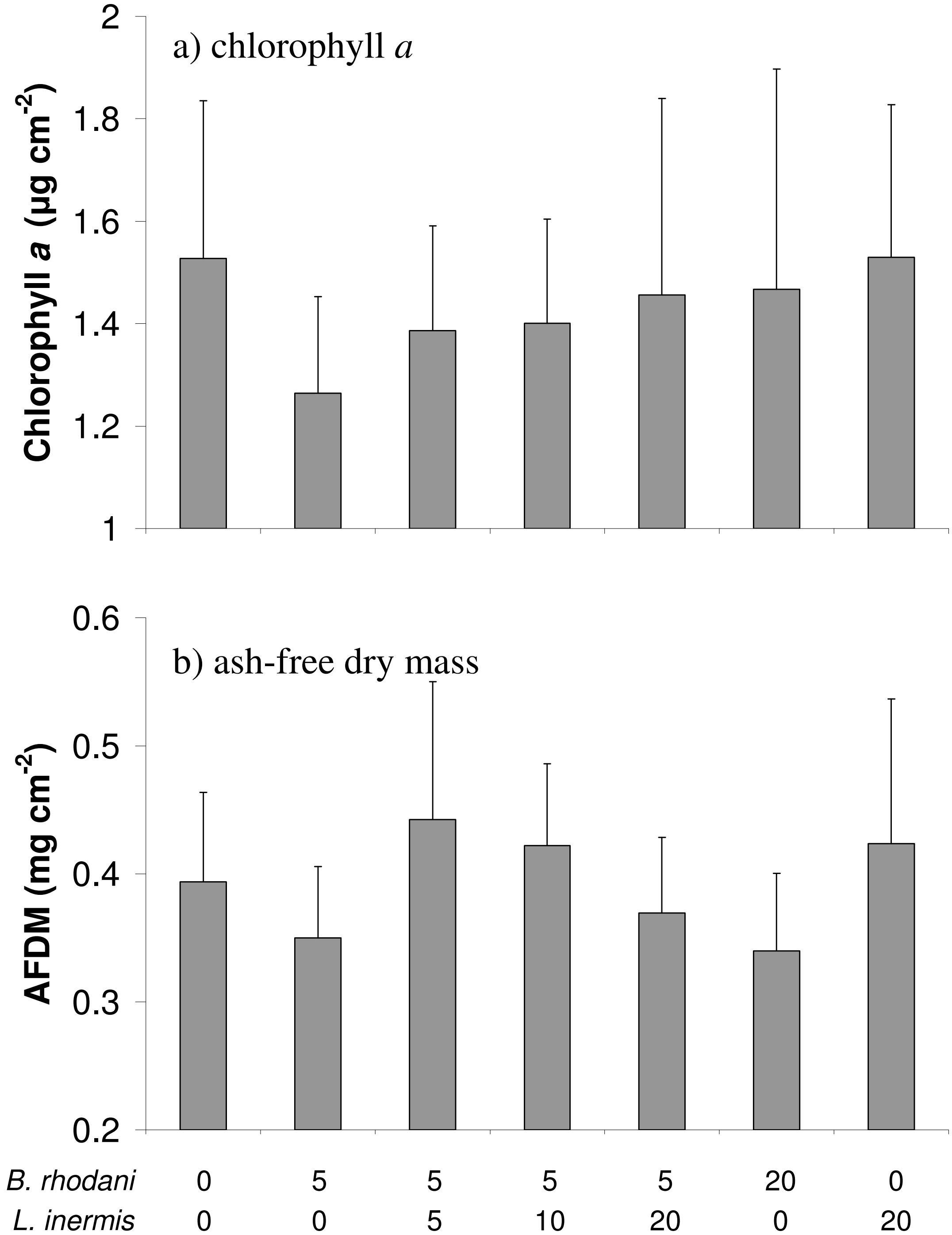

Figure 5.4 Mean (+ 1 SE) chlorophyll *a* (panel a) and ash-free dry mass (panel b) of benthic algae collected from the upper surfaces of stones in each of the seven experimental treatments.

Table 5.1 Description of four experimental treatments exposing *Baetis rhodani* (at low density) to different densities of its competitor, *Leuctra inermis*.

Treatment (multiples of ambient density)	Number of individuals in enclosure	
	Baetis rhodani	*Leuctra inermis*
0 × *Leuctra*	5	0
0.25 × *Leuctra*	5	5
0.5 × *Leuctra*	5	10
1 × *Leuctra*	5	20

CHAPTER SIX

SUCCESS OF AN ACID-SENSITIVE GRAZER WHEN FED ALGAL BIOFILMS FROM LIMED STREAMS

6.1 SUMMARY

1. Liming programmes have reduced the severity of surface water acidification in treated catchments. However, despite successful elevation of pH above the tolerance limits of acid-sensitive macroinvertebrate species, many are failing to return to chemically restored sites.
2. Here I test the hypothesis that the success of recolonists in restored sites is limited by low quantity or nutritional quality of available food. Specifically, I tested whether the growth rate of an acid-sensitive grazer, *Baetis rhodani* (Pictet), was affected by the provenance (acid vs. limed streams) of benthic algal layers. I compared the quantity (chlorophyll *a*, ash-free dry mass) and quality (C:N ratio, autotrophic index) of algal biofilms from limed and naturally circumneutral streams, and conducted a growth experiment using in-stream enclosures to compare the fitness of nymphs fed biofilms from these two stream types.
3. Algal standing crop was similar in limed and circumneutral streams, and no difference in the nutritional value of biofilms from each stream type was evident. However, growth and survival of *B. rhodani* was significantly lower when fed limed biofilms.

4. The results suggest that some aspect of algal chemical composition or palatability in limed streams is having a negative impact on the success of *B. rhodani*. I speculate that failure of acid-sensitive grazers to recolonise chemically restored streams may in part be due to phosphorus limitation, or toxic levels of aluminium in biofilms resulting from lime treatment.

6.2 INTRODUCTION

The addition of neutralising agents such as lime and dolomite to hydrological source areas has been successful in ameliorating the effects of surface water acidification across sensitive regions. Case studies in the UK (Ormerod & Durance, 2009), Scandinavia (Löfgren *et al*., 2009), mainland Europe (Lorz *et al*., 2003) and North America (Eggleton *et al*., 1996; Menendez *et al*., 1996) demonstrate that liming applications typically increase pH and alkalinity, enhance concentrations of dissolved calcium, and reduce concentrations of dissolved metals (Mason, 2002), thereby improving water quality in formerly acidified streams (e.g. Fjellheim & Raddum, 1992; Stoddard *et al*., 1999; Evans *et al*., 2001; Bradley & Ormerod, 2002; Raddum & Fjellheim, 2003).

Despite widespread improvements in stream chemistry, there are relatively few studies that document any corresponding recovery of stream biota where water quality has apparently been restored (Alewell *et al*., 2000; Driscoll *et al*., 2001; Moerke & Lamberti, 2003; Pretty *et al*., 2003; Yan *et al*., 2003; Monteith *et al*., 2005). Even where water quality has improved over several decades, evidence for sustained biological recovery in limed streams has been disappointing, with only limited persistence of acid-sensitive species, such as herbivorous mayflies (e.g. Soulsby *et al*., 1997; Gee, 2001; Bradley & Ormerod, 2002; Lewis *et al*., 2007). The continued absence of mayflies and other sensitive taxa from limed streams has been the subject of some debate (e.g. Fjellheim & Raddum, 2001; Bradley & Ormerod, 2002; Monteith *et al*., 2005). For example, it has been postulated that delays in biological recovery may be caused by continued acid episodes (abiotic resistance), strong competitive or predatory effects (biotic resistance), an absence of local colonists (dispersal limitation), or poor food quality (resource limitation).

Implicit in the resource limitation hypothesis is that the successful reestablishment of herbivorous species, such as mayfly larvae, is limited from the 'bottom-up' via water quality effects on food supply. The quality of benthic algae as a food resource for macroinvertebrate grazers is governed by the concentration of essential elements (e.g. carbon, nitrogen) and organic compounds (e.g. fatty acids, amino acids etc.) in algal tissue (Sterner & Hessen, 1994; Brett & Müller-Navarra, 1997). Algae grown under nutrient limiting conditions can develop high carbon to nutrient ratios that may constrain growth rates of consumers (Allan & Castillo, 2008). The significance of nutrient limitation in constraining benthic algal production has been demonstrated in stream fertilisation experiments, many of which report a marked increase in the abundance of algae after the addition of nutrients, with a consequent increase grazer abundance, growth and production (Hershey *et al*., 1988; Lamberti *et al*., 1989; Hart & Robinson, 1990; Peterson *et al*., 1993; Rosemond *et al*., 1993; Sterner, 1993).

Acidification of streams can result in algal biofilms with reduced organic content (Sutcliffe & Hildrew, 1989) as a consequence of lower availability of essential elements (Ca, Na, K) and nutrients (NO_3) (Alewell *et al*., 2000). Shifts in diatom composition at low pH (Hirst *et al*., 2004) may also result in biofilms that are unpalatable to macroinvertebrates. It is therefore possible that, despite the higher quantities of algae often found in some acid streams (e.g. Allard & Moreau, 1985; Parent *et al*., 1986; Planas *et al*., 1989), reduced biofilm quality and palatability leads to the exclusion of grazer species (Townsend *et al*., 1983; Winterbourn *et al*., 1985; Sutcliffe & Hildrew, 1989; Winterbourn *et al*., 1992).

Although liming increases base-flow pH and dissolved calcium levels above the tolerance limits of most aquatic organisms (Fjellheim & Raddum, 2001), it can result in reduced organic carbon availability (Kullberg *et al*., 1993), and suspension of large quantities of particulate inorganic material (Kullberg, 1987), which could reduce the organic content of algal biofilms. Furthermore, precipitation of aluminium oxides in liming mixing zones (Rosseland *et al*., 1992; Teien *et al*., 2004) may cause fouling of plant and algal material. Although negative associations between liming and invertebrate assemblage structure and biodiversity have been documented (D'Amico *et al*., 2004), it is unclear whether this is due to alterations in food supply. To date, however, there has been no formal comparison of the composition and nutritional quality of benthic algae from circumneutral and limed streams.

The effect of acidification on food quality has been investigated in the context of detritus-detritivore interactions in upland streams. For example, experiments with detritivorous stoneflies *Leuctra nigra* and *Nemurella pictetii* have revealed that the growth of nymphs fed acid-conditioned leaves with impoverished microbial layers was lower than those supplied with circumneutral-conditioned material (Groom & Hildrew, 1989; Thomsen & Friberg, 2002). By comparison, analyses of pH effects on algal resource quality are scarce. However, Winterbourn *et al*. (1985) fed several common macroinvertebrate species (*Leuctra nigra*, *Nemurella pictetii*, *Ecdyonurus dispar*, *Baetis* spp., *Sericostoma personatum*, Tipulinae, *Gammarus pulex*) algal biofilms from streams of contrasting pH, and found that biofilms from acid sites contained few algae, and therefore constituted a relatively poor quality food source for aquatic invertebrates. More recently, Stelzer & Lamberti (2002) found that the growth of the snail *Elmia* was directly related to the nutritional quality (evidenced by ratios of C, N and P) of its algal food source. The authors suggest that the limitation of primary consumer growth by algal chemical composition may be a widespread phenomenon, particularly in oligotrophic streams. To my knowledge however, research has not been undertaken to test the hypothesis that food quality limits biological recovery in neutralised upland streams through negative effects on growth rates and survivorship of acid-sensitive recolonists.

The research presented in this chapter aims to bridge this research gap and examines the relative nutritional value of epilithic biofilm from a series of limed and circumneutral streams in mid-Wales. The putative nutritional value of the algal layers from these two stream types was measured using a set of biomass (chlorophyll *a*, AFDM) and biochemical descriptors (C:N ratio, autotrophic index). A growth experiment conducted in the River Bidno, a tributary of the River Wye, assayed the growth of a test organism, the mayfly *Baetis rhodani*, fed biofilms from a suite of sites with contrasting water quality and liming histories. Where reduced success of grazers in limed streams is related to impoverished algal quality, I predicted, first, that the quantity and quality of benthic algae in limed streams would be lower than those in circumneutral streams, and second, that growth and survivorship of *B. rhodani* nymphs would track resource availability.

6.3 METHODS

6.3.1 Site description

The experiment was conducted in a stretch of the River Bidno (WY31), which is a tributary of the upper Wye near Llangurig, mid-Wales (52º24´78´´N, 3º37´90´´W). Catchment land use is semi-improved and improved grassland used for sheep grazing in the lower reaches, and coniferous plantations in the headwaters. The stream bed at the study site is relatively unstable (Pfankuch Index of 58) and consists predominantly of large boulders, cobbles and gravels. In July 2003, the Wye and Usk Foundation began a liming programme in the upper Wye catchment, including the headwaters of the Bidno. Water chemistry data collected in 2006 showed that this stream is calcium rich (1.98 and 1.80 mg L^{-1} in spring and summer, respectively), and moderately circumneutral (mean annual pH > 6.0). Dissolved aluminium concentrations are relatively low (mean 75 µg L^{-1} in 2006). Dissolved sulphate levels are also comparatively low (mean 2.96 mg L^{-1} in 2006).

The macroinvertebrate assemblage in the River Bidno is the most taxonomically rich (spring 33, summer 35 taxa) of the 15 sites sampled across the upper Wye catchment during this study. The site contains abundant populations of several acid-sensitive species throughout the year, including *Baetis rhodani*, *Baetis scambus*, *Alainites muticus*, *Rhithrogena semicolorata*, *Electrogena lateralis* and *Ancylus fluviatilis*. The abundance of acid-sensitive grazers in the Bidno throughout the year reflects adequate water quality, making it the ideal site to conduct this experiment. Algal assemblages in the Bidno are dominated by the diatom *Achnanthidium minutissimum* (I. Jüttner and S. Ormerod *pers. comm.*).

Six further sites (three limed, three circumneutral) were used as sources of algal biofilm. The chosen circumneutral streams (WY44, WY46, WY51; minimum pH ≥ 6.0, mean pH ≥ 6.5) all supported abundant populations of acid-sensitive species, including *Baetis rhodani* (Chapter 2). The limed streams (WY33, WY34, WY52; minimum pH ≤ 5.5, mean pH < 6.5) had either calcium carbonate deposited directly at source from July 2003 onwards, or were subject to the cumulative influence of limed tributaries. The chosen limed streams also lacked populations of the acid-sensitive *Baetis* in spring. Recent diatom species lists for each of the six source streams were acquired from The Catchment Research Group at Cardiff University (I. Jüttner and S. Ormerod *pers. comm.*), to give an indication of the algal community composition at each of these sites (Table 6.1).

6.3.2 Experimental design

In June 2008, a growth experiment using in-stream enclosures was conducted in the River Bidno to test the hypothesis that poor quality algal food in limed streams limits the growth and survival of the mayfly *Baetis rhodani*. Six treatments were established (6 replicates, 36 enclosures in total), consisting of *Baetis rhodani* nymphs supplied with biofilm-coated cobbles collected from one of the six source streams (3 limed, 3 circumneutral). Cobbles were collected from the six sites on the day the experiment began (day 0) and inspected to ensure any attached macroinvertebrates were removed. A proportion of cobbles were sampled for biofilm (for subsequent analysis, see below), with the remainder placed in the enclosures prior to the introduction of the nymphs. Biofilm was removed from the upper surface of each cobble using a toothbrush, and the resulting slurry washed into a 24 ml polypropylene bottle.

Samples were stored in a cool box in the dark and subsequently frozen. The upper surface sampled was traced on to an acetate sheet and the area (cm^2) calculated from the mass (mg) of the tracing.

The number of nymphs introduced to each enclosure (n = 10) was calculated from the mean ambient density of *B. rhodani* (Surber samples, 0.0625 m^2, 250 µm mesh) in the River Bidno (WY31) on day 0. Nymphs for enclosures were collected from riffles downstream of the experimental area using a standard FBA pond net (250 µm mesh), and placed immediately in the enclosures. Effort was made to ensure that all individuals of *B. rhodani* were of approximately equal size (mean initial body length 4.3 mm). A sub-sample of 50 nymphs was taken for initial dry mass determination and preserved in 70% IMS.

Enclosures containing cobbles and nymphs were placed in blocks of six in well-lit stony riffles and secured to metal stakes driven in to the stream bed, resulting in a 6 × 6 randomised block design. The side-walls of each enclosure contained six circular apertures (12.6 cm^2) of 300 µm mesh, preventing *B. rhodani* emigration whilst allowing flow through the enclosures. The mesh walls were cleaned regularly during the experiment to prevent blockage. At the end of the experiment (after 8 days) the enclosures were recovered from the river, and enclosed *B. rhodani* individuals were collected and preserved immediately in 70% IMS. Biofilm was sampled from each replicate enclosure.

6.3.3 Measuring macroinvertebrate growth

In the laboratory, species identity was confirmed (Elliot *et al*., 1988), and the body length of individuals (from tip of head to base of cerci) collected at the beginning and end of the experiment was measured using a binocular microscope (Zeiss Stemi 2000) and eye-piece graticule. The length of individuals (BL, mm) was converted to dry mass (DM, mg) using length-mass regression equations previously determined for *B. rhodani* (Burgherr and Meyer, 1997):

$$\ln \text{DM} = -5.55 + 2.66 \ . \ \ln \text{BL}$$

The mean instantaneous growth rate (Gw) of *B. rhodani* in each enclosure was then calculated from the initial (W_0) and final (W_t) dry mass of individuals, by:

$$\text{Gw} \ \% \ \text{d}^{-1} = [\ln \ . \ W_t - \ln \ . \ W_0/t] \ . \ 100$$

where t is the 8 d of the experiment.

6.3.4 Estimating quantity and quality of algae

Biofilm samples were defrosted and diluted to 24 ml where necessary using deionised water. Chlorophyll pigments were extracted from 5-10 ml aliquots of biofilm slurry using 10 ml of 90% acetone (extracted overnight at 5°C, in the dark), following freeze-drying. The concentration of chlorophyll *a* was calculated after measuring absorbance of the extraction at wavelengths of 664 nm, 647 nm and 630 nm (WPA Lightwave S2000 UV/Visible Spectrophotometer, and 1 cm quartz cuvettes) and correcting for turbidity by subtracting absorbance at 750 nm (Jeffrey & Humphrey, 1975; Sterman, 1988). A further 10 ml aliquot of biofilm slurry was placed in a crucible for ashing (500°C for 4 h). Ash-free dry mass (AFDM) was estimated as the difference between oven dried (80°C) and ashed crucibles. Chlorophyll *a* and ash-free dry mass content of biofilms were used as a measure of the quantity of algae at each site.

Remaining biofilm slurry (4 ml) was freeze-dried and used for elemental analysis (carbon and nitrogen content). Between 1-2 mg of this freeze-dried biofilm was combusted at 950°C using a Carlo Erba EA1110 Flash Elemental Analyser. Gases produced in the combustion process were separated on a chromatographic column and measured to calculate the percentage of carbon and nitrogen in algal biofilm samples (0.1% error). The ratio of carbon to nitrogen is a commonly used index (e.g. McMahon *et al.*, 1974; Hunter, 1980; Bondu *et al.*, 2008) that provides a measure of the balance between carbohydrates and amino acids in sampled tissue (Gregory, 1983; Steinman, 1996). Samples with a high C:N ratio (low N) have a relatively low level of protein and are considered poor quality (Steinman, 1996).

In order to estimate the relative viability of algal biofilms from each location, the autotrophic index (AI) was calculated (AFDM (mg cm^{-2}) / chlorophyll *a* (mg cm^{-2}; APHA *et al.*, 1992; Steinman *et al.*, 2006). When an AI is lower than 100, the biofilm is dominated by algae; for an AI between 100 and 400, the biofilm is balanced between heterotrophic and autotrophic conditions; when an AI is greater that 400, the biofilm consists predominantly of heterotrophs and non-living organic material (Ameziane *et al.*, 2002).

6.3.5 Data analysis

Nested analysis of variance (ANOVA) was used to examine the effect of stream type (limed, circumneutral) and site location nested within stream type, on quantitative (chlorophyll *a*, AFDM) and qualitative (C:N ratio, autotrophic index) descriptors of biofilm. Nested ANOVA was also used to test whether *Baetis* growth, survival and the level of resource depression (% change chlorophyll *a*, AFDM) differed significantly within and between treatments. Data not conforming to normality and equality of variances (Levene's test) were $\log_{10}$ transformed prior to analysis. A pairwise comparison of means (Tukey HSD) was performed where ANOVAs were statistically significant ($P < 0.05$). Pearson's product-moment correlation was used to relate the growth and survival of *B. rhodani* to algal descriptors (chlorophyll *a*, AFDM, C:N ratio, autotrophic index) and water quality variables (pH, conductivity, TDS). Historical chemical data (pH, PO_4, NO_3) for the six source streams (2003-2006) were also related to *B. rhodani* fitness in this way.

6.4 RESULTS

Measures of biofilm quantity (chlorophyll *a*, ash-free dry mass) and quality (C:N ratio, autotrophic index) did not differ significantly between circumneutral and limed streams ($P > 0.05$; Figure 6.1, Table 6.2). Chlorophyll *a* content of biofilms and the autotrophic index differed among sites (Figure 6.1, Table 6.2), but these differences were not related to stream type. Mean initial chlorophyll *a* (Figure 6.1a) and ash-free dry mass (Figure 6.1b) of biofilms varied from 0.595 and 1.885 μg cm^{-2}, and 0.657 and 1.015 mg cm^{-2}, respectively across treatments. The mean carbon to nitrogen ratio in biofilms ranged from 7.7 to 9.1 (Figure 6.1c), and values of the autotrophic index (Figure 6.1d) ranged from 659 to 1153 (as AFDM (mg cm^{-2}) / chlorophyll *a* (mg cm^{-2})).

The mean growth rate of *Baetis rhodani* nymphs was 7.7% DM d^{-1} in circumneutral streams and 5.7% DM d^{-1} in limed streams (Figure 6.2a). Nested ANOVA revealed that the growth rates of *B. rhodani* nymphs were significantly lower when fed algae from limed streams (Figure 6.2a, Table 6.2). The mean survivorship of *B. rhodani* (Figure 6.2b) was also higher for individuals fed algal biofilms from circumneutral streams (mean 94%) compared to limed streams (mean 78%), but only at a significance level of $P < 0.1$ (Table 6.2). The level of resource depression by *B. rhodani* varied with site (Figure 6.3, Table 6.2), but not with treatment.

Pearson's product-moment correlations revealed that differences in the growth rate and survival of *B. rhodani*, and the level of resource depression by this species (as percentage chlorophyll *a* and ash-free dry mass loss) were not significantly related (*P*

> 0.05) to any of the measures of algal nutritional quality (carbon to nitrogen ratio, autotrophic index) or quantity (chlorophyll *a*, ash-free dry mass).

Stream pH (Table 6.1), conductivity and total dissolved solids concentrations in algal source streams at the time of biofilm collection ranged from 6.1 and 6.9, 43 and 82 µS cm^{-1}, and 28 and 54 mg L^{-1}, respectively. *B. rhodani* growth and survivorship did not correlate with any of these water chemistry variables, but did correlate with total dissolved phosphate concentrations (mean PO_4 sampled in 2006 and growth $r = 0.883$, $P = 0.020$; summer PO_4 and growth $r = 0.783$, $P = 0.001$), and mean and minimum pH between 2003 and 2006 (mean 2003-2006 pH and survival $r = 0.880$, $P = 0.021$; minimum 2003-2006 pH and survival $r = 0.921$, $P = 0.005$) in the food source streams. These data were collected at least two years prior to the enclosure experiment however, and not at the time of biofilm collection.

6.5 DISCUSSION

The intention of the research presented in this chapter was to examine the relative nutritional value of epilithic biofilms from a series of limed and circumneutral streams in mid-Wales, and to compare the growth and survival of the mayfly *Baetis rhodani* when fed biofilms from these streams. My results demonstrate that the quantity of algae, evidenced by chlorophyll *a* and ash-free dry mass, was not reduced in limed streams. Furthermore, measures of algal biofilm nutritional quality collected during this experiment, as revealed by the carbon to nitrogen ratio and autotrophic index, were similar for limed and circumneutral streams. Benthic algae with relatively high organic content and low carbon to nitrogen ratios are generally regarded as being of higher nutritional value as a food source for primary consumers (McMahon *et al*., 1974; McMahon, 1975; Gregory, 1983). Low ratios of carbon to nitrogen indicate a higher proportion of protein, and a lower cellulose and lignin content, and it has been proposed that for benthic algae, ratios should lie between 4 and 8 if it is of acceptable quality for grazers (Gregory, 1983). In this experiment, five out of the six algal source streams contained biofilm with a C:N ratio greater than 8, indicating that algal quality may be impoverished in the majority of streams in the upper Wye catchment, regardless of acid-base status.

The suggestion that algal quality is impoverished in the majority of upland streams is further emphasised by the extremely high autotrophic index for algae from the six source streams (range 700 to 1153 across all sites). Values above 400 indicate that biofilms contain a high proportion of non-living organic material, resulting from poor water quality (Ameziane *et al*., 2002). In stable, enriched lowland rivers, values of between 50 and 200 are common (Ameziane *et al*., 2002), whereas similarly high values to the ones reported here have been found in other upland stony streams (Hill *et al.*, 2000). Recent taxonomic investigations of benthic algae (I. Jüttner & S. Ormerod *pers. comm.*) suggest that diatom communities in my study streams consist of a mixture of acidophilic and neutrophilic species, even in the most neutral site, raising the possibility that all streams, both circumneutral and limed, are prone to periodic acid stressor fluctuations throughout the year.

Growth and survival of *Baetis rhodani* was markedly reduced when fed biofilms from limed streams. However, differences in the quantity and quality of biofilms from the two sets of sites (circumneutral and limed) were not apparent using the basic techniques employed in this study, and there was no relationship between biofilm descriptors or water quality variables collected at the time of biofilm sampling and estimates of *Baetis* growth and survival. There was also no evidence to support the idea that macroinvertebrates compensate for low food quality by increasing consumption rates (Collier & Winterbourn, 1990; Cruz-Rivera & Hay, 2000; Fink & Von Elert, 2006), as the impact of *B. rhodani* on biofilms was similar across sites and treatments. Only by looking back at historical water chemistry measurements for each stream could I find a possible explanation for the lower fitness of individuals fed limed biofilms. Analysis of the most recent physicochemical data for the biofilm source streams showed that *B. rhodani* growth rate was significantly related to 2006 dissolved phosphate concentrations, and that *B. rhodani* survival was significantly related to mean and minimum pH in 2006 and preceding years.

These findings suggest that despite improvements in the water quality of acid streams as a result of liming, water chemistry is not fully restored by this process and may continue to have damaging effects on macroinvertebrate grazers *indirectly* through alterations in food supply. This ‘bottom-up’ influence on grazers may be two-fold; first, phosphorous limitation in limed streams may reduce grazer growth rates, and second, biofilms in limed streams may contain some toxic deposited material, possibly aluminium, which results in increased grazer mortality. The latter point is difficult to prove without detailed examination of the biochemical composition of algae from limed streams. It is already known, however, that acid episodes in limed streams can lead to precipitation of aluminium (Rosseland *et al.*, 1992; Teien *et al.*, 2004), some of which may be deposited on plant and algal material and prove toxic to invertebrates. Phosphorus, on the other hand, is known to be in short-supply in benthic ecosystems, particularly to those impacted by acidification (Planas, 1996) and often limits algal abundance (Borchardt, 1996). Stream fertilisation experiments using phosphate have resulted in increased algal biomass (Stockner & Shortreed, 1978; Sterner, 1993) and subsequent increases in grazer numbers (Hart & Robinson, 1990; Peterson *et al.*, 1993), but it is unclear whether grazer communities are responding to increased nutritional quality of algae, or merely its increased quantity. Stelzer & Lamberti (2002) found that the growth of the snail *Elmia* was more strongly related to the phosphorus content of its algal food than the abundance of nitrogen, carbon, organic material or chlorophyll *a*, and suggested that dissolved phosphorus concentration limits snail growth in oligotrophic streams. Similarly, Fink & Von Elert, (2006) found that reduced availability of phosphorus in algae strongly constrained the biomass accrual of the freshwater snail *Radix*. Further research is required, however, to establish if the same is true for insect grazers, and whether the phosphorus content of algal food in limed streams is lower compared to naturally circumneutral streams. An additional hypothesis is that changes to algal taxonomic composition following liming result in biofilms that are unpalatable to macroinvertebrates, although to my knowledge this idea has not yet been tested explicitly in stream environments.

Whether failure of acid-sensitive grazers to recolonise limed streams is due to phosphorus limitation or toxic levels of aluminium in biofilms, it is clear that some aspect of the chemical or structural composition of algae in limed streams is affecting the growth and survival of *B. rhodani*. It is imperative, therefore, that further work be carried out to discover the mechanism behind this finding, in order that the process of biological recovery from acidification can be accelerated. Algal biofilms are clearly impoverished in streams across the upper Wye catchment, irrespective of acid-base status, so slight differences in their biochemical composition as a result of lime treatment could have a marked impact on the value of this already limited resource and its ability to support the metabolic requirements of aquatic macroinvertebrates.

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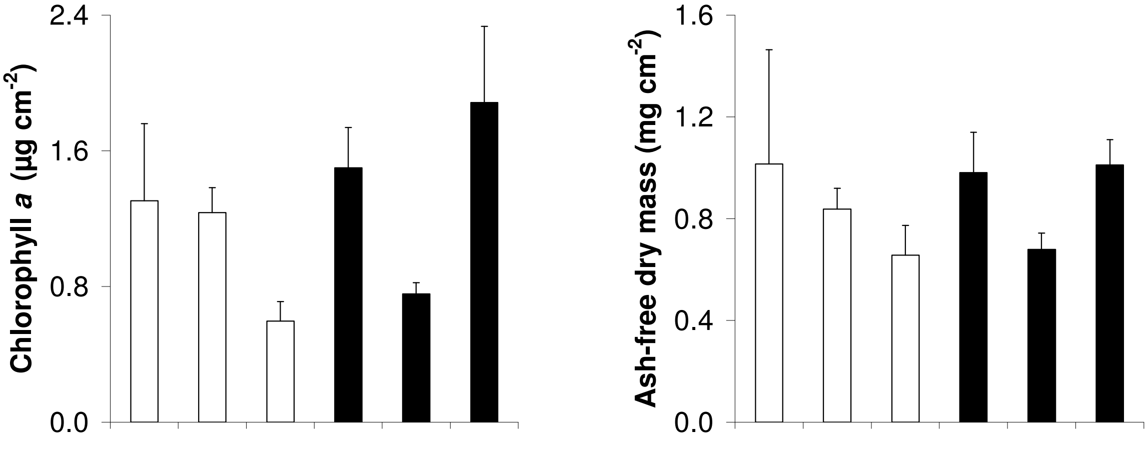
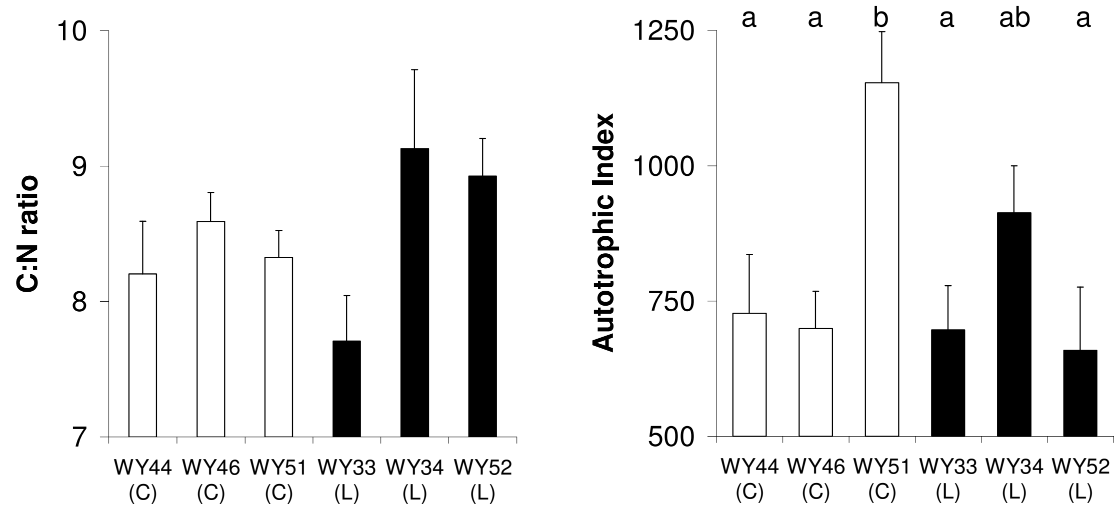


a) chlorophyll *a*

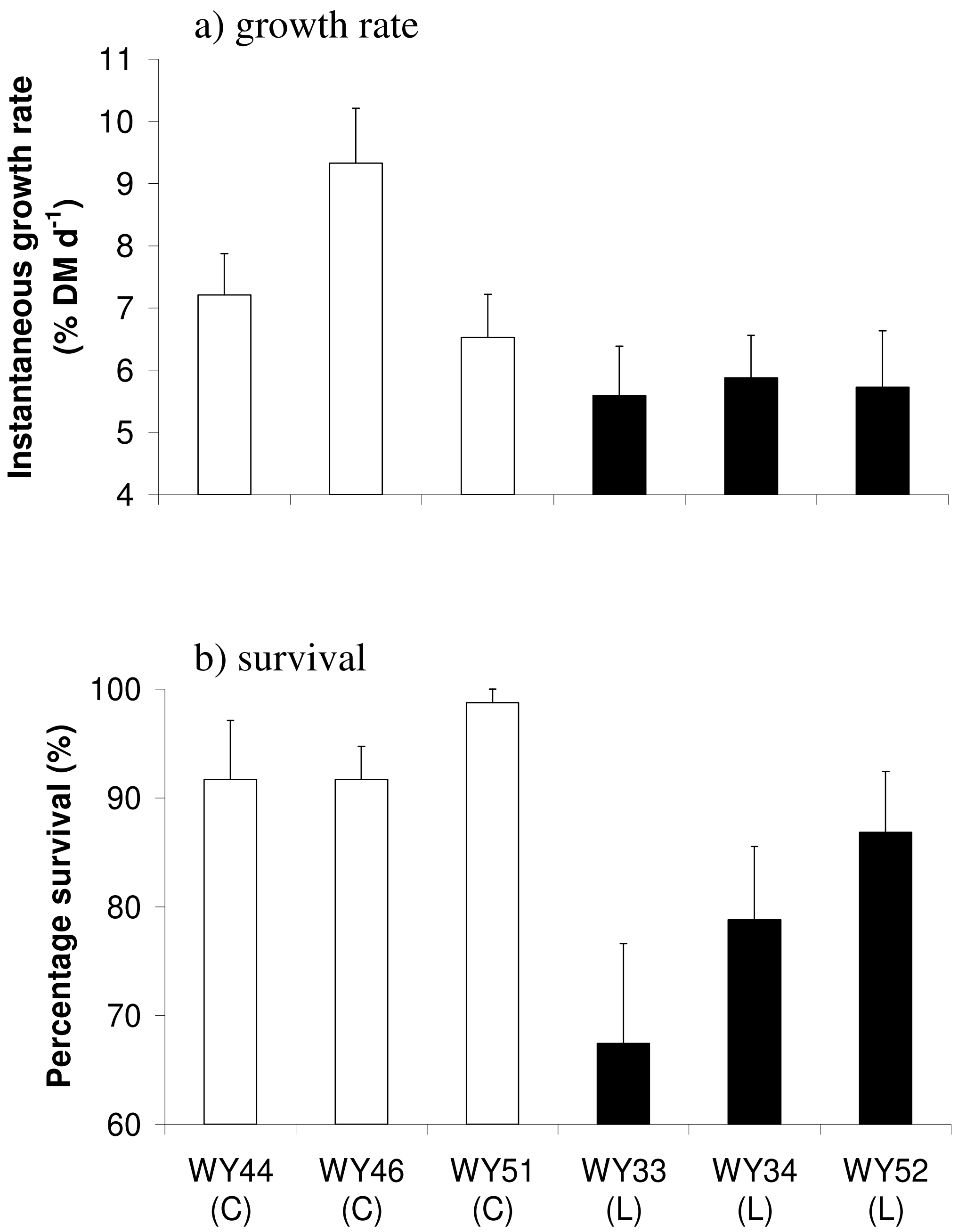
b) ash-free dry mass



c) C:N ratio

d) autotrophic index

Figure 6.1 Quantity (chlorophyll *a* and ash-free dry mass) and quality (C:N ratio and autotrophic index) of biofilm in each source stream at the onset of the experiment (C = circumneutral stream; L = limed stream). Results of two-level nested ANOVA are shown in Table 6.2. Bars with different lowercase letters above (Figure 6.1d only) are significantly different ($P > 0.05$)

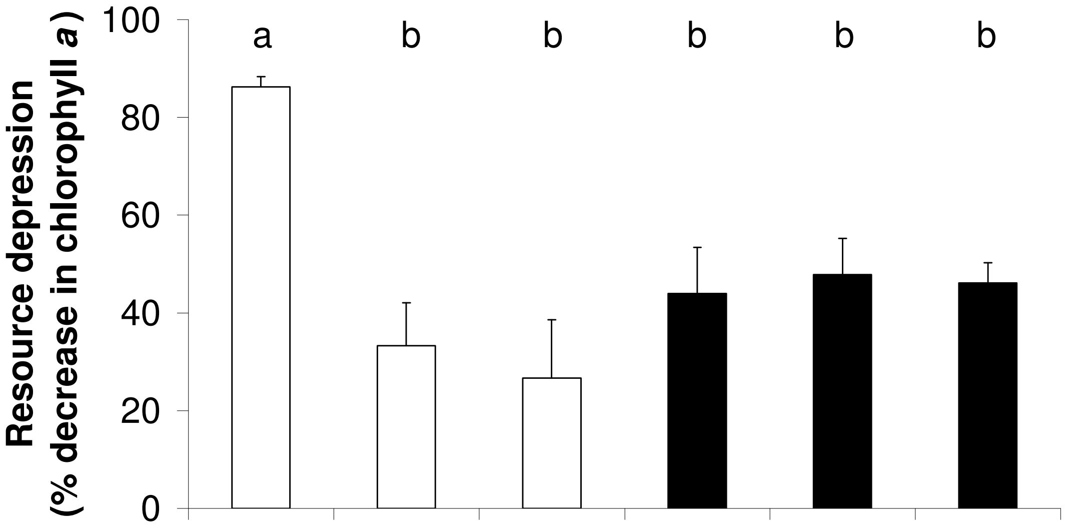


a) growth rate

b) survival

Figure 6.2 Fitness of *Baetis rhodani*, expressed as instantaneous growth rate (% increase in dry mass per day) and percentage survival (%) for nymphs fed biofilm from each source stream (C = circumneutral streams; L = limed streams). Results of two-level nested ANOVA are shown in Table 6.2.

a) chlorophyll *a*



b) ash-free dry mass

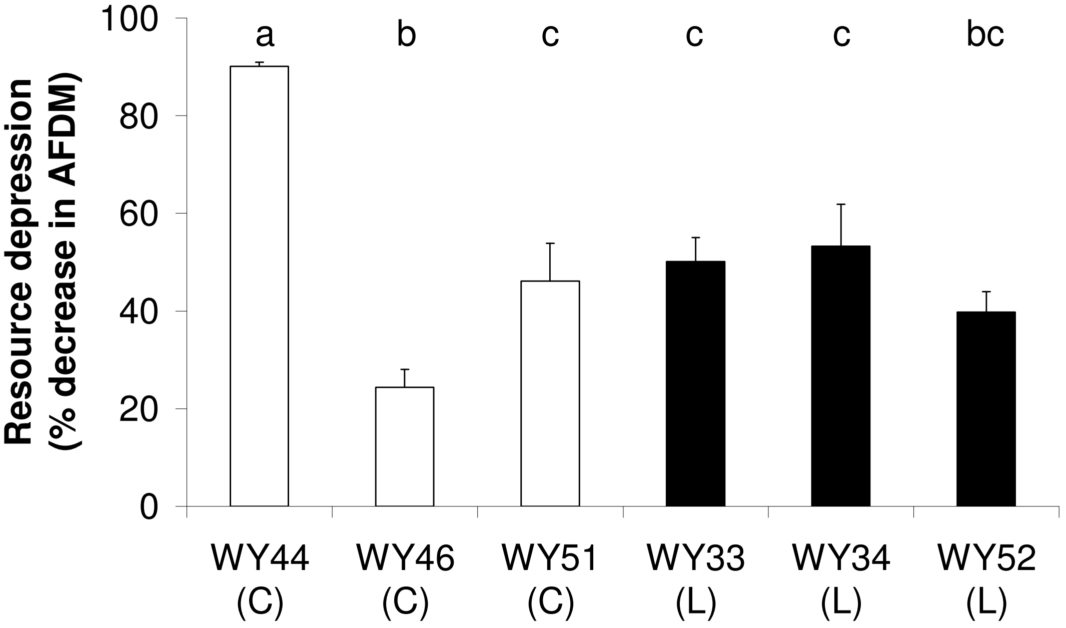


Figure 6.3 Resource depression (percentage decrease in chlorophyll *a* and ash-free dry mass) by *Baetis rhodani* when given biofilm from each source stream (C = circumneutral; L = limed). Results of nested ANOVA shown in Table 6.2. Groups with different lowercase letters above have significantly different means ($P < 0.05$).

Table 6.1 Composition of biofilm collected from each source stream (I. Jüttner *pers. comm.*), and fed to test animals (*Baetis rhodani*) at experimental site (WY31). Stream pH was measured at time of biofilm collection.

Site	Status	pH 2003 - 2006		pH (at collection)	Composition of biofilm (dominant diatom species)
		Mean	Minimum		
WY44	Circumneutral	6.7	6.3	6.6	*Achnanthidium minutissimum* *Eunotia exigua* *Fragilaria* cf. *capucina* var. *gracilis*
WY46	Circumneutral	6.5	6.1	6.9	*Achnanthidium minutissimum* *Eunotia exigua* *Fragilaria* cf. *capucina* var. *gracilis*
WY51	Circumneutral	6.7	6.3	6.1	*Achnanthidium minutissimum* *Fragilaria* cf. *capucina* var. *gracilis* *Eunotia exigua*
WY33	Limed	5.7	5.1	6.7	*Eunotia exigua* *Tabellaria flocculosa*
WY34	Limed	5.9	5.5	6.5	*Achnanthidium minutissimum* *Eunotia exigua* *Fragilaria* cf. *capucina* var. *gracilis*
WY52	Limed	5.9	5.5	6.3	*Eunotia exigua* *Eunotia subarcuatiodes* *Tabellaria flocculosa*

Table 6.2 Results of two-level nested ANOVA exploring the impact of treatment, and biofilm source site within treatment, on macroinvertebrate and algal variables. Results of pairwise comparisons shown where ANOVA provided a significant result (C = circumneutral; L = limed). Tests considered significant at $P < 0.05$.

Source of variation	*df*	SS	MS	*F*	*P*	Pairwise
Initial chlorophyll *a*						
Treatment	1	1.012	1.012	0.700	0.450	
Site within treatment	4	5.783	1.446	2.873	0.040	(WY)51 < 33 & 52
Error	30	15.096	0.503			(WY)34 < 52
Total	35					
Initial ash-free dry mass						
Treatment	1	0.026	0.026	0.133	0.734	
Site within treatment	4	0.791	0.198	0.758	0.561	
Error	30	7.831	0.261			
Total	35					
Initial carbon to nitrogen ratio ($\log_{10}$ transformed data)						
Treatment	1	0.001	0.001	0.137	0.730	
Site within treatment	4	0.017	0.004	2.481	0.071	
Error	24	0.040	0.002			
Total	29					
Initial autotrophic index						
Treatment	1	96930	96930	0.388	0.567	
Site within treatment	4	999699	249924	4.691	0.005	Figure 6.1d
Error	30	1598357	53277			
Total	35					
Growth rate of *Baetis rhodani* ($\log_{10}$ transformed data)						
Treatment	1	0.171	0.171	8.223	0.046	C > L
Site within treatment	4	0.083	0.021	0.974	0.437	
Error	30	0.642	0.021			
Total	35					
Survival of *Baetis rhodani*						
Treatment	1	2400.9	2400.9	7.174	0.055	(C > L)
Site within treatment	4	1338.7	334.7	1.659	0.185	
Error	30	6050.7	201.7			
Total	35					
Resource depression by *Baetis rhodani* (% decrease in chlorophyll *a*)						
Treatment	1	69.696	69.696	0.022	0.890	
Site within treatment	4	12842.5	3210.6	8.337	< 0.001	Figure 6.3a
Error	30	11553.5	385.12			
Total	35					
Resource depression by *Baetis rhodani* (% decrease in ash-free dry mass)						
Treatment	1	306.09	306.09	0.087	0.818	
Site within treatment	4	14079.4	3519.8	18.263	< 0.001	Figure 6.3b
Error	30	5781.7	192.7			
Total	35					

CHAPTER SEVEN

GENERAL DISCUSSION

7.1 DISCUSSION

The impact of acid deposition on freshwater biota has been widely researched in sensitive regions of the northern hemisphere since the 1980s (e.g. Haines, 1981; Dillon *et al*., 1984; Hildrew & Ormerod, 1995; Mason, 2002). During this time, a number of catchment-scale monitoring programmes have been set up to record the response of acid-sensitive communities to the reduction in acid deposition resulting from improved pollution control legislation (e.g. Fjellheim & Raddum, 2001; Tipping *et al*., 2002; Ormerod & Durance 2009), and although there is gathering evidence to suggest that biological recovery of sensitive communities is occurring as a result of improvements in water chemistry of streams and rivers (see Monteith *et al*., 2005), there are several examples where only minor improvements in biological status have been recorded over the last two decades. In a review of 25 years of chemical and macroinvertebrate data for streams in the Llyn Brianne catchment of mid-Wales, Ormerod & Durance (2009) concluded that the ecological response to reduced acid deposition was negligible, and that catchment liming has done little to accelerate improvements in acid-base status of upland streams. Similarly, in the nearby upper Wye catchment, despite significant investment in liming programmes to improve water quality for macroinvertebrates and salmonid fish, there is little evidence to suggest that acid-sensitive communities are responding (Lewis *et al*., 2007). These recent studies demonstrate the continued damage acid deposition is doing to aquatic ecosystems in the UK, and emphasise the importance of furthering our understanding of this complex pollution issue.

7.1.1 Impact of acidification on ecosystem processes

The first aim of my study was to provide an up-to-date picture of the impact of acidification on macroinvertebrate communities in three acid-sensitive catchments of mid-Wales, and examine the effect of surface water acidification on ecosystem function in these upland streams. Historically, the primary aim of freshwater ecologists researching the topic of acidification was to document shifts in taxonomic composition that occur as pH declines (e.g. Weatherley *et al.*, 1989; Peterson & Eekhaute, 1992; Guérold *et al.*, 2000; Petrin *et al.*, 2007), although more recently attention has turned to the consequences of these shifts for ecosystem stability, function and health (Gessner & Chauvet, 2002; Simon *et al.*, 2009). In order to establish whether acidification has a detrimental impact on ecosystem processes in streams in mid-Wales, I examined changes in the functional composition of macroinvertebrate communities across an acidity gradient. I then explored the impact of acidity on two key ecosystem processes; herbivory and leaf decomposition.

Physicochemical measurements from the thirty study streams in the upper Wye, Irfon and Llyn Brianne catchments revealed the harsh and oligotrophic nature of these upland sites (Chapter 2). Low nutrient availability ($NO_3 < 1.0$ mg L^{-1}, $PO_4 < 0.3$ mg L^{-1}, Ca < 2.0 mg L^{-1}) in the majority of streams, coupled with a high level of streambed instability (mean Pfankuch Index of 55) resulted in the exclusion of important macroinvertebrate groups such as crustaceans and gastropods. Furthermore, low pH (< 5.5) and high dissolved aluminium (> 0.1 mg L^{-1}) in spring exceeded the tolerance limits of acid-sensitive taxa, and resulted in reduced richness and abundance of taxa belonging to the filter-feeder and grazer functional groups. Reduction in grazer numbers in acid streams was potentially the most significant finding, due to the crucial role this group of macroinvertebrates plays in algal biomass regulation and nutrient recycling (Wallace & Webster, 1996). However, due to the physical instability of the study streams, coupled with the low initial biomass of algae and the small size of grazers, I failed to detect a consistent impact of the grazer functional group on algal abundance (Chapter 3). Only in spring, in a subset of circumneutral streams, was herbivore impact on algal abundance detected. In acid streams, where grazer numbers were reduced, no corresponding proliferation of algae was found, possibly as a result of turnover in taxonomy of grazers and the presence of the acid-tolerant stonefly *Amphinemoura*. Furthermore, algal biomass in the absence of grazers was not influenced by pH, although there was some evidence to suggest that low nutrient availability limited algal growth in the most acidic streams. These findings suggest that although macroinvertebrate grazers are largely absent from acidic headwater streams, their scarcity has no bearing on algal abundance, and that instead, algal biomass is regulated primary by hydrological regimes in upland catchments.

In comparison to the grazer functional group, taxon richness and density of macroinvertebrate shredders were largely unaffected by low pH (Chapter 4). However, the oligotrophic nature of the study streams and their inability to retain detritus, coupled with the low quality of deposited allochthonous material, meant that shredder richness was universally low. Furthermore, low nutrient availability and high levels of physical disturbance meant that the majority of these upland streams were not able to support populations of powerful shredders such as *Gammarus*, and instead contained relatively feeble shredder taxa including Plecoptera and Trichoptera. Consequently, the contribution of macroinvertebrate shredders to the breakdown process was low, and instead, the decomposition of leaf litter was controlled primarily by microbes. However, microbially-mediated breakdown was severely impacted by low pH, due to the sensitivity of fungal and bacterial communities to hydrogen ions and labile aluminium.

7.1.2 Biological barriers to recovery

The delayed recolonisation of acid-sensitive macroinvertebrate species following chemical restoration and recovery of formally acidic streams (e.g. Alewell *et al*., 2000; Driscoll *et al*., 2001; Monteith *et al*., 2005) was largely unexpected, as it was assumed that once the acid-base status of surface waters was within the tolerance limits of sensitive organisms, biological recovery would promptly follow (Weatherley & Ormerod, 1992; Rundle *et al*., 1995). In many regions however, this has not been the case (Monteith *et al*., 2005), and in upland Welsh catchments there is currently little evidence to suggest that biological recovery is underway (Lewis *et al*., 2007; Ormerod & Durance, 2009). A number of hypotheses have been proposed to explain this delay (see Monteith *et al*., 2005), but in this thesis I investigated the two hypotheses that have received the least attention in riverine habitats. Firstly, I explored the idea that ecological interactions, namely competition, close communities in chemically restored streams to acid-sensitive recolonists, by examining the impact of competition from an acid-tolerant generalist (*Leuctra inermis*) on the success of an acid-sensitive grazer (*Baetis rhodani*). Although evidence of competition exclusion of acid-sensitive species has been found in lotic communities (e.g. Keller & Yan, 1998;

Vinebrooke *et al.*, 2003; Frost *et al.*, 2006) this hypothesis has not been formally tested in stream habitats. Secondly, I explored the idea that algal food in limed streams is of reduced quality, and that this has a negative impact on the growth and survivorship of acid-sensitive recolonists. It is known that there is a discrepancy in the water quality of limed and naturally circumneutral streams (Rosseland *et al.*,1992; Kullberg *et al.*, 1993; Teien *et al.*, 2004), but the consequences of this discrepancy for algal nutritional quality has not yet been investigated.

Exploration of the *competition* hypothesis (Chapter 5) revealed that the potential for competition between natural populations of *L. inermis* and *B. rhodani* does exist, due to the significant overlap in diet of these two species. However, results of my in-stream mesocosm experiment found no impact of *Leuctra* on the growth of *Baetis*, possibly because algal resources in the enclosures were not limiting to either species. The growth of *Baetis* (2.29% DM d^{-1}) was low however in comparison to published growth rates for this genus, which suggested that delayed recolonisation of acid-sensitive grazers in limed streams may in fact be due to reduced *quality* of available algal food. Indeed, direct comparison of *Baetis* fitness when fed periphyton from limed and circumneutral streams (Chapter 6) revealed that the growth and survival of this species was markedly reduced after consuming limed biofilms (5.7% DM d^{-1}, 78% survival; compared to 7.7% DM d^{-1} and 94% survival when fed circumneutral biofilms). Although I could find no difference in measures of algal nutritional quality (C:N ratio, autotrophic index) between the two biofilm types (circumneutral, limed), I propose that phosphorus limitation in limed streams may result in poor-quality biofilms that are unable to support the nutritional needs of grazers, and that lime treatment causes fouling of the periphyton with aluminium, which may prove toxic to sensitive invertebrates.

7.2 CONCLUSIONS

Upland river catchments contain a valuable source of physicochemical and ecological diversity (Duigan, 2004), so it is imperative that these fragile ecosystems are fully protected from anthropogenic influences. It is clear from the research presented in this thesis that atmospheric deposition of acidifying pollutants continues to have a damaging impact on macroinvertebrate communities in acid-sensitive headwater streams in mid-Wales, but that this damage does not necessarily translate to impaired ecosystem function. On the whole, due to the oligotrophic and hydrodynamic nature of these streams and the high level of physical disturbance they experience, biotic interactions are weak, both within macroinvertebrate communities and between macroinvertebrates and their resources. As a result, competition over resources is unlikely to provide a significant barrier to the recolonisation of acid-sensitive species. Impoverished food supply as a result of poor water quality, on the other hand, may constrain growth rates of grazer species, providing an explanation for the delayed recolonisation of acid-sensitive grazers in streams where neutral status had been restored artificially through liming. It is already known that chronic and episodic acidity have direct toxic effects on sensitive macroinvertebrates (Kowalik & Ormerod, 2006), but my research is the first to give strong support to the idea that poor water quality in acid-sensitive catchments acts indirectly on the grazer community by damaging food supply.

7.3 SYNTHESIS

The research presented in this thesis provides a current assessment of the status of benthic communities and water quality in the upper Wye, Irfon and Llyn Brianne catchments of mid-Wales, and in accordance with the findings of Ormerod & Durance (2009) and Lewis *et al*. (2007), demonstrates that acid deposition continues to have a damaging effect on the biodiversity of freshwater communities in these catchments. Since surveys began in the Llyn Brianne catchment in 1981, there has been little improvement in the status of acid-sensitive macroinvertebrate species, with many streams remaining too acidic for their survival (Ormerod & Durance, 2009). However, my research expands on existing descriptions of the effect of acidity on macroinvertebrate diversity, by examining the impact of low pH on important ecosystem processes. My research, for example, was the first to formally test the 'grazer-release' hypothesis in natural stream systems, and provide evidence that algal biomass in headwater streams is regulated primary by hydrological conditions rather than grazing. Furthermore, my research questions the common notion that detrital breakdown rates are controlled primarily by the action of macroinvertebrate shredders, and provides a clearer picture of the impact of acidity on breakdown rates in upland headwater streams.

Recovery of acid-sensitive macroinvertebrate communities, either in limed streams or those that are recovering naturally as a result of reduced acid-deposition, has undoubtedly been slow. Previously in upland headwater streams in Wales, only two hypotheses explaining this delay have been tested; firstly, that acid episodes continue to exclude acid-sensitive species (abiotic resistance), and secondly, that acid-sensitive species are unable to disperse to streams that they have previously been eliminated from (dispersal limitation). The research presented in Chapters 5 and 6 of this thesis was the first to test two further hypotheses that have not been formally investigated in upland streams; that a) strong competitive interactions with resident species delay the recolonisation process (biotic resistance), and that b) acid-sensitive grazers do not persist in chemically recovering streams due to the impoverished food supply found within them (food limitation). My results were the first to provide experimental evidence that the growth and survival of an acid-sensitive grazer is reduced when fed biofilms from limed streams, giving compelling support to the *food limitation* hypothesis as a mechanism explaining delayed recovery of acid-sensitive macroinvertebrate communities upland Welsh streams.

7.4 FUTURE RESEARCH IDEAS

The findings presented in Chapter 5 of this thesis suggest that biotic interactions between macroinvertebrates in upland streams are weak, and that the strength of competition from *Leuctra inermis* has no bearing on the reinvasion success of acid-sensitive *Baetis rhodani*. However, the interactions between *B. rhodani* and *L. inermis,* and *Baetis* conspecifics tested in my experiment are only a small subset of the possible competitive interactions between species that may occur in my study stream, and I cannot therefore refute the hypothesis that strong competitive interactions prevent recolonisation of acid-sensitive macroinvertebrate species to chemically restored streams completely. Furthermore, the competition hypothesis is a subset of the 'biotic resistance' hypothesis, and does not take into account the possible impact of other biotic interactions, namely predation, on the success of acid-sensitive species in chemically restored streams. The presence of predators has been found to have a negative impact on populations of *Baetis*, for example, both directly through their consumption, and indirectly through sub-lethal effects of the predator's presence (Kratz, 1996; McPeek & Peckarsky, 1998). Predation pressure may, therefore, contribute in some way to the delayed recolonisation of acid-sensitive species in chemically recovered streams, and is worthy of further investigation.

The benefits of liming to benthic communities in acid upland streams has recently been questioned (Ormerod & Durance, 2009), and the findings presented in Chapter 6 of this thesis suggest that lime treatment may in fact harm populations of sensitive species indirectly through altered food quality. It remains unclear, however, whether lime treatment affects the phosphate content of algal biofilms, causes fouling with toxic metals, or reduces biofilm palatability. Further work is therefore required to fully describe and compare the structure and biochemistry of algal biofilms from limed and circumneutral streams, and discover how any differences affect the recolonisation success of acid-sensitive species.

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APPENDICES

Appendix 1. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in spring (April) 2006.

	AC15	AC16	AC17	AC18	CI2	IF2	IF6	IF8	IF9	IF24	LI1	LI2	LI6	LI7	LI8
Planariidae	0	0	0	3.2	0	0	0	0	0	3.2	3.2	0	0	0	0
Planaria torva	0	141	9.6	3.2	0	3.2	51.2	0	3.2	9.6	28.8	6.4	83.2	3.2	6.4
Ancylus fluviatilis	0	3.2	6.4	3.2	0	0	0	0	0	0	0	0	0	0	0
Sphaeridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	12.8	528	80	333	48	9.6	76.8	349	99.2	57.6	16	16	12.8	6.4	32
Hydracarina	22.4	3.2	19.2	259	16	28.8	83.2	89.6	44.8	3.2	12.8	6.4	0	6.4	0
Ostracoda	0	9.6	12.8	0	0	0	3.2	0	12.8	0	0	0	0	0	0
Gammarus pulex	0	957	3.2	0	0	0	0	0	0	0	0	0	0	0	0
Niphargus aquilex	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0
Siphlonurus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Siphlonurus lacustris	0	0	0	0	0	3.2	0	0	0	0	0	0	12.8	0	0
Baetis spp.	12.8	480	333	3.2	3.2	0	0	0	320	0	0	0	3.2	51.2	0
Baetis rhodani	38.4	294	240	25.6	0	0	0	3.2	141	0	0	0	0	246	0
Heptageniidae	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0
Rhithrogena semicolorata	48	250	60.8	12.8	0	0	0	0	259	0	0	0	0	67.2	0
Ecdyonurus sp.	0	0	6.4	3.2	0	0	0	0	9.6	0	0	0	0	3.2	0
Electrogena lateralis	3.2	9.6	28.8	70.4	0	0	0	0	16	0	0	0	3.2	179	0
Paraleptophlebia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Caenis rivulorum	3.2	0	0	19.2	3.2	0	0	0	0	0	0	0	0	0	0
Plecoptera indet	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0	3.2
Brachyptera spp.	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0
Brachyptera putata	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0	0
Brachyptera risi	9.6	0	6.4	3.2	0	3.2	44.8	0	9.6	0	0	0	9.6	6.4	0
Nemouridae indet	0	0	0	0	0	0	0	0	0	0	0	9.6	3.2	0	0
Protonemura meyeri	0	0	9.6	0	0	0	6.4	0	9.6	54.4	0	25.6	0	3.2	12.8
Amphinemura sulcicollis	0	0	3.2	0	0	0	16	0	25.6	211	38.4	147	96	19.2	76.8
Nemurella pictetii	0	0	0	0	0	0	0	0	0	6.4	0	0	9.6	0	0
Nemoura spp.	0	0	0	0	0	0	0	0	0	0	6.4	6.4	28.8	0	3.2
Nemoura avicularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemoura cinerea	0	0	0	0	0	0	0	0	0	3.2	0	0	6.4	0	0
Leuctra spp.	6.4	12.8	70.4	28.8	0	6.4	6.4	3.2	35.2	0	6.4	0	6.4	12.8	0
Leuctra hippopus	0	0	12.8	9.6	3.2	0	0	0	6.4	0	3.2	6.4	0	0	9.6
Leuctra inermis	221	28.8	410	122	150	275	464	28.8	496	0	0	0	76.8	246	3.2
Leuctra nigra	0	0	92.8	0	0	0	16	0	0	28.8	48	9.6	0	0	3.2
Perlodidae indet	0	0	0	3.2	0	0	0	3.2	0	0	0	0	0	0	0
Isoperla grammatica	6.4	6.4	3.2	0	89.6	16	25.6	12.8	60.8	0	0	0	3.2	22.4	0
Chloroperlidae indet	0	0	6.4	6.4	0	19.2	0	6.4	0	0	0	3.2	0	0	0
Chloroperla tripunctata	0	35.2	102	0	0	76.8	6.4	3.2	0	0	0	0	0	19.2	0
Siphonoperla torrenentium	112	259	125	528	554	262	448	317	237	64	6.4	12.8	189	44.8	67.2
Cordulegaster boltonii	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0
Velia sp.	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0	0
Velia caprai	3.2	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0
Coleoptera indet	0	0	0	0	0	0	0	6.4	0	0	0	0	0	0	0
Scirtididae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dytiscidae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stichtonectes lepidus	0	0	0	0	9.6	0	0	0	0	0	0	0	6.4	0	0
Graptodytes flavipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oreodytes sanmarkii	0	0	0	3.2	0	0	3.2	28.8	0	0	0	0	0	3.2	3.2
Agabus didymus	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0
Agabus guttatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrinus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orechtochilius villosus	0	0	0	3.2	0	0	0	9.6	0	0	0	0	0	0	0
Hydraena gracilis	3.2	0	6.4	0	0	3.2	0	0	9.6	0	0	0	0	6.4	0

Appendix 1 *continued*. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in spring (April) 2006.

	AC15	AC16	AC17	AC18	CI2	IF2	IF6	IF8	IF9	IF24	LI1	LI2	LI6	LI7	LI8
Elmis aenea	6.4	38.4	28.8	12.8	6.4	3.2	0	41.6	128	0	0	0	6.4	22.4	0
Esolus parallelepipedus	0	0	0	51.2	3.2	9.6	0	16	3.2	0	0	0	0	9.6	0
Limnius volckmari	12.8	16	6.4	64	25.6	3.2	3.2	115	9.6	0	0	0	0	3.2	0
Oulimninius sp.	0	0	0	32	202	6.4	0	67.2	3.2	0	0	0	0	0	0
Curculionidae indet	0	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0
Trichoptera indet	3.2	0	0	0	0	0	0	0	9.6	0	0	0	0	6.4	0
Rhyacophila sp.	0	0	3.2	6.4	0	0	0	0	22.4	0	0	0	0	0	0
Rhyacophila dorsalis	0	0	3.2	0	3.2	0	12.8	0	12.8	3.2	0	0	0	0	0
Glossosoma spp.	0	0	9.6	0	0	0	0	0	6.4	0	0	0	0	19.2	0
Glossosoma boltonii	0	0	32	0	0	0	0	0	41.6	0	0	0	0	22.4	0
Glossosoma conformis	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Agapetus fuscipes	0	102	9.6	0	0	0	0	0	0	0	0	0	3.2	0	0
Oxyethira sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philapotamus montanus	0	0	19.2	0	0	0	0	0	3.2	0	0	0	0	6.4	0
Wormaldia occipitalis	0	0	19.2	0	0	0	0	0	0	0	0	0	0	16	0
Psychomyiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metalype fragilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tinodes sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polycentropodidae indet	0	0	0	0	0	0	0	0	0	6.4	0	0	0	0	0
Plectrocnemia spp.	0	0	9.6	12.8	3.2	6.4	6.4	0	3.2	19.2	28.8	12.8	12.8	0	0
Plectrocnemia brevis	0	0	3.2	0	3.2	3.2	6.4	0	0	3.2	3.2	0	3.2	0	0
Plectrocnemia conspersa	0	0	0	0	0	0	0	0	0	0	19.2	0	3.2	0	0
Plectrocnemia geniculata	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Chaetopteryx villosa	0	0	0	0	3.2	0	0	0	6.4	0	0	0	6.4	0	0
Hydropsyche sp.	0	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche siltalai	12.8	0	0	32	16	12.8	0	3.2	3.2	0	0	0	0	0	0
Diplectrona felix	0	35.2	70.4	0	0	0	3.2	0	3.2	0	0	0	0	0	0
Lepidostomatidae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepidostoma hirtum	0	0	3.2	0	0	0	0	3.2	0	0	0	0	0	0	0
Limnephnephilidae indet	0	0	0	0	6.4	0	6.4	0	22.4	0	0	9.6	41.6	3.2	0
Drusus annulatus	0	6.4	0	0	12.8	12.8	48	0	3.2	3.2	0	0	25.6	0	0
Ecclisopteryx guttulata	0	0	0	6.4	0	0	0	0	0	0	0	0	0	0	0
Halesus sp.	3.2	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0
Halesus radiatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Micropterna sequax	0	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0
Potamophylax cingulatus	0	0	6.4	0	0	0	3.2	0	3.2	0	0	0	3.2	3.2	3.2
Potamophylax latipennis	3.2	3.2	0	0	0	0	3.2	0	0	0	0	0	0	0	0
Potamophylax rotundipennis	0	3.2	0	0	0	0	0	0	0	0	0	0	0	0	3.2
Goera pilosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Silo pallipes	12.8	3.2	0	0	0	25.6	22.4	12.8	9.6	0	0	0	12.8	0	0
Beraea sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Sericostoma personatum	0	3.2	6.4	3.2	0	0	0	0	3.2	0	0	0	0	0	0
Odontocerum albicorne	0	22.4	12.8	0	0	0	0	0	51.2	0	0	0	0	3.2	0
Leptoceridae indet	0	0	0	9.6	0	0	0	0	0	0	0	0	0	0	0
Athripsodes sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera indet	0	0	6.4	0	0	0	16	0	0	3.2	0	3.2	0	0	0
Tipulididae	16	54.4	51.2	0	6.4	6.4	3.2	0	38.4	19.2	16	9.6	0	9.6	3.2
Psychodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Ceratopogonidae	3.2	0	22.4	12.8	0	3.2	3.2	9.6	0	0	0	0	0	0	0
Simuliidae	12.8	3.2	32	6.4	6.4	3.2	384	6.4	9.6	3.2	6.4	432	16	3.2	6.4
Chironomidae	16	89.6	374	355	122	70.4	237	73.6	1507	173	166	19.2	76.8	76.8	6.4
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	0	298	0	0	0	32	0	0	0	0	0	0	0
Empididae	12.8	6.4	28.8	41.6	9.6	6.4	9.6	9.6	38.4	3.2	9.6	0	0	6.4	0

Appendix 1 *continued*. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in spring (April) 2006.

	WY31	WY33	WY34	WY39	WY44	WY45	WY46	WY47	WY48	WY49	WY50	WY51	WY52	WY55	WY56
Planariidae	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0
Planaria torva	0	3.2	25.6	3.2	0	3.2	0	0	12.8	6.4	0	16	3.2	6.4	106
Ancylus fluviatilis	6.4	0	0	0	57.6	0	0	0	0	0	0	0	0	9.6	0
Sphaeridae	0	0	0	0	3.2	3.2	3.2	0	0	0	0	0	0	0	0
Oligochaeta	330	22.4	35.2	9.6	32	38.4	150	0	12.8	211	115	19.2	25.6	80	118
Hydracarina	57.6	35.2	9.6	0	22.4	6.4	25.6	6.4	12.8	9.6	12.8	12.8	22.4	51.2	0
Ostracoda	0	0	0	0	0	0	6.4	0	0	3.2	0	0	0	6.4	0
Gammarus pulex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Niphargus aquilex	0	6.4	9.6	0	0	0	0	0	0	0	0	0	0	0	0
Siphlonurus sp.	0	0	0	0	3.2	0	0	0	0	0	0	0	0	0	0
Siphlonurus lacustris	0	3.2	0	0	3.2	16	3.2	0	0	3.2	6.4	0	0	0	0
Baetis spp.	51.2	0	0	0	44.8	3.2	0	0	0	0	0	240	0	19.2	0
Baetis rhodani	125	0	9.6	0	83.2	0	3.2	0	0	0	0	339	0	25.6	0
Heptageniidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhithrogena semicolorata	64	0	3.2	0	0	0	0	0	0	0	0	54.4	0	0	0
Ecdyonurus sp.	16	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0
Electrogena lateralis	3.2	28.8	16	16	122	0	0	0	0	0	0	170	0	67.2	0
Paraleptophlebia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caenis rivulorum	0	19.2	0	0	0	0	0	0	0	0	0	0	0	0	0
Plecoptera indet	0	0	0	0	0	0	3.2	0	9.6	0	0	0	0	0	0
Brachyptera spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachyptera putata	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0
Brachyptera risi	3.2	0	0	3.2	0	0	0	0	0	0	0	0	0	9.6	0
Nemouridae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28.8
Protonemura meyeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4
Amphinemura sulcicollis	0	0	0	12.8	0	0	0	6.4	0	0	6.4	51.2	0	0	630
Nemurella pictetii	0	0	0	0	0	3.2	0	0	0	0	0	0	0	0	22.4
Nemoura spp.	0	0	0	0	0	3.2	0	0	0	0	0	0	0	0	131
Nemoura avicularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.8
Nemoura cinerea	0	0	0	0	0	22.4	0	0	0	0	0	0	0	0	3.2
Leuctra spp.	16	16	9.6	9.6	3.2	3.2	0	19.2	12.8	35.2	25.6	192	122	6.4	230
Leuctra hippopus	0	6.4	3.2	9.6	6.4	115	0	9.6	3.2	12.8	3.2	9.6	3.2	3.2	32
Leuctra inermis	80	150	86.4	118	186	0	12.8	115	86.4	25.6	73.6	787	73.6	41.6	0
Leuctra nigra	0	6.4	0	12.8	9.6	6.4	0	0	0	0	0	3.2	0	0	38.4
Perlodidae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isoperla grammatica	3.2	106	0	28.8	19.2	0	35.2	19.2	32	35.2	134	25.6	157	38.4	0
Chloroperlidae indet	0	0	3.2	0	0	0	6.4	6.4	16	0	3.2	12.8	0	9.6	0
Chloroperla tripunctata	0	3.2	0	9.6	9.6	0	0	9.6	6.4	0	0	25.6	0	0	0
Siphonoperla torrenentium	432	1024	189	336	371	99.2	374	99.2	371	96	467	237	355	672	6.4
Cordulegaster boltonii	0	0	0	0	6.4	0	3.2	0	0	0	0	6.4	0	0	0
Velia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Velia caprai	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera indet	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0	0
Scirtididae indet	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0
Dytiscidae indet	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0	3.2
Stichtonectes lepidus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Graptodytes flavipes	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0	0
Oreodytes sanmarkii	0	32	0	0	0	0	0	0	0	0	12.8	0	0	0	0
Agabus didymus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agabus guttatus	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0	0
Gyrinus sp.	0	0	0	0	0	3.2	0	0	0	0	0	0	0	0	0
Orechtochilius villosus	12.8	0	6.4	6.4	0	0	0	0	0	0	0	0	3.2	0	0
Hydraena gracilis	6.4	0	0	3.2	6.4	0	6.4	0	0	0	0	0	0	0	0

Appendix 1 *continued*. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in spring (April) 2006.

	WY31	WY33	WY34	WY39	WY44	WY45	WY46	WY47	WY48	WY49	WY50	WY51	WY52	WY55	WY56
Elmis aenea	3.2	3.2	3.2	6.4	80	0	0	0	0	0	3.2	44.8	3.2	0	0
Esolus parallelepipedus	9.6	9.6	0	0	6.4	0	3.2	0	0	6.4	0	6.4	3.2	3.2	0
Limnius volckmari	22.4	19.2	16	3.2	0	0	12.8	0	3.2	0	0	67.2	3.2	16	0
Oulimninius sp.	44.8	16	9.6	0	0	0	28.8	0	3.2	28.8	0	0	0	83.2	0
Curculionidae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichoptera indet	0	0	0	0	0	0	3.2	0	0	0	0	0	0	3.2	0
Rhyacophila sp.	0	0	0	0	0	0	3.2	0	0	0	0	0	0	3.2	0
Rhyacophila dorsalis	9.6	6.4	0	0	0	0	0	0	0	0	0	3.2	0	0	0
Glossosoma spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossosoma boltonii	3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossosoma conformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agapetus fuscipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxyethira sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4
Philapotamus montanus	0	0	0	0	6.4	0	0	0	0	0	0	0	0	0	0
Wormaldia occipitalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psychomyiidae	0	0	0	0	0	0	0	0	0	0	3.2	0	3.2	0	0
Metalype fragilis	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0	0
Tinodes sp.	0	0	0	0	0	0	0	0	0	0	0	0	12.8	0	0
Polycentropodidae indet	0	0	0	0	0	6.4	3.2	0	0	0	0	0	3.2	0	0
Plectrocnemia spp.	0	12.8	0	22.4	6.4	25.6	3.2	3.2	0	6.4	3.2	28.8	3.2	0	141
Plectrocnemia brevis	0	0	0	6.4	0	28.8	0	0	6.4	6.4	0	0	0	0	32
Plectrocnemia conspersa	0	0	0	0	0	0	0	0	0	3.2	3.2	0	0	0	16
Plectrocnemia geniculata	0	0	0	0	22.4	0	3.2	0	0	0	6.4	3.2	0	0	73.6
Chaetopteryx villosa	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Hydropsyche sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche siltalai	32	6.4	3.2	0	9.6	0	0	3.2	12.8	9.6	0	51.2	51.2	48	0
Diplectrona felix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepidostomatidae indet	22.4	3.2	12.8	0	0	0	0	0	0	0	0	0	0	6.4	0
Lepidostoma hirtum	19.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limnephnephilidae indet	9.6	0	0	0	0	6.4	0	0	0	0	3.2	3.2	0	3.2	134
Drusus annulatus	0	19.2	0	3.2	0	0	0	0	0	0	0	0	0	35.2	0
Ecclisopteryx guttulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Halesus sp.	0	0	0	0	3.2	0	0	0	0	0	0	0	0	0	0
Halesus radiatus	0	0	0	0	0	3.2	0	0	0	0	0	0	0	0	0
Micropterna sequax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamophylax cingulatus	0	0	0	0	3.2	0	0	0	0	0	0	3.2	0	0	0
Potamophylax latipennis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamophylax rotundipennis	3.2	0	0	3.2	3.2	3.2	0	0	0	0	0	0	0	6.4	0
Goera pilosa	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0	0
Silo pallipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Beraea sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostoma personatum	12.8	0	0	0	0	0	0	0	0	0	0	0	0	9.6	0
Odontocerum albicorne	3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae indet	6.4	12.8	0	3.2	0	0	3.2	0	3.2	0	0	0	0	0	0
Athripsodes sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4	0
Diptera indet	3.2	0	0	3.2	3.2	0	16	0	0	0	9.6	0	6.4	0	6.4
Tipulididae	32	9.6	0	0	51.2	3.2	6.4	3.2	6.4	19.2	0	3.2	6.4	9.6	12.8
Psychodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	6.4	0	0	0	0	0	0	0	0	3.2	0	3.2	3.2	6.4	0
Simuliidae	3.2	0	0	22.4	12.8	0	16	25.6	0	3.2	12.8	48	3.2	83.2	166
Chironomidae	787	154	35.2	51.2	32	230	659	25.6	67.2	611	864	160	1635	282	218
Tabanidae	0	0	3.2	0	0	0	6.4	0	0	0	0	0	0	0	0
Athericidae	6.4	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0
Empididae	3.2	9.6	0	6.4	3.2	12.8	51.2	3.2	9.6	19.2	44.8	6.4	32	12.8	0

Appendix 2. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in summer (July) 2006.

	AC15	AC16	AC17	AC18	CI2	IF2	IF6	IF8	IF9	IF24	LI1	LI2	LI6	LI7	LI8
Planaria torva	6.4	0	0	9.6	0	3.2	0	0	0	6.4	3.2	9.6	19.2	6.4	0
Ancylus fluviatilis	0	3.2	6.4	3.2	0	0	0	0	0	0	0	0	0	0	0
Pisidium sp.	0	0	3.2	0	0	0	0	0	0	0	0	3.2	0	0	0
Oligochaeta	121.6	1894	332.8	905.6	198.4	208	403.2	812.8	268.8	128	16	230.4	224	134.4	80
Hydracarina	528	16	32	611.2	284.8	518.4	1194	534.4	204.8	118.4	0	0	0	54.4	25.6
Ostracoda	3.2	3.2	25.6	0	0	9.6	3.2	3.2	115.2	0	0	0	32	16	0
Gammarus pulex	0	3696	3.2	0	0	0	0	0	0	0	0	0	0	0	0
Niphargus aquilex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Siphlonurus armatus	0	0	0	0	0	0	0	0	0	0	0	0	19.2	0	0
Siphlonurus lacustris	0	0	0	0	0	0	0	0	0	0	0	0	54.4	0	0
Baetis spp.	592	150.4	230.4	1994	748.8	454.4	9.6	460.8	473.6	6.4	0	19.2	169.6	937.6	38.4
Baetis rhodani	73.6	12.8	22.4	300.8	12.8	112	0	38.4	230.4	0	0	9.6	0	896	0
Baetis scambus	73.6	25.6	6.4	22.4	86.4	19.2	6.4	41.6	0	0	0	0	214.4	0	0
Baetis vernus	0	0	0	0	0	0	0	0	6.4	19.2	0	0	0	0	0
Alainites muticus	3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heptageniidae indet	16	0	3.2	16	9.6	12.8	0	12.8	35.2	0	0	0	0	6.4	0
Rhithrogena semicolorata	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Ecdyonurus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Electrogena lateralis	0	9.6	6.4	0	0	0	0	0	0	0	0	0	0	153.6	0
Ameletus inopinatus	0	0	0	0	0	6.4	0	0	0	0	0	0	0	0	0
Seratella ignita	147.2	192	64	185.6	268.8	342.4	96	284.8	678.4	0	0	0	0	240	0
Nemouridae indet	0	3.2	0	0	0	0	0	0	3.2	3.2	0	0	0	0	6.4
Protonemura sp.	25.6	3.2	0	0	0	9.6	3.2	3.2	6.4	16	0	0	0	0	0
Protonemura meyeri	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0
Amphinemoura sulcicollis	0	0	0	0	0	0	0	0	0	0	0	0	6.4	0	0
Nemurella pictetii	0	0	0	0	0	0	0	0	0	0	3.2	0	76.8	0	0
Nemoura sp.	0	0	0	0	0	0	0	0	0	6.4	0	0	0	0	0
Leuctra spp.	0	0	0	0	0	0	12.8	0	0	0	0	0	0	0	0
Leuctra hippopus	643.2	496	1226	217.6	412.8	387.2	64	230.4	800	48	6.4	12.8	67.2	259.2	25.6
Leuctra nigra	3.2	0	0	0	0	0	0	0	0	9.6	3.2	0	3.2	0	0
Perlodidae indet	9.6	0	6.4	0	3.2	3.2	0	0	9.6	0	0	0	0	0	0
Diura bicaudata	0	0	0	0	28.8	0	0	0	3.2	0	0	0	0	3.2	3.2
Isoperla grammatica	0	0	6.4	3.2	0	0	0	0	0	0	0	0	0	12.8	0
Siphonoperla torrentium	38.4	22.4	32	0	28.8	96	51.2	22.4	22.4	0	0	0	220.8	28.8	12.8
Coenagrion mercuriale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cordulegaster boltonii	0	0	0	0	6.4	0	0	0	0	0	0	0	9.6	0	0
Velia sp	0	0	0	3.2	0	3.2	0	0	0	12.8	0	0	3.2	3.2	0
Gerridae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corixididae indet	0	0	0	0	0	0	0	0	0	0	16	3.2	0	0	0
Micronecta sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera indet	0	0	3.2	0	0	0	0	0	0	0	0	0	3.2	0	0
Dytiscidae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stictonectes lepidus	0	0	0	0	0	0	0	0	0	0	64	0	25.6	6.4	6.4
Oreodytes sanmarkii	0	0	0	3.2	51.2	6.4	41.6	32	0	0	3.2	0	0	6.4	0
Platambus sp.	0	0	0	0	9.6	3.2	0	0	0	0	0	0	0	0	0
Agabus sp.	0	0	0	0	3.2	0	3.2	0	0	3.2	22.4	19.2	3.2	0	9.6
Gyrinus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helophorus obscurus	9.6	0	0	0	0	0	6.4	0	0	28.8	0	0	51.2	9.6	38.4
Hydrophilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2 *continued*. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in summer (July) 2006.

	AC15	AC16	AC17	AC18	CI2	IF2	IF6	IF8	IF9	IF24	LI1	LI2	LI6	LI7	LI8
Anacaena globulus	0	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0
Cercyon sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0
Hydraena gracilis	16	0	0	0	6.4	0	3.2	9.6	3.2	0	0	0	3.2	3.2	3.2
Scirtidae indet	0	0	0	0	0	0	0	0	0	0	0	0	3.2	3.2	0
Elmis aenea	22.4	48	44.8	6.4	41.6	6.4	0	192	297.6	0	0	3.2	6.4	220.8	9.6
Esolus parallelepipedus	38.4	16	0	92.8	41.6	6.4	3.2	44.8	6.4	3.2	0	0	0	19.2	12.8
Limnius volckmari	25.6	41.6	0	28.8	80	22.4	3.2	54.4	0	0	3.2	0	0	0	0
Oulimnius tuberculatus	12.8	3.2	0	48	1389	105.6	3.2	188.8	12.8	3.2	9.6	3.2	54.4	6.4	3.2
Curculionidae indet	12.8	0	0	0	0	3.2	0	0	0	3.2	19.2	19.2	0	3.2	243.2
Sialis sp.	0	0	0	0	0	0	0	3.2	3.2	3.2	0	9.6	0	0	0
Tricoptera indet	16	0	0	0	0	3.2	35.2	0	0	0	0	0	0	0	0
Rhyacophila sp.	12.8	6.4	12.8	25.6	70.4	54.4	83.2	19.2	32	6.4	3.2	0	0	6.4	3.2
Rhyacophila dorsalis	16	6.4	3.2	22.4	28.8	9.6	28.8	25.6	6.4	0	0	0	0	16	0
Rhyacophila munda	0	0	0	0	0	3.2	0	3.2	0	0	0	0	0	0	0
Rhyacophila obliterata	3.2	0	9.6	0	0	6.4	0	0	3.2	0	0	0	0	6.4	0
Rhyacophila fasciata	0	0	0	3.2	0	6.4	12.8	0	0	0	0	0	0	6.4	6.4
Oxyethira sp.	3.2	0	0	0	9.6	0	3.2	0	0	28.8	0	6.4	0	0	3.2
Wormaldia occipitalis	3.2	0	0	0	0	0	0	0	0	0	0	0	0	19.2	0
Plectrocnemia sp.	3.2	9.6	3.2	0	35.2	3.2	0	0	3.2	16	28.8	118.4	44.8	3.2	25.6
Plectrocnemia brevis	0	0	0	0	0	0	0	0	0	3.2	0	0	0	0	3.2
Plectrocnemia conspersa	0	0	0	0	0	0	0	0	3.2	9.6	12.8	0	3.2	0	3.2
Plectrocnemia geniculata	0	0	0	0	0	0	0	0	3.2	6.4	0	0	6.4	16	3.2
Polycentropus sp.	0	0	0	0	19.2	0	0	0	0	0	0	0	0	0	0
Polycentropus flavomaculatus	0	0	0	0	0	9.6	9.6	19.2	0	0	0	0	0	0	0
Chaetopteryx villosa	0	83.2	16	0	3.2	0	0	3.2	9.6	3.2	0	0	89.6	0	0
Hydropsyche sp.	0	0	0	0	41.6	0	0	0	0	0	0	0	0	0	0
Hydropsyche pellucidula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche siltalai	54.4	3.2	0	6.4	86.4	9.6	0	9.6	9.6	0	0	0	0	3.2	0
Diplectrona felix	6.4	0	3.2	3.2	0	0	0	0	0	0	0	0	0	0	0
Limnephilidae indet	0	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0
Drusus annulatus	89.6	0	3.2	150.4	16	22.4	44.8	0	0	6.4	0	3.2	3.2	3.2	0
Halesus digitatus	0	19.2	6.4	0	0	0	0	0	0	0	0	0	0	0	0
Halesus radiatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potmaophylax cingulatus	0	28.8	35.2	0	0	0	0	0	0	0	0	0	0	0	3.2
Potmaophylax rotundipennis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Silo pallipes	9.6	0	0	0	0	12.8	35.2	0	3.2	0	0	0	3.2	6.4	0
Sericostoma personatum	0	0	0	3.2	3.2	0	0	153.6	3.2	0	0	0	0	3.2	0
Odontocerum albicorne	70.4	153.6	214.4	6.4	0	0	3.2	60.8	51.2	0	0	0	0	16	0
Leptoceridae indet	3.2	0	0	0	0	0	0	3.2	0	0	0	0	0	0	0
Mystacides azurea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipulididae	80	22.4	41.6	38.4	38.4	89.6	166.4	44.8	76.8	144	9.6	9.6	38.4	60.8	35.2
Psychodidae	0	3.2	0	3.2	0	3.2	3.2	0	0	0	0	0	0	0	0
Dixidae	16	0	0	3.2	0	0	3.2	3.2	12.8	3.2	0	0	3.2	3.2	0
Thaumaleidae	6.4	3.2	19.2	0	0	0	0	0	3.2	0	0	0	0	83.2	0
Ceratopogonidae	6.4	9.6	9.6	6.4	0	0	0	12.8	3.2	0	0	0	0	0	0
Simuliidae	268.8	38.4	51.2	73.6	265.6	387.2	35.2	150.4	259.2	508.8	16	889.6	3.2	41.6	944
Chironomidae	1898	1274	1898	1411	1107	2819	7299	2902	1437	2771	3811	3043	1680	896	1386
Rhagionidae	0	0	0	76.8	0	0	0	35.2	3.2	0	0	0	0	0	0
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	0	188.8	0	0	0	0	0	0	0	0	0	0	0
Empididae	60.8	12.8	38.4	35.2	12.8	32	160	44.8	86.4	38.4	3.2	9.6	6.4	44.8	9.6

Appendix 2 *continued*. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in summer (July) 2006.

	WY31	WY33	WY34	WY39	WY44	WY45	WY46	WY47	WY48	WY49	WY50	WY51	WY52	WY55
Planaria torva	3.2	0	3.2	0	3.2	0	0	0	0	0	6.4	0	0	0
Ancylus fluviatilis	25.6	0	0	0	204.8	0	92.8	0	0	0	0	0	0	3.2
Pisidium sp.	0	0	0	0	3.2	3.2	3.2	0	0	0	0	0	0	0
Oligochaeta	1091	124.8	38.4	76.8	35.2	76.8	316.8	3.2	86.4	2666	176	16	0	182.4
Hydracarina	736	153.6	105.6	28.8	35.2	67.2	521.6	121.6	803.2	112	240	67.2	16	112
Ostracoda	0	0	0	0	0	0	54.4	0	0	22.4	0	0	0	0
Gammarus pulex	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Niphargus aquilex	0	0	0	0	0	0	3.2	0	0	0	6.4	0	0	0
Siphlonurus armatus	0	0	0	0	0	6.4	0	0	0	0	0	0	0	0
Siphlonurus lacustris	0	0	0	0	3.2	0	0	0	0	44.8	0	0	0	9.6
Baetis spp.	3392	358.4	1242	0	44.8	35.2	620.8	0	0	16	38.4	662.4	99.2	32
Baetis rhodani	76.8	28.8	0	0	28.8	12.8	0	0	0	0	0	57.6	9.6	0
Baetis scambus	211.2	51.2	70.4	0	0	0	102.4	0	0	3.2	0	0	22.4	3.2
Baetis vernus	0	0	0	0	0	0	9.6	0	0	80	73.6	0	0	0
Alainites muticus	6.4	0	0	0	0	0	0	0	0	0	0	0	0	0
Heptageniidae indet	160	102.4	0	0	9.6	0	0	3.2	0	0	0	3.2	0	3.2
Rhithrogena semicolorata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ecdyonurus sp.	0	16	0	0	0	0	0	0	0	0	0	0	0	0
Electrogena lateralis	76.8	0	0	0	22.4	0	0	0	0	0	0	121.6	0	0
Ameletus inopinatus	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4
Seratella ignita	995.2	86.4	384	0	6.4	76.8	371.2	0	16	0	25.6	252.8	57.6	60.8
Nemouridae indet	0	0	0	0	0	0	0	0	0	0	0	0	0	35.2
Protonemura sp.	0	6.4	0	0	0	25.6	0	0	0	0	0	0	0	0
Protonemura meyeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphinemoura sulcicollis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemurella pictetii	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemoura sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leuctra spp.	0	0	0	0	3.2	0	0	0	0	0	0	64	9.6	0
Leuctra hippopus	144	489.6	19.2	22.4	73.6	179.2	38.4	28.8	67.2	195.2	3.2	902.4	352	89.6
Leuctra nigra	0	0	0	0	3.2	0	0	0	0	0	0	0	3.2	0
Perlodidae indet	19.2	16	0	0	0	0	3.2	0	0	0	0	0	0	0
Diura bicaudata	3.2	9.6	0	0	0	0	3.2	0	3.2	16	9.6	9.6	22.4	3.2
Isoperla grammatica	9.6	0	0	0	0	0	0	0	0	3.2	3.2	0	6.4	0
Siphonoperla torrentium	16	25.6	3.2	6.4	12.8	0	0	3.2	3.2	3.2	22.4	67.2	0	0
Coenagrion mercuriale	0	0	0	0	3.2	0	3.2	0	0	0	0	0	0	3.2
Cordulegaster boltonii	0	0	0	0	0	6.4	0	0	0	0	0	0	0	0
Velia sp	0	0	0	3.2	6.4	19.2	3.2	0	0	9.6	0	0	0	0
Gerridae indet	3.2	6.4	0	0	0	0	0	0	3.2	0	0	0	0	0
Corixididae indet	0	0	0	0	0	0	0	6.4	0	0	0	0	0	0
Micronecta sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	41.6
Coleoptera indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dytiscidae indet	0	0	0	0	0	0	0	0	0	0	0	0	6.4	0
Stictonectes lepidus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oreodytes sanmarkii	0	19.2	0	0	0	3.2	0	0	6.4	0	9.6	3.2	12.8	0
Platambus sp.	0	6.4	0	0	0	12.8	0	12.8	0	0	0	0	0	0
Agabus sp.	0	0	0	0	0	0	0	0	0	9.6	0	0	3.2	0
Gyrinus sp.	0	0	0	0	0	25.6	0	0	0	0	0	0	0	0
Helophorus obscurus	0	38.4	0	6.4	32	9.6	0	6.4	0	48	0	12.8	6.4	22.4
Hydrophilidae	0	0	0	0	0	0	0	0	0	0	0	9.6	0	0

Appendix 2 *continued*. Mean density of macroinvertebrate taxa in thirty study streams in mid-Wales, in summer (July) 2006.

	WY31	WY33	WY34	WY39	WY44	WY45	WY46	WY47	WY48	WY49	WY50	WY51	WY52	WY55
Anacaena globulus	0	0	0	0	12.8	0	0	0	0	0	0	0	0	3.2
Cercyon sp.	0	0	0	0	0	6.4	0	0	0	0	0	0	0	3.2
Hydraena gracilis	32	12.8	0	0	0	0	9.6	6.4	3.2	3.2	3.2	0	0	0
Scirtidae indet	0	0	0	0	3.2	0	0	0	0	0	0	3.2	0	0
Elmis aenea	16	51.2	0	6.4	137.6	0	28.8	121.6	3.2	0	6.4	108.8	16	3.2
Esolus parallelepipedus	99.2	25.6	0	9.6	0	6.4	22.4	12.8	6.4	3.2	6.4	16	0	0
Limnius volckmari	25.6	32	12.8	9.6	3.2	0	76.8	38.4	0	3.2	28.8	51.2	0	3.2
Oulimnius tuberculatus	99.2	121.6	12.8	28.8	12.8	3.2	128	64	19.2	124.8	12.8	9.6	0	118.4
Curculionidae indet	0	6.4	0	272	0	6.4	0	0	0	0	0	0	0	0
Sialis sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricoptera indet	48	9.6	0	0	0	0	0	3.2	0	0	0	0	0	0
Rhyacophila sp.	19.2	22.4	16	0	0	3.2	35.2	12.8	0	0	57.6	35.2	22.4	0
Rhyacophila dorsalis	25.6	35.2	16	0	0	12.8	41.6	6.4	0	0	54.4	9.6	60.8	0
Rhyacophila munda	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhyacophila obliterata	0	0	0	0	0	0	0	0	0	0	3.2	3.2	0	0
Rhyacophila fasciata	0	0	0	0	0	0	6.4	0	0	0	0	0	0	0
Oxyethira sp.	0	38.4	0	0	0	9.6	0	19.2	9.6	9.6	118.4	0	3.2	0
Wormaldia occipitalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plectrocnemia sp.	0	6.4	0	0	6.4	28.8	41.6	0	0	6.4	16	16	9.6	3.2
Plectrocnemia brevis	0	0	0	6.4	0	0	0	3.2	0	0	0	3.2	0	0
Plectrocnemia conspersa	0	0	0	0	0	22.4	0	0	0	28.8	0	0	6.4	0
Plectrocnemia geniculata	0	0	0	0	12.8	6.4	3.2	0	0	3.2	0	3.2	0	0
Polycentropus sp.	0	0	0	0	0	0	0	3.2	0	0	0	6.4	0	0
Polycentropus flavomaculatus	3.2	28.8	0	0	0	0	32	0	0	9.6	0	6.4	0	0
Chaetopteryx villosa	0	0	0	9.6	6.4	0	0	0	0	0	0	0	0	0
Hydropsyche sp.	6.4	0	3.2	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche pellucidula	6.4	0	6.4	0	0	0	0	0	3.2	0	0	0	0	0
Hydropsyche siltalai	265.6	41.6	598.4	0	6.4	41.6	92.8	6.4	32	0	0	76.8	3.2	124.8
Diplectrona felix	3.2	0	0	0	0	0	6.4	0	0	0	0	0	0	0
Limnephilidae indet	25.6	6.4	0	0	0	0	0	0	0	0	0	0	6.4	0
Drusus annulatus	0	9.6	0	0	3.2	9.6	12.8	0	3.2	0	0	0	0	0
Halesus digitatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Halesus radiatus	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2
Potmaophylax cingulatus	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2
Potmaophylax rotundipennis	3.2	0	0	0	0	0	0	0	0	0	0	0	0	0
Silo pallipes	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0
Sericostoma personatum	6.4	0	0	0	0	0	0	0	3.2	0	0	0	0	0
Odontocerum albicorne	3.2	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae indet	0	6.4	0	0	0	0	0	0	0	0	0	0	0	0
Mystacides azurea	0	0	0	0	0	0	0	0	16	0	0	0	0	9.6
Diptera indet	0	3.2	0	0	0	0	0	0	0	0	0	0	0	0
Tipulididae	195.2	35.2	147.2	6.4	19.2	16	108.8	35.2	70.4	28.8	92.8	0	67.2	16
Psychodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	3.2
Dixidae	0	0	0	0	0	3.2	0	0	0	0	3.2	0	0	3.2
Thaumaleidae	0	0	0	0	0	3.2	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	6.4	0	0	3.2	3.2	0	0	0	0	0	0	0
Simuliidae	195.2	86.4	208	22.4	134.4	496	310.4	156.8	38.4	1674	2166	1632	588.8	9.6
Chironomidae	2477	2330	1661	739.2	2099	2531	6925	931.2	4371	6707	1542	486.4	4282	2301
Rhagionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	3.2	0	3.2	0	0	0	0	0	0	0	0	0	0	0
Athericidae	16	0	0	0	0	0	0	0	9.6	0	0	0	0	0
Empididae	0	0	3.2	0	6.4	83.2	230.4	3.2	16	9.6	19.2	3.2	9.6	35.2