

**THE ROLE OF ACID EPISODES IN OFFSETTING THE
RECOVERY OF STREAMS FROM ACIDIFICATION**

A Thesis Submitted for the Degree of Doctorate of Philosophiae

by

Renata A. Kowalik

School of Biosciences

Cardiff University

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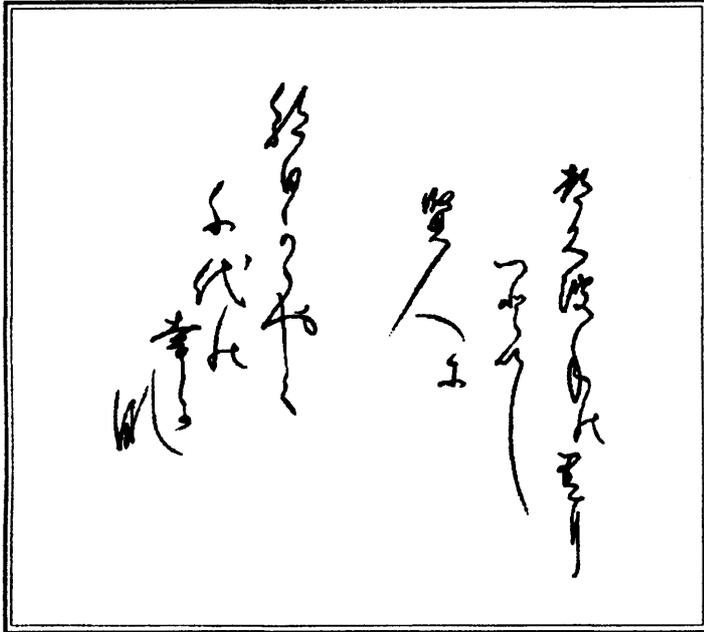
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Acid rain 2000



The wise of the world, gathered at Mt. Tsukuba,
for the well-being of mankind, now and in the future
The bright light of the rising sun, may it bless us,
for a thousand years

Kenichi Satake

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SUMMARY

1. A current need in freshwater acidification research is to explain why biological recovery has not matched unequivocal evidence of chemical reversal. A leading hypothesis is that acid-sensitive species are prevented from recolonising recovering streams because of anthropogenic acid episodes – transient periods of reduced pH during rainstorms or snowmelt (hours to weeks).
2. Intra-annual variation in invertebrate abundance and survival during episodes were assessed in episodic streams at the Llyn Brianne experimental catchments. Survival patterns produced clear evidence that acid episodes are still toxic to sensitive species.
3. Using large-scale surveys data from 89 streams spread across Wales and Scotland, I next tested five predictions required to support the ‘episodic acidification’ hypothesis: i) there should be differences in invertebrate composition between episodic and well-buffered streams; ii) any such effects should differentiate sites where episodes are driven by anthropogenic acidification as opposed to base-cation dilution; iii) chemical variation between base- and storm-flow should be greater in regions receiving the most acid deposition (Wales and Galloway > the NW Highlands); iv) strong variations in invertebrate assemblages should result and v) they should reflect storm-flow (i.e. episode) chemistry more than base-flow.
4. Strong acid anions –dominantly non-marine sulphate – still contribute to episodic acidification in sensitive areas. pH fell during storm-flow by 0.5-1.8 units in acid and episodic sites everywhere. However, aluminium concentrations were significantly greater, or increased more between flows, in Galloway and Wales than in the NW Highlands. Loss of ANC at acid or episodic sites was greatest in Galloway whereas mean values became negative only in Wales. Variations in invertebrate assemblages between acid, episodic and circumneutral streams were most marked in Wales and Galloway and effects were greatest in streams with episodes driven largely by non-marine sulphate. Although these patterns

reflected both base-flow and storm-flow chemistry, correlations between assemblage structure and aluminium were strongest at storm-flow.

5. Regression models were used to project the effects of base-flow or storm-flow pH on invertebrates using trends apparent across the UK Acid Waters Monitoring Network (UKAWMN). Episode chemistry provided the best predictions of acidification effects on stream invertebrates, but model projections were affected by critical assumptions about future recovery process.

6. These data illustrate that acid episodes still affect stream organisms in spite of continued recovery in air and water quality and this may be a sufficient explanation for slow biological recovery in many locations. Episodic effects may impose important controls on organisms as recovering sites reach the base-flow pH range 5.5-7. The strongest recommendation is for better understanding and monitoring of the hydrochemistry and biological effects of acid episodes as acid deposition abates.

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CHAPTER 1. GENERAL INTRODUCTION

1.1. PREFACE

Although acidification due to acid deposition formerly affected streams across large areas of North America and Europe, evidence is now emerging from chemical data that this trend is reversing (Fowler *et al.*, 2001; Harriman *et al.*, 2001; Fölster & Wilander, 2002). Sulphate concentrations in deposition and streamwater have declined increasingly across Europe over a period of more than 20 years (Moldan *et al.*, 2001; Fölster & Wilander, 2002). This reduction in deposition of acidifying compounds has led to signs of chemical recovery in freshwaters with increases in pH and acid neutralising capacity (Evans & Jenkins, 2000; Evans *et al.*, 2001). In contrast, evidence for any accompanying and sustained biological recovery is patchy (Tipping *et al.*, 2002), for example acid-sensitive invertebrates occur in recovering streams often only sporadically (Soulsby *et al.*, 1997; Collier & Quinn, 2003). In addition, although mitigation measures against acidification, such as liming have proven to be successful in creating the conditions suitable for acid-sensitive invertebrates e.g. baetid mayflies, and fish e.g. juvenile Atlantic salmon, *Salmo salar* L. and brown trout *Salmo trutta* L., conditions are not consistent enough for acid-sensitive taxa to persist (Bradley & Ormerod, 2002b).

One of the current needs in acidification research is to explain current limits on biological recovery from acidification relative to unequivocal evidence of chemical reversal. One of the possible hypotheses for the sporadic and slow biological response to chemical recovery is that acid-sensitive species might be prevented from recolonising or sustaining populations in recovering streams because of acid episodes – transient periods of reduced pH (hours to weeks), for example during rainstorms or snowmelt (Bradley & Ormerod, 2001, 2002b). Such episodes were strongly implicated in the initial biological effects of acidification (Davies *et al.*, 1992). At the onset of reduced acidic deposition the effects of episode chemistry on freshwater biota was noted and it was suggested that, if they continued, episodes could prevent biological recovery even as mean pH increased (Lacroix & Townsend, 1987).

An assessment of the current understanding of episodic acidification has been used here to identify those areas that need to be addressed in order to answer some of the questions on the biological effects of episodes. The review considers both the past work and present status of studies into episodic acidification. It aims to identify the underlying mechanisms driving episodic acidification from the basic chemical processes including soil chemistry and the relative contribution of sulphur and nitrogen through to catchment vegetation and seasonal effects from both a biological and chemical perspective.

1.2. A REVIEW OF PAST AND PRESENT WORK ON 'ACID EPISODES'

1.2.1. *Introduction to 'acid episodes'*

Acid episodes are an important part of the complex abiotic interactions within surface waters and are considered to have a major effect on the biology of freshwater systems. Since acidification of freshwaters became an issue, episodicity has been studied to identify a number of aspects, from a range of physicochemical characteristics and geographical differences through to the toxicological effects on the fauna and flora (Herricks & Cairns, 1974; Björnberg, 1983; Stoner *et al.*, 1984; Burton *et al.*, 1985; Lacroix & Townsend, 1987; Ormerod, 1987; Hopkins *et al.*, 1989). Although episodic acidification occurs in all surface waters to some degree, the extent to which an episode influences the chemistry and biology varies greatly, for example permanently acidified waters with a depauperate acid-tolerant fauna will be unaffected by the change in chemistry, yet can cause major episodic disturbances in circumneutral waters where acid-sensitive fauna will respond negatively to reduced alkalinity and pH. Chemical recovery from an episode can occur in a matter of hours or days with biological recovery usually following in response to a return to pre-disturbance conditions (Herricks & Cairns, 1974). However, the magnitude of an episode and time to recovery will be dependant on the mechanisms driving the episode (Hopkins *et al.*, 1989; Matthaei *et al.*, 1997).

1.2.2. Mechanisms of episodic acidification

Acid episodes are depressions in alkalinity and pH which are usually associated with declines in base cations and increased H⁺ ions. These changes in chemistry can be attributed to a range of factors including dilution of base cations and the addition of acid anions, organic acids and seasalts (Kahl *et al.*, 1992). Dilution of base cations is caused by increased input of rainwater or snowmelt, which effectively reduces the acid neutralising capacity (ANC) of the water body. ANC is determined by changes in the concentration of ionic solutes, the basic relationship is the difference in charge balance between the sum of base cations (sodium, potassium, calcium and magnesium) and sum of acid anions (sulphate, nitrate and chloride), expressed in micro equivalents L⁻¹ (*Equation 1*).

$$\text{ANC} = ([\text{Na}^+] + [\text{K}^+] + 2[\text{Ca}^{2+}] + 2[\text{Mg}^{2+}]) - (2[\text{SO}_4^{2-}] + [\text{NO}_3^-] + [\text{Cl}^-])$$

(*Equation 1*)

There are variations to this equation that incorporate aluminium (n[Alⁿ⁺]), ammonium ([NH⁴⁺]), fluoride ([F⁻]) and sometimes organic anions that help to explain the relative concentration of all the major ions (Schaefer *et al.*, 1990; DeWalle & Swistock, 1994). Nevertheless, the concentration of these ions can be difficult to determine, often resulting in over-estimated or under-estimated values of ANC.

The effect of dilution can cause major declines in ANC, which is usually associated with elevated levels of pre-episode base cations i.e. well buffered streams. In contrast, the effect of dilution is usually far less important in streams with low levels of base cations (DeWalle & Swistock, 1994). In conjunction with reduced buffering capacity due to dilution and declines in alkalinity, there is also an inevitable decline in pH (Molot *et al.*, 1989). These are usually accompanied by the addition of acid anions, organic acids and seasalts in varying combinations and concentrations that are dependant on a range of factors including soil chemistry, riparian vegetation, geology, climate, seasonality and antecedent conditions (O'Brien *et al.*, 1993; Laudon *et al.*, 2000).

Soil chemistry makes a highly significant contribution to episode chemistry because ions within rainwater and snowmelt percolate through the soil layers to interact with cations and metals held in the soil matrix (Drever, 1997). The highly variable composition of soils and underlying geology give rise to a wide range of responses, for example, thin soils overlying base-poor bedrock can result in poorly buffered waters that can lead to pronounced episodic effect during periods of increased discharge (Jenkins, 1985). Alternatively, the surrounding soil may be calcareous resulting in well buffered waters that for the most part withstand increased discharge and neutralise acid anion addition (Björnberg, 1983). Thus, acidic soils in combination with mobile acid anions will enhance the effect of episodic acidification (Feger, 1990).

Sea-salts that occur through deposition of marine-based ions can have an impact on acid episodes occurring in coastal areas or catchments subject to orographic rainfall (Kahl *et al.*, 1992). Seasalts enter rainwater within coastal areas when seaspray, evaporation of seawater and solid aerosol particles are transported by winds until dissolved by rainfall (Drever, 1997). Seasalts occurring further inland are usually a result of orographic rainfall over the uplands because winds carry the marine aerosols which are then scavenged by hill top cloud (Dore *et al.*, 1992; Fowler *et al.*, 2001). In addition, dry deposition of sea-salts build up on vegetation and forest canopy later to be scavenged during rainfall, which increases the concentration of marine-derived ions in surface waters and soils (Inglis *et al.*, 1995; Juggins *et al.*, 1996). The resulting drops in pH and alkalinity caused by sea-salts is derived from cation-exchange that occur between sodium ions and hydrogen, aluminium and base cations held in the soil (Wright *et al.*, 1988).

The most important mobile anions contributing to episodic acidification are sulphate and nitrate, originating from wet and dry deposition and biogeochemical cycling within catchments and water bodies (Miegroet, 1994). Both these acid anions are major contributors to ANC and pH decline in surface waters. However, anthropogenically derived sulphate, its retention time within a watershed and removal process is usually the most significant factor during acid episodes. Recent work has shown that sulphate

concentrations in surface waters are still higher than levels in rainfall (Reynolds *et al.*, 1999; Kvaalen *et al.*, 2002). Although the cause is a result of reduced sulphur emissions (Soulsby *et al.*, 1997), it is also a combination of the accumulation of dry and occult deposition by vegetation, evapo-transpiration, oxidation of sulphur in drained soils (Stoner *et al.*, 1984) and the export of previously deposited sulphur (Laudon & Bishop, 2002a). However, wet deposition of sulphur can still play a major role during acid episodes (Reynolds *et al.*, 1997).

Nitrate, a strong acid anion, is generally not considered a dominant control on acidity, however, concentrations in surface waters can be highly correlated with nitrogen deposition (Wright *et al.*, 2001; Lepori *et al.*, 2003a). The severity of episodes has also been shown to increase with pulses of nitrate that are linked to soil saturation and acidic deposition of nitrogen (Wigington *et al.*, 1996b; Evans & Jenkins, 2000). In addition, elevated levels of nitrate are often linked with land-use such as forestry, because of enhanced deposition, or catchments with land improvement, which are linked to fertiliser use (Evans *et al.*, 2000).

During episodic acidification, the relative effects of these strong acid anions, base cations and metals such as aluminium are fundamentally linked with hydrological pathways caused by rainstorms or snowmelt (Davies *et al.*, 1992). These hydrological pathways affect episodes by controlling the hydrochemistry of storm runoff, particularly in areas where high rainfall causes saturated soils (Soulsby & Reynolds, 1993; Soulsby, 1995). During rainstorms, subsurface flow-paths through acid soils elevate concentrations of acid anions or metals, while rapid runoff of precipitation results in low alkalinity (DeWalle & Swistock, 1994). Stream channel chemistry then reflects variable end-member mixing between these acid, metal-rich and low alkalinity components. In addition, high altitude headwaters can be particularly sensitive when dominated by acid-rich soils that generate runoff of low pH leading to more acid streamwater during peak flow (Bird *et al.*, 1990). Extreme runoff can be characteristic of the uplands making streamwater more sensitive to acidic precipitation (Björnberg, 1983). In contrast, runoff that slowly percolates through to groundwater level often results in well buffered waters that feed streams during base-flow

conditions. In a comparison of mid-Atlantic and coastal plain streams it was concluded that storm flow chemistry and sources of acidity and alkalinity loss during episodes, were highly influenced by shallow subsurface processes combined with bedrock geology (O'Brien *et al.*, 1993).

In addition to the direct influence of soils and related hydrochemistry, water runoff can retain the chemical signature of catchment vegetation (Smart *et al.*, 2001). Riparian and catchment vegetation can be efficient at scavenging pollutants such as sulphate, particularly during dry periods, which can result in dry and occult deposition (Stoner *et al.*, 1984) later to be washed off during rainfall causing increased acidic deposition beneath the canopy (Mason, 1991). Afforestation also reduces the water content of soils, which increases the concentration of pollutants such as sulphate. In addition, afforestation can also create the conditions in the soil substrate for near surface flow paths that are responsible for generating acid, aluminium-rich storm runoff (Soulsby & Reynolds, 1993). Catchment vegetation can also be a significant indicator of hydrochemistry, for example Smart *et al.* (2001) accurately predicted stream water chemistry over a range of flows and spatial scales using riparian vegetation and geochemistry.

Differences in seasonal deposition and hydrochemistry have been found to be important factors in the change between autumn and spring episodes (Bird *et al.*, 1990). The differences in alkalinity loss between dilution and titration have been examined in the Canton Ticino region of Switzerland, and it was found that autumn rainfall episodes were driven by dilution while spring snowmelt episodes involved both dilution and acid anion titration (Lepori *et al.*, 2003a). Similarly, correlations were strong between the sulphur in snow and subsequent spring snowmelt decline in alkalinity (Bishop *et al.*, 2001).

Antecedent conditions including climatic factors, occult and dry deposition and seasonal drought are all contributors to episodic acidification. Increased acidity and alkalinity loss during episodes is highly correlated with climate (O'Brien *et al.*, 1993). Areas with major differences in physicochemistry and sensitivity to episodic acidification are often associated with climatic trends in wet deposition (Benzie *et al.*, 1991). In effect, areas with

significant rainfall, and those experiencing major snowmelt events will be expected to experience frequent acid episodes (Bird *et al.*, 1990). Drought conditions preceding storm events can also have a strong influence on episode chemistry, for example, low ground water levels following a summer drought have been found to cause oxidation of sulphur (Laudon & Bishop, 2002a).

1.2.3. Regional differences in episodicity

Episodic acidification is widespread and well documented in North America and Europe and although these episodes are common, their physical and hydrochemical character will vary within and between these regions. In North America the Episode Response Project (ERP) was established to characterise and assess the biological effects of episode chemistry and to determine the physicochemical changes that accompany hydrological events (Wigington Jr. *et al.*, 1993). Although episodic acidification is of concern within any susceptible areas within the United States, the study focused on three priority regions: the northern Appalachian plateau of Pennsylvania and the Catskill and Adirondack Mountains of New York (Wigington *et al.*, 1996a; Wigington *et al.*, 1996b). They expected that increased alkalinity following recovery from acidification would reflect strong regional declines in sulphate concentrations. However, sulphate in Pennsylvania and nitrate in the Catskills and Adirondack Mountains continued to contribute to the severity of episodes. This is caused by wet deposition and large baseline concentrations of both these anions and reduced episodic ANC minima, even when ionic concentrations remained unchanged during episodes.

The headwater streams of the northern Appalachian Plateau are especially vulnerable to episodic acidification with streams in this region strongly influenced by sulphate input (DeWalle & Swistock, 1994). However, dilution was also a major contributor to declines in pH and alkalinity during these episodes. Differences in the importance of sulphate and nitrate affecting episodicity across these regions show that increases in snowpack driven nitrate dominate the declines in alkalinity in the Adirondack and Catskill Mountains and

Maine (Schaefer *et al.*, 1990; Kahl *et al.*, 1992; Murdoch & Stoddard, 1992). In contrast, mid Atlantic streams flowing into Chesapeake Bay, experience declines in alkalinity as a result of sulphate (O'Brien *et al.*, 1993).

In Europe, regional differentiation between acid episodes is strongly associated by both anthropogenic and natural influences. Regions in the UK sensitive to episodic acidification include the uplands of central and north Wales, southwest Scotland and the Pennines in northern England. However, any area where freshwaters are recovering from acidification can be particularly sensitive. In Wales, the magnitude and quality of rainstorm events are highly influenced by extensive afforestation, acid-sensitive soils and geology and orographic rainfall, which all impact on episode chemistry (Soulsby, 1995). Forestry in this region, and similarly in Galloway in Scotland, impacts on the hydrochemical character of episodes by generating near-surface flow paths that generate acid, aluminium-rich storm runoff (Soulsby & Reynolds, 1993). In northern Sweden, the regularity and intensity of episodic acidification has generated a strong research history in the area. Research has identified the driving mechanisms of episodicity that include significant sources of natural acidity in addition to the deposition of anthropogenic sulphur (Bishop *et al.*, 2001). Natural organic acids in combination with dilution of the buffering capacity can also be one of the major driving mechanisms during spring episodes in boreal regions (Laudon, 2000; Laudon *et al.*, 2000). However, anthropogenically driven episodic acidification as a result of sulphate deposition is declining in this region (Laudon & Hemond, 2002), although deposition of sulphur continues to drive severe acid episodes in southern Norway (Sandoy & Romundstad, 1995).

A study assessing regional trends across North America and Europe found significant declines in sulphate during episodes (Skjelkvale *et al.*, 2001). Nevertheless, sulphate pulses continue to be important in Europe and Canada and nitrate pulses more important to episodic acidification in the northeastern United States. In addition, a comparison of the characteristics of episodic acidification concluded that a major determinant of episodicity common to all these regions was the change in flowpath during hydrological events (Wigington *et al.*, 1992).

1.2.4. *Biological effects of acid episodes*

An integral line of research into episodicity has been the assessment of the biological consequences (Hopkins *et al.*, 1989; Bernard *et al.*, 1990; Weatherley & Ormerod, 1991; Brewin *et al.*, 1996). Although, much of this research has examined the damaging effects of both natural and anthropogenically driven episodes (Matthaei *et al.*, 1997; Gibbins *et al.*, 2001), more recently the emphasis has been to further understand the mechanisms affecting recovery (Masters, 2002). In addition, the conservation and economic value of freshwaters, such as salmonid fisheries, warrants a more comprehensive understanding of episodic effects. A wide range of freshwater organisms and methods have been employed to examine the toxicological and ecological effects of episodicity, including laboratory and field studies. Both of these approaches have inherent problems, for example, laboratory experiments cannot replicate the complex chemical or ecological interactions within freshwaters and conversely field studies lack adequate controls and precision (Ormerod & Jenkins, 1994). The biological response to episodes also varies across a range of individual and ecological factors including species type, community structure, population abundance, life-cycle, habitat availability, capacity for colonisation and seasonality.

Episodic acidification affects a wide range of freshwater species that spend either part or their entire life-cycle within various freshwater bodies including ponds, streams and lakes. This includes species affected indirectly through aquatic food abundance, for example dippers (*Cinclus cinclus* L.) (Ormerod *et al.*, 1986). The effects of acidic surface waters on fish and invertebrate species have been extensively studied and some of the major fish species used to date include rainbow trout (*Oncorhynchus mykiss* (Walbaum)), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) (Witters, 1986; Whitehead & Brown, 1989; Ingersoll *et al.*, 1990; Staurnes *et al.*, 1993). Studies of benthic invertebrates usually involve whole communities or individually selected acid-tolerant and acid-sensitive species (Ormerod *et al.*, 1987; Merrett *et al.*, 1991; Matthaei *et al.*, 1997). In addition, diatoms have been found to be good indicators of episodicity using species such as *Eunotia exigua* and *Achnantheidium minutissimum* (Hirst *et al.*, 2004).

Laboratory bioassays are used to investigate the toxic effects of freshwater acidification at the physiological and cellular level on a range of freshwater biota (Buikema & Cairns, 1980; Taylor, 1996). Physiological tolerances (survival and mortality) and specific effects can be more precisely observed and tested under laboratory conditions. However, the complexity of natural freshwaters are difficult to evaluate in this way, thus, laboratory studies usually benefit from complimentary field experiments (Vangenechten, 1989). A range of approaches are used in the field to examine the biological effects of episodicity, they include population surveys or monitoring in relation to hydrochemical data (Hamalainen & Huttunen, 1996); experimental designs, for example artificially acidifying stream sections (Ormerod *et al.*, 1987), or *in situ* bioassays such as transplantation of species into waters of differing ionic chemistry and pH (Masters, 2002; Lepori *et al.*, 2003b).

During episodic acidification, the relative concentration of base cations, acid anions and aluminium have been found to have a highly significant effect on freshwater fish and invertebrate species. For example, although a set of streams had the same concentration of water hardness, the streams subject to higher sulphate deposition could not support equivalent fish and invertebrate populations (Stoner *et al.*, 1984). In general, these studies found densities of fish species in response to episodic acidification are fairly consistent with the absence of acid-sensitive species and the reduced abundance of the more tolerant species such as trout (Baker *et al.*, 1996). Similarly, invertebrate community structure is usually depressed, causing reduced abundance and diversity in streams that experience episodic acidification (Kimmel *et al.*, 1996).

At the species level, *in situ* bioassays have identified the acute acidic conditions (e.g. low pH and elevated aluminium) that can inhibit swimming performance, growth and feeding in rainbow and brown trout and Atlantic salmon (Lacroix & Townsend, 1987; Ormerod *et al.*, 1987; VanSickle *et al.*, 1996; Wilson, 1996). The increasing magnitude of these episodes usually leads to increased mortality. Similarly, acid-sensitive invertebrates (e.g. mayflies *Baetis* spp.) may not be significantly affected when exposed to short episodes, with mortality rates only increasing when these episodes increased in time and intensity

(Merrett *et al.*, 1991). In contrast, acid-tolerant stonefly and some caddisfly species (e.g. *Amphinemura sulcicollis* and *Hydropsyche instabilis*) can exhibit high survival rates during severe acid episodes (Merrett *et al.*, 1991). There is little experimental information on how diatom assemblages respond to episodic acidification, however, there is some evidence that diatoms respond rapidly and directly to changes in acid-base status and that short-term acidification affect diatoms more rapidly than subsequent recovery from episodes (Hirst *et al.*, 2004).

Behavioural responses of fish and invertebrates are important mechanisms for avoiding the toxic effects of acid episodes. Animals may respond to an episode by moving or drifting in order to find refugia and thereby mitigating the adverse affects of episodicity (Ormerod *et al.*, 1987; Bernard *et al.*, 1990; Hall *et al.*, 1990; Baker *et al.*, 1996). Drift downstream can be a behavioural response (Kratz *et al.*, 1994), particularly for invertebrate species, or animals may be accidentally dislodged during increased flow (Hopkins *et al.*, 1989). However, refugia can also be limited to the local substrate, with animals moving into deeper sediments (Giberson & Hall, 1988).

The hydrochemical differences of episodicity (e.g. increasing concentrations of aluminium) can also affect the magnitude and timing of drift across different taxonomic groups (Bernard *et al.*, 1990). For example, depressions in pH alone may delay the response of acid-tolerant stoneflies until runoff delivers acid-aluminium rich water. In addition, habitat degradation as a result of physicochemical and hydrological changes can severely compromise community composition through lack of suitable habitat and refugia (Laudon, 2000).

Sensitivity of fish and invertebrates to the toxic effects of episodes can be reduced when the interval between successive acid episodes is increased. This period between episodes allows for recovery and possible adaptation i.e. increased resistance to episodic conditions (Morris & Reader, 1990; McDonald & Wood, 1992; Vuori, 1995). Similarly, when acid-sensitive invertebrate taxa are exposed to multiple episodes there is evidence that drift

response is less pronounced after repeated episodes compared to a single event, suggesting acclimation (Kratz *et al.*, 1994).

The movement and drift of species results in reduced densities in areas affected by episodic acidification that are later recolonised after a return to pre-disturbance conditions. The interval between disturbance and recolonisation can vary between species, although evidence suggests that drift from upstream areas is probably the most important pathway of recolonisation in invertebrate species (Matthaei *et al.*, 1997). In contrast, fish have been known to recolonise from alkaline tributaries or habitats that are able to maintain low densities during acid episodes (Baker *et al.*, 1996).

Ecological change, as a result of episodic acidification, can have consequences to a range of organisms that are not directly influenced by the hydrochemistry or physicochemical effects of episodicity. However, these effects are difficult to quantify and there are few studies relating the secondary effects of episodic acidification to catchment ecology (Ormerod & Jenkins, 1994). However, community structure has been shown to reflect the effects of episodicity (Baker *et al.*, 1996; Kimmel *et al.*, 1996; Gibbins *et al.*, 2001) with differences in biomass, species densities and diversity. These changes can then have an effect on the complexity of food webs eliminating species such as top predators that results in further disruption to the distribution and abundance of other species (Hall *et al.*, 1980). For example, the dipper *Cinclus cinclus*, a top predator, suffered a decline in population numbers as a result of reduced invertebrate species and densities caused by acidification (Ormerod *et al.*, 1986; Ormerod & Tyler, 1991; Buckton *et al.*, 1998). In effect, numbers of dippers may not increase unless streams can recovery invertebrate populations over the effects of episodic acidification.

1.2.5. Conclusions

An extensive range of work has been undertaken to develop the knowledge and understanding of the physicochemical characteristics of episodes. However, even though

the chemistry of episodes is increasingly well understood the biological effects are still poorly quantified (Bradley & Ormerod, 2002b). Yet, it is only recently that work on the biological effects of episodes has developed in relation to biological recovery from acidification. There is also, in addition to behavioural and physiological adaptation to changes in chemistry, growing evidence that the chemical effects of episodicity can alter biological community structure in freshwaters.

There is difficulty in assessing directly the biological effects of acid episodes or of inferring effects from survey data (Weatherley & Ormerod 1991). Nevertheless, in order to address the questions that support the hypothesis, that acid-sensitive species might be prevented from recolonising or sustaining populations in recovering streams because of acid episodes, some approaches are available. For example intensive year-round sampling of invertebrates in recovering streams might reveal not only how distribution changes seasonally in response to episodes (Lepori *et al.*, 2003b) but also may identify those species at increased risk because of their annual phenology. For example, snowmelt in spring or orographic rainfall during autumn and winter can produce episodes of contrasting duration, chemistry and severity (Davies *et al.*, 1992; Gibbins *et al.*, 2001; Lepori *et al.*, 2003a). Biological effects might therefore depend on which species have exposed stages during the most likely episodic periods. In addition to intensive sampling, exposure experiments might reveal whether conditions during episodes are toxic to sensitive organisms. Early *in situ* designs used acid or metal additions to streams to mimic acid-episodes, but many were characterised by artefacts because episodes were induced without accompanying high-flow or because metal speciation probably differed from real acid episodes (Hall *et al.*, 1980; Burton *et al.*, 1985; Allard & Moreau, 1987; Ormerod *et al.*, 1987). More recently, transplantations into real episodic streams exposed organisms to true episode chemistry (Hall *et al.*, 1988; MacNeil *et al.*, 2000). Such work shows that recovery can, indeed, be delayed after brief acid exposure even in organisms with short generation times (Hirst *et al.*, 2004).

Regional differences in invertebrate assemblage and the relative importance of changes in stream-flow – high-flow (episodes) versus base-flow – and associated effects on the biota

need to be assessed. For example, large-scale regional surveys can reveal not only contrasting sensitivity to acidification and deposition climate (Courtney & Clements, 1998), but also contrasting sources of acid precursors. Such comparisons of the chemistry, severity and effect of episodes across regions of contrasting deposition could indicate how episodes might continue to be important as deposition reductions continue. In addition, large-scale regional surveys might reveal differential influences on organisms from episodes driven by acid anion addition in contrast to base cation dilution (Davies *et al.*, 1992). Previous work has shown that it is possible to identify those factors contributing to the character of episodes, for example the relative contribution of acid anions and dilution to the overall chemistry of an episode (Kahl *et al.*, 1992). Once this has been done it is then possible to associate those factors with biological community structure and species abundances and assess the overall risk to the biota of continued episodicity. Regional surveys might also reveal contrasts in the organisms at risk (Malmqvist, 2002).

Finally, although much evidence indicates that many organisms in acid-sensitive streams are affected by episodic acidification, effective biological models that predict the effects of episodic acidification have proved elusive. This is despite previous efforts at modelling relationships between chemistry and stream organisms (Rutt *et al.*, 1990; Weatherley & Ormerod, 1992) and is a major problem that affects attempts to predict future recovery as acid-deposition declines. If such models were available, they would not only augment previous biological models of acidification, but they would also allow better links with important dynamic hydrochemical models such as PEARLS (Cooper *et al.*, 2000) or MAGIC (Cosby *et al.*, 1985a, b). The latter, in particular, has figured strongly in predicting regional or catchment-level recovery and susceptibility to acidification that has informed UK and European policy on air quality (Allott *et al.*, 2001; Reynolds *et al.*, 2002). Without good links to biological effects models, however, hydrochemical models are limited in scope. Therefore the large-scale surveys carried out here can be used to develop models using contrasting invertebrate community composition and species abundance in order to determine whether high-flow chemistry is a better predictor than base-flow chemistry.

1.3. AIMS

The aim of this thesis was to examine the effects of acid episodes on freshwater benthic invertebrates and determine if biological recovery from acidification in upland streams is being hindered by episodes. The objectives were to:

1. Assess the current understanding of episodic acidification and identify those areas that need to be addressed by reviewing past and present work on 'acid episodes' (Chapter 1);
2. Assess episodic effects on an acid-sensitive indicator species in the Llyn Brienne experimental catchment (Chapter 2) using a combination of intra-annual data of invertebrate abundance and survival during experimental transplantations;
3. Assess variations in the chemical signature of episodic acidification in order to identify factors influencing the character of episodes and their risk to the biota (Chapter 3);
4. Determine the relative importance of high-flow (episodic) over base-flow chemistry on invertebrate assemblages (Chapter 4) using intensive survey data of benthic macroinvertebrate assemblages and chemistry data taken at base-flow and high-flow from streams in areas of contrasting deposition in Scotland and Wales;
5. Evaluate whether high-flow chemistry better predicts invertebrate community composition than base-flow chemistry (Chapter 5) using invertebrate and chemistry data from streams in Wales and Scotland.

All chapters presented here are self-contained as potential papers for submission to journals and have their own reference lists.

1.4. STUDY AREAS

Surface waters in Scotland and Wales have been impacted by acidification in the past and in some areas deposition, afforestation and intensive livestock farming continue to influence the physicochemistry of streams and other water bodies. The study areas for this work cover four regions of varying land-use and landscape types within Scotland and Wales encompassing catchments and streams of varying sensitivity to episodic acidification. The regions in Scotland include the northwest Highlands and Galloway in the southwest and the regions in Wales were Conwy in the north and the Llyn Brianne and Irfon catchments in mid Wales. All these areas are upland sites of varying geology, soils and land-use and highly influenced by orographic rainfall due to westerly airflow coming in from the Atlantic.

The northwest Highlands were of interest because sites were expected to be unaffected by rainfall pollutants such as anthropogenic emissions of SO_x and NO_x. In addition, the ecology and hydrochemical nature of streams in the area are poorly known. Galloway, in the southwest, contrasts with the northwest Highlands having been impacted by extensive afforestation and acidic deposition. The streams and lochs in this region are well documented as a result of acidification, having been surveyed for fish populations and chemical data since 1978 (Harriman *et al.*, 1987; Harriman, 2001). The site locations in Scotland covered an extensive area, approximately 10,400 km² of the northwest Highlands and 4500 km² of Galloway, although the number of sites was similar in both these regions and the regions in Wales (20-25).

Conwy in north Wales has a combination of moorland, forestry and farmland, and has historically received high levels of sulphate deposition which is the main contributor to non-marine acid anion concentrations (Jenkins *et al.*, 1996). This area is approximately 253 km², however, although researched well with regards to the hydrochemical status of acidification, knowledge of the effects on benthic invertebrates is limited. In contrast, research and monitoring of stream invertebrate ecology in the Llyn Brianne catchment in

mid Wales has been ongoing since the 1980's (Stoner, 1984; Donald & Gee, 1992; Bradley & Ormerod, 2002b). The study sites for this mid Wales region cover an area of approximately 99 km² and incorporate both the Llyn Brienne and Irfon catchments. Both catchments have been impacted by acidic deposition in the past, however the Irfon catchment is another area where data are limited (Tanner, 2001).

1.5. STUDY ORGANISMS

Benthic macroinvertebrate species are regularly used in studies of freshwaters as a result of the predictable nature of community composition and response to changes in stream hydrology and physicochemistry. The relative dominance of invertebrate species can give an indication of the status or changes in stream chemistry (Burton *et al.*, 1985; Simpson *et al.*, 1985), for example increased acidity through deposition of pollutants such as SO_x and NO_x has been detrimental to invertebrate community structure (Engblom & Lingdell, 1984; Stoner *et al.*, 1984). Stream acidification has been found to decrease species diversity, increase representation of community dominant species and decrease the complexity of the food web (Hall *et al.*, 1980). As a consequence studies have made use of these findings and models developed in order to make preliminary assessments of stream status using invertebrates as indicator species (Rutt *et al.*, 1990; Moss, 1999; Clarke *et al.*, 2002).

The work here incorporates benthic invertebrate assemblages sampled from stream riffle and margins using the semi-quantitative method of kick sampling and quantitative Surber sampling. Invertebrates were sampled intensively from all regions over a spring period and then selected sites monthly over a 12 month period. Whole assemblages down to single species were used depending on the particular study.

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**CHAPTER 2. THE EFFECTS OF EPISODIC ACIDIFICATION ON THE DENSITY
AND SURVIVAL OF ACID-SENSITIVE MAYFLIES SHOWN BY INTENSIVE
SAMPLING AND TRANSPLANTATION**

2.1. SUMMARY

1. The response of benthic invertebrates to chemical change in streams recovering from acidification is slow. One hypothesis is that acid episodes continue to restrict biological recovery despite increased mean pH. In this chapter, transplantation and intensive quantitative sampling were used to assess possible episodic effects on acid-sensitive indicator species in the Llyn Brianne experimental catchments.

2. By assessing intra-annual variation in invertebrate abundance in the episodic streams, I aimed to assess possible exposure to episodic effects quantified from a risk index based on long-term discharge. These effects were then tested experimentally using short-term transplantations between acid and circumneutral streams to mimic short-term acid episodes.

3. Six streams were sampled quantitatively over a 12 month period for benthic invertebrates, with contemporaneous measurements of pH, conductivity and temperature. Complete base-flow and high-flow chemistry were also evaluated at each site. Streams classified as episodic, acidic and circumneutral differed in pH, alkalinity, calcium, aluminium and nitrate between high- and low-flow.

4. Acid-sensitive mayflies were found mostly in the circumneutral streams (*Ephemerella ignita*, *Baetis rhodani*, *B. muticus*, *B. vernus*, *Rhithrogena semicolorata*, *Ecdyonurus* spp. and *Heptagenia lateralis*) and at least four species had life-cycles that, if repeated elsewhere, would expose them to the increased risk of acid episodes in autumn/winter. Only *B. rhodani* occurred in the episodic streams, but densities declined substantially in autumn. No mayflies occurred in the acid streams.

5. *B. rhodani* were transplanted during base-flow (September 2003) and high-flow (April 2004) from the circumneutral into the acidic streams and survival compared with circumneutral reference sites. Animals were exposed to two treatments involving 1) continual (chronic) exposure in the acid streams over the 16 days of the experiment and 2) repeated short-term (episodic) exposure to acid conditions for 2x4 day periods interspersed with 4-day recovery periods in the reference streams.

6. During the transplantation experiment at low-flow, only conductivity differed between acid and reference streams. pH, conductivity and temperature then fell everywhere at high-flow, and accentuated differences in acidity between stream types. *Baetis* survival in the reference streams remained high during both low- and high-flow experiments. By contrast, mortality in the chronic and episodic exposure to low pH (mean low-flow = 5.5-5.8 and high-flow = 3.8-3.9) increased significantly ($P < 0.01$) but only during the high-flow experiment. Mortality at high-flow varied significantly in the order chronic (80-89%) > episodic (28-43%) > reference (8-10%).

7. These data illustrate that despite national and Europe-wide trends towards recovery from acidification, it is not possible to refute the hypothesis that acid episodes still affect aquatic invertebrates at Llyn Brianne. Most acid-sensitive mayflies are still scarce in chronically and episodically acidified Welsh streams. Several species have life-cycles that could lead to acid exposure during the autumn and winter, and this is sufficient to explain their current distribution. Moreover, high-flow events in acid-sensitive streams are accompanied by pH sufficiently low to reduce survival significantly even during episodes as short as 4 days.

2.2. INTRODUCTION

Where acidification in the past altered the chemical and biological status of streams, evidence is now emerging for chemical recovery in some locations (Fowler *et al.*, 2001; Harriman *et al.*, 2001; Fölster & Wilander, 2002). However, evidence for associated biological recovery is patchy. Invertebrate communities have responded to amelioration in streams treated with lime, although effects are sometimes modest (Bradley & Ormerod, 2002b). Elsewhere, acid-sensitive invertebrate species occur in recovering streams often only sporadically (Soulsby *et al.*, 1997; Collier & Quinn, 2003). Among the possible hypotheses to explain the shortfall in biological recovery, one currently favoured is that acid-sensitive species might be recolonising acid-sensitive streams in recovery but failing to persist because of acid episodes. Such episodes were strongly implicated previously as key features of acidified streams and, if they continued, could affect biological recovery even as mean pH increased. So far, evidence to support this hypothesis is scarce, but some experimental and observational approaches are possible.

Detailed sampling of invertebrates through time allows an assessment of how their distribution changes seasonally (Furse *et al.*, 1984; Ormerod, 1987; Brewin *et al.*, 2000), for example in response to episodic effects in acid-sensitive streams, changes through time might indicate episodic effects or alternatively might reveal species at risk (Wilkinson & Ormerod, 1994). Lepori *et al.* (2003b) used this approach to identify species that might be sensitive to episodes and then used transplantation experiments to assess postulated effects (Lepori, 2003). This work focused particularly on mayflies since they are among the most acid-sensitive of invertebrates (Elliott *et al.*, 1988; Fjellheim & Raddum, 1992).

Transplantation and *in situ* toxicity testing in freshwaters is being utilised increasingly to evaluate the response of a range of organisms to both natural and anthropogenic ecological change. In order to assess the effects of acidification on freshwater organisms some of the earliest *in situ* designs used experimentally acidified streams to mimic short acid-episodes through artificially reduced pH and increased metal concentrations (Hall *et al.*, 1980; Burton *et al.*, 1985; Allard & Moreau, 1987; Ormerod *et al.*, 1987). However, hydrological and chemical artefacts might have resulted, for example because episodes were induced with accompanying high-flow or because metal speciation might differ from

real acid episodes. Transplantation, on the other hand, allows organisms to be exposed to real episode chemistry that incorporates changes in physicochemical conditions such as lowered pH with increased flow (Hall *et al.*, 1988; MacNeil *et al.*, 2000).

This chapter uses a blend of intensive sampling and transplantation in streams to evaluate possible episodic effects on benthic invertebrates. By assessing seasonal variation in mayfly densities, the risk of exposure to episodic effects would be apparent. Transplantation was then used to examine in more detail any short-term or chronic acidification effects. All the work was carried out at Llyn Brianne in mid Wales, where streams contrast between chronically acid, episodic and circumneutral.

2.3. METHODS

In outline, this study involved two stages. First, intensive sampling was used to assess year-round variation in benthic invertebrates in circumneutral, episodic and chronically acid streams. Patterns were compared with the long-term risk of exposure to acid episodes as calculated using long-term data on river discharge. Second, transplantation experiments were undertaken between streams at base-flow (September 2003) and high-flow (April 2004) i.e. episodic, and involved exposing the mayfly, *Baetis rhodani* (Pictet), to episodically acid conditions for varying durations. Survival was compared with circumneutral references.

2.3.1. Site descriptions

The sites were situated in the experimental catchments of the rivers Camddwr and Tywi draining into the Llyn Brianne reservoir in mid Wales (52°8'N, 3°45'W). A full description of the geology, topography, soil types and chemistry can be found in Chapter 2.0 and in other papers (Stoner, 1984; Stoner *et al.*, 1984; Rutt *et al.*, 1989; Merrett *et al.*, 1991). The Camddwr is under moorland vegetation and parts of it were artificially limed during 1987/88. As a result, the two streams involved, CI 2 (SN765575) and CI 5 (SN775557), are now re-acidifying and have episodically acid chemistry (Bradley & Ormerod, 2002b). Two streams of the Tywi catchment, LI 1 (SN809530) and LI 2

(SN881516), are situated in conifer forest and have acidic water. The two moorland streams, LI 6 (SN822496) and LI 7 (SN818493), are circumneutral. Each pair of streams (acidic, episodic and circumneutral) were considered replicate sites based on long-term data (approx. 15 years) (Weatherley & Ormerod, 1990; Merrett *et al.*, 1991). For the transplantation studies, the acidic streams LI 1 and LI 2 were used as acid (test) sites and the circumneutral streams LI 6 and LI 7 as the reference sites.

2.3.2. Intensive benthic sampling

Benthic invertebrates were sampled over a 12 month period between February 2002 and January 2003 from LI 1, LI 2, LI 6, LI 7, CI 2 and CI 5 at approximately four week intervals. Invertebrates were sampled quantitatively using a Surber sampler of surface area 0.25 m² and 1 mm mesh size (HMSO, 1982). Five samples were taken from each stream on each occasion from the riffles where possible. In each case the substrate was disturbed by hand to a depth of 10 cm. The samples were preserved on site using 100% IMS (Industrial Methylated Spirit). They were then sorted in the laboratory by initially rinsing through a 500 µm sieve, immersing in fresh water and removing the macroinvertebrates by hand. The sorted samples were preserved in a 70% solution of IMS. A wide range of invertebrates were identified, but only species-level data for the ephemeropterans *Baetis rhodani* (Pictet), *B. muticus* (L.), *B. vernus* Curtis, *Ephemerella ignita* (Poda), *Rhithrogena semicolorata* (Curtis), *Heptagenia lateralis* (Curtis) and *Ecdyonurus* spp. Eaton are presented here.

2.3.3. Water chemistry

Stream chemistry was monitored for pH, electrical conductivity (EC) and temperature at the same frequency as invertebrate sampling using a hand-held combined pH/EC/TDS meter (Hanna Instruments HI 991300). Chemistry data from a full ionic analysis were also available to represent base-flow (low-flow August 2002) and episodic conditions (high-flow February 2003). The water samples taken during these low- and high-flow events were analysed for ionic concentrations of the chemical variables sodium, potassium, calcium, magnesium, ammonium, chloride, nitrate, phosphate, sulphate, silica and Al (Na⁺,

K⁺, Ca²⁺, Mg²⁺, Cl⁻, NO₃⁻, SO₄²⁻ and Alⁿ⁺), as well as dissolved organic carbon (DOC). Anions were determined by ion chromatography (Dionex), base cations and metals by Atomic Absorption Spectrophotometry (AAS) (Perkin Elmer) and Si by the molybdenum blue method. DOC was determined by continuous flow colorimetry with UV digestion (Skalar autoanalyser System) and Al colorimetrically using the pyrocatechol violet method (Dougan & Wilson, 1974). pH was measured with a hand-held combined pH/EC/TDS meter and alkalinity (ANC) was calculated by the charge balance method i.e. sum of base cations minus the sum of acid anions (Na⁺ + K⁺ + Ca²⁺ + Mg²⁺ - NO₃⁻ - SO₄²⁻ - Cl⁻).

2.3.4. Risk of exposure to acid episodes

An index of risk of exposure to acid episodes was calculated using hydrological data from the National River Flow Archive collected between 1958-2001 at the Plynlimon Flume on the River Severn (SN 853 872; 331 m OD), within 40km of the experimental sites at Llyn Brianne. For each month, long-term average daily discharge values were divided by the lowest monthly discharge (May) and then the relative difference from this low value scaled between 0 and 10 (= greatest risk). This approach assumes that i) acid episodes are driven essentially by increased discharge and ii) current average risks of episodes reflect discharge data collected over the last 43 years. The index calculated for Plynlimon correlated very highly significantly with indices calculated from an equally long-term rainfall record from Plynlimon, and also from discharge data from the main Afon Tywi (both $r > 0.95$); application of an index from the upper river Severn to the upper Tywi is therefore justifiable.

2.3.5. Transplantation and test animals

The test animals for the transplantation experiment were nymphs of the ephemeropteran, *B. rhodani*, an acid-sensitive species well known in acidification studies. *B. rhodani* is abundant in the circumneutral sites LI 6 and LI 7 where it forms over 90% of the baetid assemblage (CRG Cardiff University unpublished data). However, it is not possible to distinguish between *B. rhodani*, *B. muticus* and *B. vernus* in the field, and although it was expected that *B. rhodani* would make up the majority of test animals sampled (Masters,

2002), all animals were preserved in 70% IMS for subsequent formal identification on completion of the experiment. Subsequently, all the sampled mayflies were positively identified as *B. rhodani*.

Animals collected for the exposure studies were selected by gently disturbing the substrate and holding a standard kick net downstream to retain those drifting. The contents of the net were emptied into a tray containing streamwater and *Baetis* mayfly nymphs were selected from the tray using a wide mouth plastic pipette. A total of 240 (160 test and 80 reference) animals were placed straight into eight containers holding streamwater in batches of 20 preventing further stress by handling. The containers were placed inside a further container holding streamwater to insulate during transportation. The animals were then placed in the cages at the test and reference sites.

During all exposure periods, pH, EC and temperature were recorded using a hand-held combined pH/EC/TDS meter (Hanna Instruments HI 991300). These measurements were taken at the beginning of the experiment and each day the cages were checked. The combined pH/EC meter was calibrated several hours prior to use in the field with standard buffer solutions (pH 4 and 7 and EC 1014 $\mu\text{S cm}^{-1}$) at room temperature ($\sim 21^\circ\text{C}$). The experiments were carried out in September 2003 during what was considered base-flow conditions (low-flow) and again in April 2004 during increased rainfall (high-flow).

The test cages used to house the mayfly nymphs were cylindrical with a plastic base, mesh (560 μm) sides and lid, were 10 cm in height and had a 16 cm diameter base (Plate 1). The same designs were used previously in experiments by (Masters, 2002; Lepori, 2003). The cages were placed in the streams two days prior to commencement of transplantation to allow them to settle through stream movement and check for free movement of water through them. Metal stakes were hammered into the substrate and the cages were attached with clips to enable relocation during the course of the experiment.

Prior to any manipulation, the animals were left in their cages in the two replicate reference (LI 6 and LI 7) and acid test streams (LI 1 and LI 2) for four days (Figure 1a) and monitored for mortality and emergence every 24 hours. On day four, the second stage of the experiment, four cages with their animals were removed from each acid stream and

placed in each of the reference streams (Figure 1b). These animals were then assumed to have been subjected to a short acid episode, and each cage was monitored for survival every 48 hours. On day eight, the four episode cages were returned to each of the test streams for a further four days (Figure 1c). This pattern of periodic exposure was then continued for 16 days to simulate exposure to a series of acid episodes typical of frontal weather systems (Soulsby, 1995). Throughout, mortality and emergence were compared to the reference cages retained in the circumneutral LI 6 and LI 7 and the test cages in the chronically acidified LI 1 and LI 2. Those animals that reached emergence were included in the survival data. In addition, all animals in both reference and test cages were sampled by the same method, and treated to the same transportation treatments at all stages of the experiment to ensure they were subject to a comparable handling.



Plate 1. Transplantation cages in a reference stream (LI 7) at Llyn Brianne.

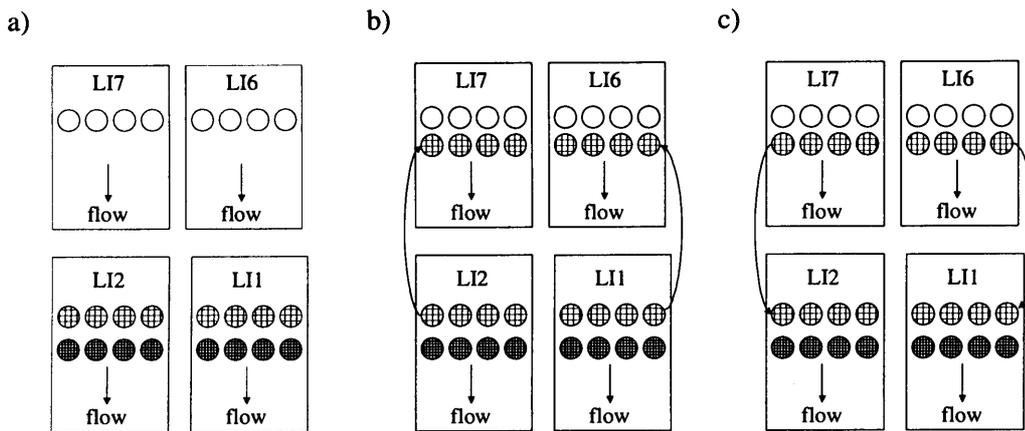


Figure 1. Experimental design for the transplantation of *Baetis* spp. from circumneutral reference streams (LI 6 and LI 7) to acid test streams (LI 1 and LI 2). First stage a) 8 test cages (4 episodic = ⊕, 4 chronic = ●) were placed in each test stream and 4 references (○) in each reference stream; second stage b) after day 4, the 4 episodic cages from each test stream were transferred to the reference streams for a further 4 days; third stage c) after day 8, the cages from the second stage were moved back to the test streams. This was repeated twice covering a 16 day period. The whole procedure was repeated during low-flow (September 2003) and a high-flow (April 2004).

2.3.6. Data analysis

For the transplantations, mortalities were compared between bioassays (reference, chronic and episodic exposure) and high- and low-flow using ANOVA and Tukeys pairwise comparisons. Two-sample T-tests were used to determine any significant increase or decrease in pH, EC and temperature over the two transplantation experiments (low-flow September 2003 versus high-flow April 2004). Prior to any analysis all macroinvertebrate data were $\log_{10}(x + 1)$ and EC and temperature \log_{10} transformed to homogenise variances.

2.4. RESULTS

2.4.1. Stream chemistry

As expected, chemistry differed substantially between streams and between flows (Table 1). pH, ANC and Ca^{2+} fell at all sites during high-flow, but most pronouncedly in the acidic stream where Al^{n+} increased substantially. Across the entire year, pH varied between stream types but not between replicate streams where changes were highly

synchronous (Figure 2). In general, circumneutral streams always had pH >5.9 while episodic streams fluctuated widely over the range 4.4 – 7.3. Acid streams had pH <5.5 in all months except October with the lowest values of all (pH 4 – 5) in the winter-spring months of November – May.

2.4.2. Variation in mayfly densities

Acid-sensitive mayfly species were present in both circumneutral streams, but none occurred in acid streams and only *B. rhodani* occurred in the two episodic streams (Table 2). In general variation in density across streams in each pair was highly synchronous. In all cases, peak densities occurred in the period between July and November and thereafter declining (Figure 3). This seasonal decline occurred in both episodic and circumneutral streams, and in itself provided no indication of episodic effects. However, *B. rhodani*, *B. muticus*, *R. semicolorata* and *H. lateralis* all had life cycles that meant nymphs were exposed to any increased discharge during the wetter months of November – April when the long-term risk of episodes was greatest (see Figure 3e). Despite persisting year-round in the circumneutral streams at >200 – 2000 individuals per square metre, *B. rhodani* was undetectable in the episodic CI 2 from December – April and in CI 5 during January – February. In each case, disappearance accompanied the lowest spot pH values recorded in these streams. On these grounds, *B. rhodani* was selected for investigation of episodic effects using transplantation experiments by virtue of its life cycle, possible exposure to acid episodes in CI 2 and CI 5, and the possibility that acid episodes might reduce densities during the autumn/winter period.

Table 1. Chemistry (pH, Al^{III}, Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, NO₃⁻, SO₄²⁻, DOC and ANC) of six streams (LI 1, LI 2, CI 2, CI 5, LI 6 and LI 7) from mid Wales during low-flow (August 2002) and high-flow (February 2003). All concentrations in mg L⁻¹, except ANC (µeq L⁻¹), Al^{III} (µg L⁻¹) and pH.

	Acidic				Episodic				Circumneutral			
	Low-flow		High-flow		Low-flow		High-flow		Low-flow		High-flow	
	LI 1	LI 2	LI 1	LI 2	CI 2	CI 5	CI 2	CI 5	LI 6	LI 7	LI 6	LI 7
pH	5.5	5.4	4.5	4.5	6.3	6.5	5.0	5.7	7.5	7.6	6.4	6.8
Al ^{III}	<10.0	20.0	490.0	510.0	<10.0	<10.0	80.0	20.0	<10.0	<10.0	<10.0	<10.0
Na ⁺	6.9	5.0	4.6	4.8	3.3	2.9	3.3	3.2	4.1	3.4	3.3	3.5
K ⁺	0.19	0.11	0.25	0.16	0.17	0.07	0.20	0.20	0.19	0.14	0.28	0.25
Ca ²⁺	2.9	1.5	0.7	0.8	1.6	1.7	0.9	2.1	8.8	8.7	1.8	3.0
Mg ²⁺	1.3	0.8	0.6	0.7	0.8	0.6	0.5	0.6	4.4	3.3	1.2	1.3
Cl ⁻	10.2	9.9	8.0	8.3	6.0	4.4	6.3	6.0	5.4	4.9	6.0	6.3
NO ₃ ⁻	0.34	0.10	0.51	0.37	0.10	0.08	0.25	0.45	0.01	0.05	0.13	0.15
SO ₄ ²⁻	5.8	5.2	4.7	5.1	2.0	3.3	2.3	3.7	3.4	4.7	3.4	3.5
DOC	2.4	2.4	1.8	1.8	7.5	3.3	2.3	1.4	0.8	1.4	1.2	1.1
ANC	126.4	-29.1	-64.8	-58.5	81.0	66.7	-3.8	17.8	767.3	619.5	86.5	157.6

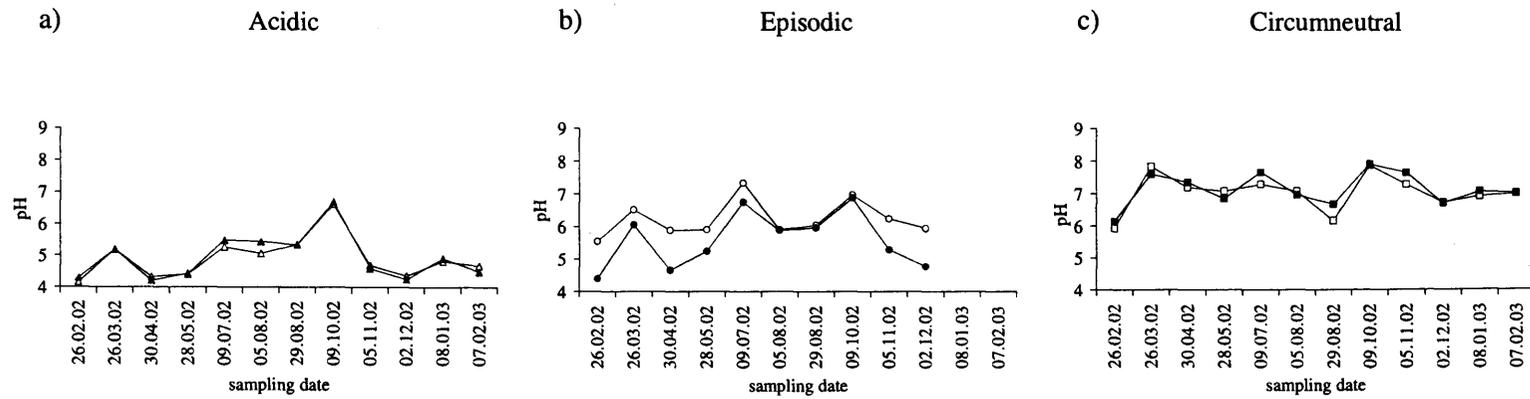
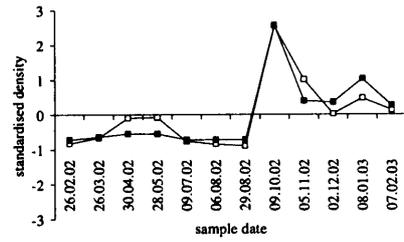


Figure 2. Change in pH over a 12 month period for a) acidic streams LI 1 (▲) and LI 2 (Δ), b) episodic streams CI 2 (●) and CI 5 (○) and c) circumneutral streams LI 6 (■) and LI 7 (□). Missing data at CI 2 and CI 5 (b) during the last two months was due to access problems as a result of weather

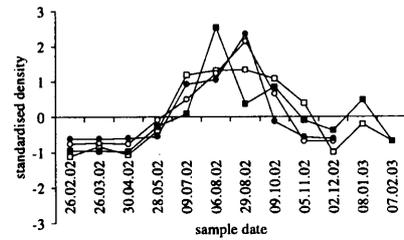
Table 2. Densities (m⁻²) of acid-sensitive mayflies (*Baetis muticus*, *B. rhodani*, *Heptagenia lateralis*, *Rhithrogena semicolorata* and *Ephemerella ignita*) over a 12 month period from circumneutral streams, LI 6 and LI 7 and episodic streams CI 2 and CI 5 (*B. rhodani* only, none of the other species occurred in these two streams during the 12 month sampling period).

Date	<i>Baetis muticus</i>		<i>Baetis rhodani</i>				<i>Heptagenia lateralis</i>		<i>Rhithrogena semicolorata</i>		<i>Ephemerella ignita</i>	
	LI 7	LI6	LI 7	LI6	CI 5	CI 2	LI 7	LI6	LI 7	LI6	LI 7	LI6
26.02.02	13	0	64	29	3	0	29	0	48	45	0	0
26.03.02	38	6	387	26	16	0	0	0	141	262	0	0
30.04.02	134	13	118	10	3	3	0	0	13	10	0	0
28.05.02	138	13	902	554	288	13	128	58	48	42	0	0
09.07.02	22	0	2730	819	528	317	122	51	3	3	224	234
06.08.02	10	0	2877	2694	835	342	86	26	0	0	122	61
29.08.02	0	0	2902	1037	1216	611	182	106	0	170	16	51
09.10.02	579	246	2608	1408	595	99	218	64	557	1443	0	0
05.11.02	320	83	1808	682	35	10	38	51	704	381	0	0
02.12.02	154	80	192	464	32	0	10	32	186	352	0	0
08.01.03	230	131	1120	1123	0	0	0	0	381	445	0	0
07.02.03	173	74	566	234	0	0	16	10	326	445	0	0

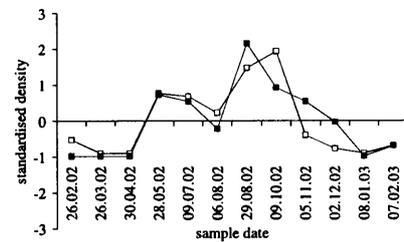
a) *Baetis muticus*



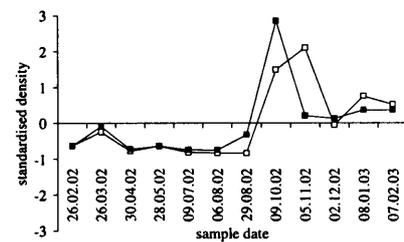
b) *Baetis rhodani*



c) *Heptagenia lateralis*



d) *Rhithrogena semicolorata*



e) *Ephemerella ignita*

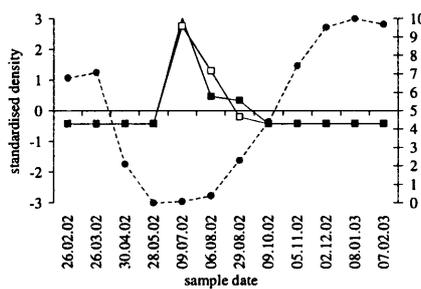


Figure 3. Variations in the density of mayflies over a 20 month period in the Llyn Brianne streams LI 6 (■), LI 7 (□), CI 2 (●) and CI 5 (○). Densities for each stream sampled have been standardised by subtraction of the mean and division by the standard deviation. The risk of acid episodes (long-term average daily discharge values divided by the lowest monthly discharge (May)) and then the relative difference from this low value scaled between 0 and 10 (= greatest risk) is shown on e) (●).

2.4.3. Stream chemistry during transplantation

In the two periods involved in the transplant experiments, pH, EC and temperature were highly significantly lower ($P < 0.001$) at high-flow during February 2002 than low-flow in August 2002 with the exception of EC in the acid streams (Table 3 and Figure 4). EC in LI 2 increased significantly ($P < 0.001$) during high-flow, but in LI 1 there was no significant change. Differences between the acid and reference streams in pH were particularly accentuated at high-flow (Figure 4).

Table 3. A comparison of low-flow (September 2003) and high-flow (April 2004) pH, electrical conductivity (EC) and stream-water temperature ($^{\circ}\text{C}$) taken during the transplantation experiments using two-sample t-tests within streams. T value is shown (d.f. = 12) and significance level ($P < 0.001$ *** and ns=not significant) with mean low- and high-flow respectively in parenthesis. See Figure 4 for source data.

	LI 7	LI 6	LI 2	LI 1
pH	11.82*** (7.4,6.2)	15.26*** (7.2,5.9)	18.13*** (5.8,3.8)	11.04*** (5.5,3.9)
EC	8.22*** (115,68)	14.79*** (106,49)	-6.22*** (55,63)	-0.31ns (58,59)
Temperature	9.47*** (12,8)	9.61*** (13,8)	9.51*** (12,7)	9.32*** (11,7)

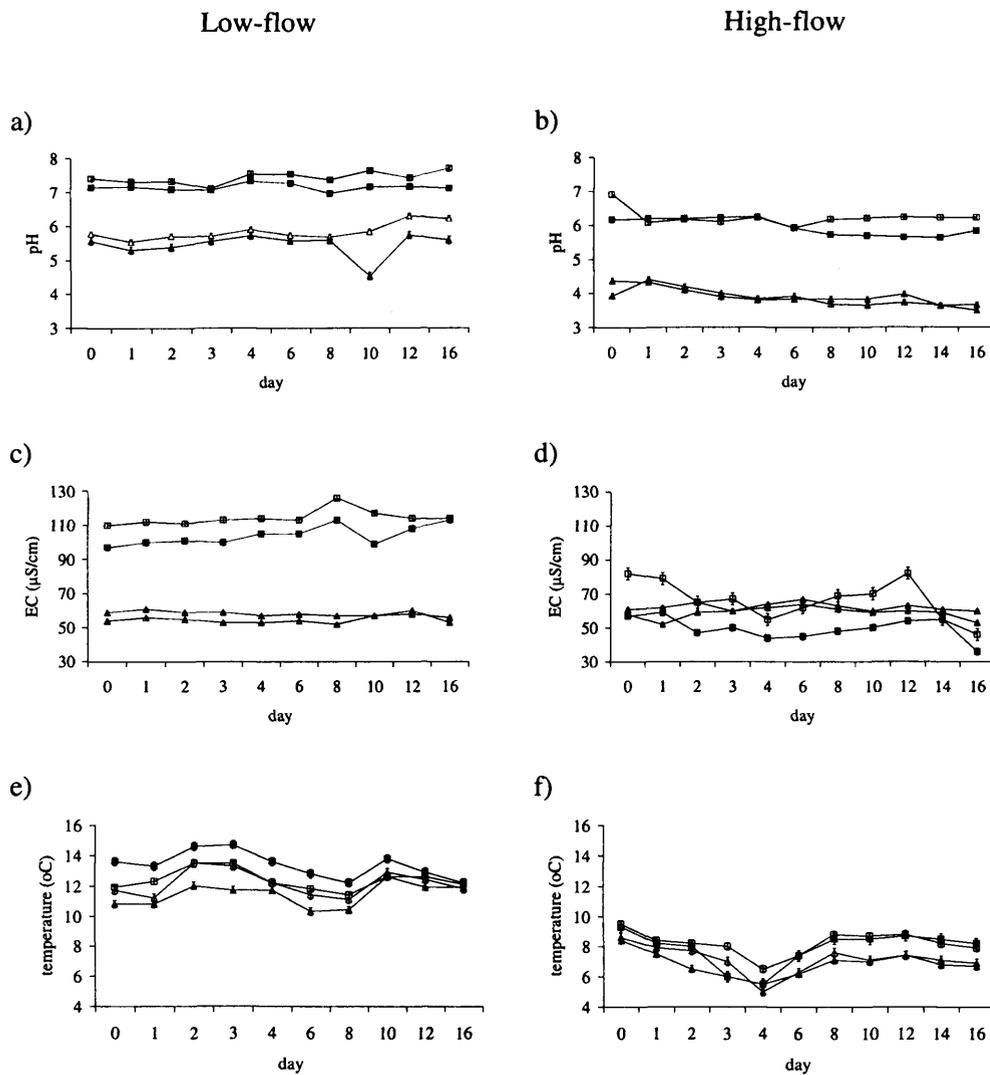


Figure 4. Changes in pH, electrical conductivity (EC) and stream-water temperature during transplantation experiments at low-flow (September 2003 - a, c and e) and high-flow (April 2004 - b, d and f) in the Llyn Brienne streams LI 7 (\square), LI 6 (\blacksquare), LI 2 (\triangle) and LI 1 (\blacktriangle). See Table 3 for analysis.

2.4.4. Survival during the transplantation experiments

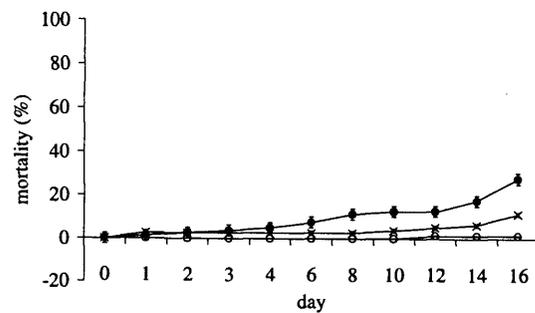
During the course of the experiments (high-flow and low-flow) mortality in the reference streams (LI 6 and LI 7) remained low and constant (Figure 5a and c). In both chronic and episodic exposure to acid streams during low-flow, mortality also remained <30% with small gradual increases in the latter part of the experiment. By contrast, mortality increased at high-flow in both episodic and chronic acid exposure (Figure 5b and d).

Overall, survival was significantly higher during low-flow (Table 4) in the reference circumneutral streams and both chronic and episodic bioassays ($F_{1,11} = 11.39$, $P < 0.05$). Survival, although moderately lower in the episodic and chronic exposure at low-flow was not significantly different from the reference sites ($F_{2,5} = 3.75$, $P = 0.153$). However, survival during high-flow was extremely low in the chronic compared to episodic exposure and reference sites ($F_{2,5} = 56.32$, $P < 0.01$). Survival was higher, nevertheless, in the reference streams than in the episodic exposure ($F_{1,11} = 17.57$, $P < 0.01$) but there was no significant flow*bioassay interaction ($F_{1,11} = 0.38$, $P = 0.70$).

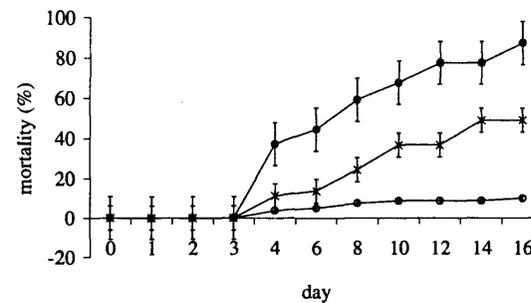
Table 4. Cumulative survival (%) for each bioassay (r = reference, c = chronic and e = episodic) across replicate sets of streams (LI 6/LI 1 and LI 7/LI 2) and for each transplantation experiment (low-flow during September 2003 and high-flow during April 2004).

Flow	LI 7(r)	LI 6(r)	LI 1(c)	LI 2(c)	LI1(e)	LI 2(e)
Low-flow	99%	91%	79%	72%	80%	89%
High-flow	90%	92%	20%	11%	57%	72%

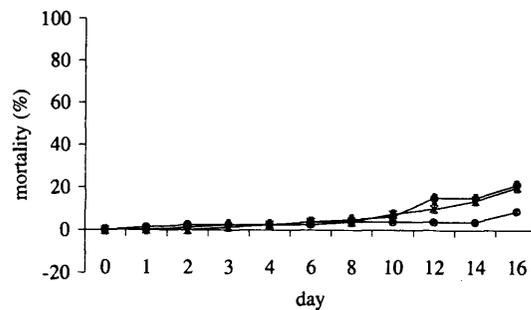
a) LI 7 and LI 2 (low-flow)



b) LI 7 and LI 2 (high-flow)



c) LI 6 and LI 1 (low-flow)



d) LI 6 and LI 1 (high-flow)

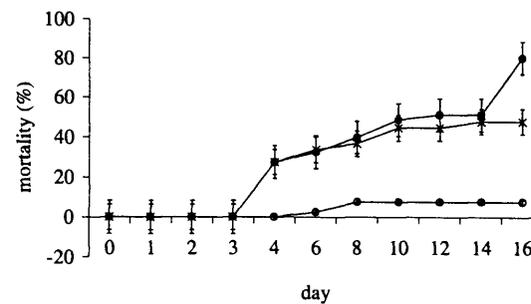


Figure 5. Cumulative (%) mortality of *Baetis* spp. in each of the transplantation experimental types at Llyn Brianne: chronic exposure (●), episodic exposure (x) and reference (○). See Figure 1 for experimental design.

2.5. DISCUSSION

2.5.1. *Acidification effects persist at Llyn Brianne*

These year-round data from streams at the experimental Llyn Brianne catchments confirm the previous observations well known from here and elsewhere that many nymphal Ephemeroptera are absent from acidic streams and scarce in episodic streams (Sutcliffe & Carrick, 1973; Weatherley & Ormerod, 1987). Typically, the threshold for presence/absence is mean pH 5.7 – 6.0, and all the species recorded in this study are affected: *E. ignita*, *B. rhodani*, *B. muticus*, *B. vernus*, *R. semicolorata*, *Ecdyonurus* spp. and *H. lateralis* (Wade *et al.*, 1989; Weatherley *et al.*, 1989). These effects have now persisted throughout 20 years of annual sampling at Llyn Brianne (1984 – 2004) despite the widespread chemical reversal of acidification increasingly apparent across large areas of Europe (Evans *et al.*, 2001). Moreover, because ephemeropterans are indicators of acid-base conditions affecting the entire invertebrate assemblage (Rutt *et al.*, 1990), at Llyn Brianne they represent limited biological recovery among a wider array of taxa – at least if recovery is defined as the re-establishment of extirpated, acid-sensitive species (Bradley & Ormerod, 2002b; Palmer *et al.*, 2005). So far, biological data on recovery from acidification elsewhere are fewer than hydrochemical data (Skjelkvale *et al.*, 2003), but those available from streams suggest that response has been partial, patchy or site-specific (Soulsby *et al.*, 1995; Tipping *et al.*, 2002; Yan *et al.*, 2003), even where chemical recovery has been accelerated by liming (Fjellheim & Raddum, 1992; Bradley, 2002). The ecological impacts of acidification are therefore still apparent in streams in many geographical locations.

While a range of hypotheses are developing to explain the apparent mismatch between biological and chemical recovery from acidification (Yan *et al.*, 2003; Ledger & Hildrew, in press), several recent papers imply that acid episodes still have biological impacts. Episodes have been implicated, for example in the limited recovery of migratory salmonids in Nova Scotia (Lacroix & Korman, 1996), in preventing recovery among invertebrates in Norwegian streams (Raddum *et al.*, 2001) and the Scottish Cairngorms (Gibbins *et al.*,

2001), in restricting the recovery of limed streams at Llyn Brienne (Bradley & Ormerod, 2002b), in nitrogen-driven effects on stream invertebrates in the Swiss Alps (Lepori *et al.*, 2003a, b) and in postulated effects that might arise from future climatic change (Skjelkvale *et al.*, 2003; Laudon *et al.*, 2004a). As with some of the earlier evidence, however, these continuing episodic effects are often inferred from toxicity, models, correlative data or speculation. The detection of real field impacts is more challenging – for example because of the difficulty of separating episodic effects from chronic acidification, or because populations and community-level responses cannot be quantified from weak inference alone (Weatherley & Ormerod, 1991; Lepori *et al.*, 2003a). In addition, there is now an additional need to understand and quantify when episodic effects still occur despite ameliorations in air and average water quality. In some regions, the contribution of anthropogenic acids to ANC loss at peak flow has decreased over recent decades and the area affected by acid spring floods has declined (Laudon & Bishop, 2002b). In other locations, episodes driven by strong acid anions still reduce pH and increase metal concentrations, particularly where soil base cations have been depleted (Campbell *et al.*, 2002; Lawrence, 2002; Armbruster *et al.*, 2003; Lepori *et al.*, 2003a; Laudon *et al.*, 2004a; Laudon *et al.*, 2004b; Wellington & Driscoll, 2004). A priority in acidification research is to link more closely this quantitative hydrochemical understanding about the current character of episodes with better evidence – rather than inference – about biological consequences.

In understanding any continuing biological effects of episodes, approaches that combine intra-annual sampling, risk assessment and field experiments have some value. Together, the data from this blend of methods at Llyn Brienne reveal four salient results: that ephemeropterans were absent from acid streams, and restricted to one species in previously limed streams now characterised by episodic acidification; that populations in episodic streams were transient – occurring during summer before being apparently extirpated when pH fell markedly in autumn or winter; that episodic exposure to acid conditions was sufficient to markedly increase mortality, but only at high-flow; and that several species overwinter as nymphs – coincident with increased risk of exposure to low pH events.

2.5.2. Toxicity during episodic exposure

The most unequivocal of all the data arose from transplantations, supporting the value of this method in detecting acidification effects (Hall *et al.*, 1988; Rosemond *et al.*, 1992; Gibbins *et al.*, 2001; Hirst *et al.*, 2004). Although the exposure of animals in cages inevitably involves artifacts, for example in preventing drift, removing access to refugia, or altering flow conditions (Kratz *et al.*, 1994; Hirst *et al.*, 2004), the results have greater realism than laboratory toxicity trials and they can aid the interpretation of field distribution. In this case, the mortality of *B. rhodani* increased significantly under chronic or episodic exposure to low pH: at low-flow and mean pH 5.5 – 5.8 in replicate acid streams, mortality nowhere exceeded 20 – 30% even with chronic exposure. By contrast, chronic high-flow exposure to pH 3.8 – 4.5 led to >80% mortality within 16 days; more importantly, over 40% of exposed individuals died even with episodic exposures of 2 x 4 days duration – roughly four times the mortality in circumneutral reference conditions. It is unfortunate that no directly contemporaneous data on metals were available, but the same experimental streams at Llyn Brianne during high-flow and pH <4.5 typically have >150 – 200 $\mu\text{g L}^{-1}$ of aluminium mostly in inorganic monomeric form (Ormerod *et al.*, 1989). Past experimental data show that *B. rhodani* is among the most acid-sensitive invertebrates to episodes with < pH 5.0 – 5.3 and exposure periods over 18 – 24h, when mortality invariably reaches values similar to those recorded here (Ormerod *et al.*, 1987; Merrett *et al.*, 1991). Many dead animals are lost through drift, and *Baetis* species from several regions show similar results. Moreover, mortalities in these conditions have typically been accompanied by depleted benthic densities or altered assemblage composition (e.g. Wales: Ormerod *et al.*, 1987; western N. America: Kratz *et al.*, 1994; eastern N. America: Hall, 1994; Switzerland: F. Lepori & S.J. Ormerod unpubl. data). It is tempting to conclude that these effects explain the autumn/winter reduction and loss of *B. rhodani* from the now episodic CI 5 and CI 2 following the sporadic reduction of pH to 4 – 5.5. Similar patterns have occurred previously in these streams, where *B. rhodani* is among those acid-sensitive taxa present only intermittently in spring samples (Rundle *et al.*, 1995; Bradley, 2002).

2.5.3. Likely life cycles and episodic exposure

Whereas the toxicological data from *B. rhodani* showed how this species is sensitive to even transient exposure to low pH, data on its apparent life cycle at Llyn Brianne illustrate why acid exposures could be a barrier to the permanent (i.e. year-round) recolonisation of episodically acid streams. Elsewhere in the UK, this species can have two generations per year of which one is completed to adulthood between April – August/September, possibly from eggs laid earlier in the same year. A second generation then over-winters as nymphs or, less likely, as eggs (Elliott *et al.*, 1988). This pattern appears to occur also at Llyn Brianne, where adults occur from March and April onwards with peak numbers in June – September (Masters, 2002). Although a facultative summer generation could colonise episodically acid streams during summer periods of low flow and increased pH, any overwintering nymphs would occur concurrently with increased flow, and hence increased likelihood of low pH (see Fig. 3). The raw density data for the two episodic streams discussed above – particularly CI 2 – reveal trends consistent with this interpretation: nymphs occurred in summer, but fell to zero during winter (see Table 2). Although intra-annual observations of density were collected over only one year, near-identical variations were apparent in previous data from these sites (Weatherley *et al.*, 1989).

As with *B. rhodani*, intra-annual density variations in other ephemeropteran species at Llyn Brianne followed expectations from their known life cycles. For example, in the UK, *B. muticus* is bivoltine, while *Rhithrogena semicolorata* and *Heptagenia lateralis* are univoltine, in all three cases with overwintering nymphs. *B. vernus* and *Ephemerella ignita* at upland sites are univoltine with overwintering eggs (Elliott *et al.*, 1988). These patterns raise interesting possibilities about the likely capacity of each of these species to colonise episodic streams. Assuming eggs could be laid by dispersing adults into acid or episodic streams (Masters, 2002; Peterson *et al.*, 2004), any acid-sensitive species with overwintering nymphs would be at risk of low winter pH. *B. vernus* and *E. ignita*, by contrast, have life cycles by which episodes would be less likely to limit distribution unless conditions were also toxic to eggs. This possibility requires investigation.

2.6. CONCLUSIONS

In combination, none of these data presented here refutes the hypothesis that acid episodes still restrict the recovery of acid-sensitive invertebrate in acidified streams at Llyn Brienne. This includes the limed, but now increasingly episodic sites appraised by Bradley & Ormerod (2002), who also provided evidence of continued episodic impacts. Thus, while subtle ecological effects might occur elsewhere as chemical recovery proceeds, for example due to restrictions on re-invasion because of competition from acid-tolerant species (Ledger & Hildrew, in press), at present hydrochemistry alone is sufficient at these sites to explain the pattern of occurrence of acid-sensitive mayflies. In subsequent chapters, I will assess the current chemical drivers of episodic acidification here and elsewhere, and assess possible consequences for stream organisms across a larger array of upland sites.

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**CHAPTER 3. ACID EPISODES RETARD THE BIOLOGICAL RECOVERY OF
UPLAND BRITISH STREAMS FROM ACIDIFICATION**

3.1. SUMMARY

1. Using data from 89 streams in Wales and Scotland, I tested two predictions required to support the hypothesis that anthropogenic acid episodes explain the continued absence of acid-sensitive species from sites otherwise recovering from acidification: i) that there should be differences in invertebrate composition between episodic and well-buffered streams and ii) that any such effects should differentiate sites where episodes are driven by anthropogenic acidification as opposed to base-cation dilution.
2. Chronic and episodically acid sites were widespread in both regions, and acidification during storm-flow overwhelmingly reflected acid titration more than dilution.
3. Chloride made up the bulk of the anion loading at all sites, but was mostly balanced by sodium. Otherwise, non-marine sulphate (16-18% vs 5-9%), and nitrate (4-6% vs 1-2%) contributed more to anion loading during episodes in Wales than Scotland, where organic acidity dominated. Reflecting current deposition quality, between one third (Scotland: 35-46%) and two-thirds (64-66%: Wales) of stream sulphate was from non-marine sources. Proportionate contributions to total sulphate in circumneutral streams everywhere declined at high flow by 10-15%, but were maintained or enhanced at episodic and acid sites.
4. Streams with episodes driven by strong anthropogenic acids had lower pH (5.0 ± 0.6) and more dissolved aluminium ($288 \mu\text{g L}^{-1} \pm 271$) during storm flow than sites where episodes were caused by dilution ($\text{pH } 5.4 \pm 0.6$; $116 \mu\text{g Al L}^{-1} \pm 110$) or those that remained circumneutral ($\text{pH } 6.7 \pm 1.0$; $50 \mu\text{g Al L}^{-1} \pm 45$).
5. Invertebrate data supported both the predictions: assemblages differed significantly among sites characterised by different episode chemistry while several acid-sensitive Ephemeroptera, Plecoptera and Coleoptera were scarce or absent only where episodes reflected anthropogenic acidification. However, other species (eg

Gammarus pulex) had reduced abundance even at naturally episodic sites so that effects require careful interpretation using supporting chemical data.

6. These data indicate that strong acid anions – and dominantly non-marine sulphate more than nitrate – still cause significant episodic acidification in acid sensitive areas of upland Britain. Episodic effects on stream organisms occur in spite of continued recovery in air and water quality and this may be a sufficient explanation for slow biological recovery in many locations.

3.2. INTRODUCTION

The acidification of sensitive freshwaters by acid precipitation is among the most intensively researched of all global change effects. At its peak during the 1970s and 1980s, large areas of Europe and North America were affected causing widespread changes in freshwater hydrochemistry, species' extirpation, altered ecological function and attendant economic damage (Muniz 1990). Although chemical data now show unequivocally that reversal is underway (Fowler *et al.*, 2001; Harriman *et al.*, 2001; Fölster & Wilander, 2002), evidence of any accompanying biological recovery is still patchy (Tipping *et al.* 2002). This mismatch is particularly marked in recovering streams and rivers - where acid-sensitive species often occur only sporadically or represent a fraction of taxa that were previously lost (Soulsby *et al.*, 1997; Bradley & Ormerod 2002a).

One of the major needs in acidification research is to explain the apparently non-convergent trends between chemical and biological recovery, and candidate hypotheses are now emerging (Yan *et al.* 2003; Ledger & Hildrew in press). One increasingly favoured is that acid-sensitive species are prevented from recolonising recovering streams because of the continued effects of acid episodes – transient periods of reduced pH generated during rainstorms or snowmelt over hours to weeks (Davies *et al.* 1992; Bradley & Ormerod 2002). By markedly increasing acidity and metal concentrations, episodes were considered key drivers of the initial biological effects of acidification, particularly in running waters. Episodes might still affect sensitive organisms despite increasing mean baseflow pH if conditions became ecotoxic at high flow – for example because strong acid anions led to the release of toxic metals such as aluminium. Antecedent deposition conditions, residual catchment pools of mobilisable anions and depleted base-cations in soils or runoff could all engender such effects (Stoddard *et al.* 1999; Alewell *et al.* 2001; Campbell & Eager 2002; Lawrence 2002).

A major recent advance in acidification research has been the development of methods to quantify contributions to acid episodes from strong anthropogenic acidity as opposed to natural organic acidity or base-cation dilution (Kahl *et al.* 1992; Wigington *et al.* 1992; 1996; Laudon & Bishop 1999; Bishop *et al.* 2000; Lepori *et al.* 2003a). These

methods have improved the understanding of chemical recovery on both sides of the Atlantic (Laudon & Bishop 2002; Laudon *et al.* 2002). They might also aid the interpretation of episodic effects on organisms (Lepori *et al.* 2003b), particularly in assessing whether anthropogenic contributions to acid runoff are still sufficient to damage biota. Data purporting to show continued episodic effects on organisms are few, mostly descriptive and provide no information about the causes, driving anions or wider ecological effects (Raddum *et al.* 2001; Bradley & Ormerod, 2002a; Kowalik & Ormerod ms submitted).

In this chapter I quantify the contributions to episodic acidification from base-cation dilution and strong acid addition in a large sample of streams across acid sensitive areas of Scotland and Wales. I also appraise consequences for aquatic invertebrates. The work was designed to test the hypothesis that anthropogenic acid episodes can explain the absence of acid-sensitive species from recovering streams and I made two predictions that would provide support. Firstly, continued episodic effects on assemblages should be accompanied by detectable differences in invertebrate assemblage composition between episodic and well-buffered streams (*prediction 1*). Second, any such effects should differentiate sites where episodes are driven by anthropogenic acidification as opposed to base-cation dilution (*prediction 2*).

The focus on aquatic invertebrate reflects their emphasis in previous acidification research, their richness and range of acid sensitivity (Wade *et al.* 1989), their life cycles and potential exposure to episodes in winter/spring (Lepori *et al.* 2003a; Kowalik & Ormerod submitted), their capacity for dispersal into recovering sites (Petersen *et al.* 2004) and their recognised value as indicator organisms (Rutt *et al.* 1990).

3.3. METHODS

3.3.1. *Sites and study areas*

Rather than determining chemical variations intensively at a small number of locations, the emphasis was on extensive comparisons across multiple sites of contrasting sensitivity to acidification and regions of contrasting acid deposition. The data were

purpose-collected from second- and third-order streams in the northwest Scottish Highlands (n = 24), Galloway in south-west Scotland (n = 25), north Wales (n = 19) and locations in central Wales centred on the Llyn Brianne experimental catchments (n = 21; Figure 1, Appendix 1). Site selection in Wales was largely restricted to landscapes expected to be acidified, while survey catchments in Galloway and the Scottish Highlands covered all landscape types. Exact site selection reflected a combination of logistics, vehicle access to low order streams and the availability of previous acidification research.

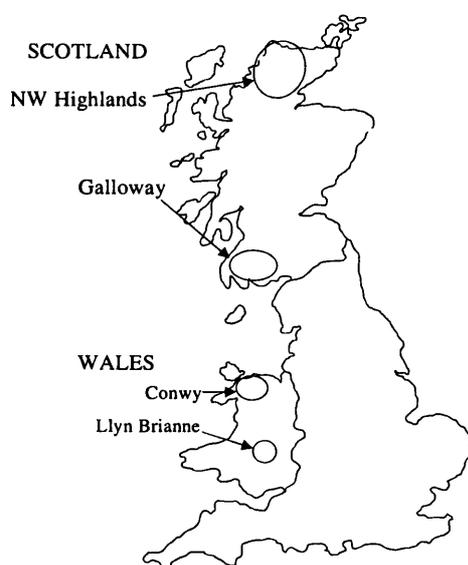


Figure 1. The survey regions in Scotland (NW Highlands and Galloway) and Wales (Conwy and Llyn Brianne).

All the target regions have been affected previously by acidification except the relatively more pristine Scottish Highlands. However, S and N deposition, and rainfall acidity, have declined since emissions peaked in the late 1970s (Fowler *et al.*, 2001). From estimates at adjacent representative locations (Conwy = 41, mid Wales = 26, Galloway = 55, and the NW Highlands = 98) in mostly standing waters (Hall *et al.*, 2004), critical loads for ANC ($<20 \mu\text{eq L}^{-1}$) are now exceeded in very few catchments in the NW Highlands (6%), and less than half or one third of those in either Wales

(30%) or Galloway (42%). Conditions across large parts of the study area are thus finely balanced towards recovery so that any episodic effects could be important.

3.3.2. *Field sampling*

Water samples were collected from each site during storm events (Galloway, October 2001; northwest Highlands, Conwy and mid Wales, January/February 2002) and at base-flow (northwest Highlands and Galloway, April 2002; Conwy and mid-Wales August 2002). This approach has been effective at characterising event chemistry in biological studies (Lepori *et al.* 2003a) and also avoids the difficulties of accurately determining mean or extreme chemistry at acid-sensitive sites from random sampling (Brewin *et al.* 1996). Samples were analysed for sodium, potassium, calcium, magnesium, ammonium, chloride, nitrate, sulphate, aluminium and dissolved organic carbon (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , NH_4^+ , Cl^- , NO_3^- , SO_4^{2-} , Al^{3+} , DOC). Alkalinity was determined by Gran titration, anions by ion chromatography (Dionex), and base cations and metals by Atomic Absorption Spectrophotometry (AAS) (Perkin Elmer) after filtration on-site at 0.45- μm into nitric acid fixative. Dissolved organic carbon was determined by continuous flow colorimetry with UV digestion (Skalar autoanalyser System) and Al colorimetrically using pyrocatechol violet (Dougan & Wilson, 1974). pH was recorded on-site using a hand-held combined pH/EC/TDS meter with an electrode specially designed for low ionic-strengths (Hanna Instruments HI 991300). Charge-balance acid neutralising capacity (ANC) was calculated as the sum of base cations minus the sum of acid anions ($\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+} - \text{NO}_3^- - \text{SO}_4^{2-} - \text{Cl}^-$). Non-marine sulphate (i.e. excess $\text{SO}_4 = \text{xSO}_4$) was determined by subtracting marine SO_4 (mSO_4) from total SO_4 based on the ratio of SO_4 to Cl^- in seawater (when computed in equivalents = 0.104).

Invertebrates were collected during April 2002 from all sites using semi-quantitative kick-samples (standard net with 1 mm mesh size) of two minutes duration in riffles and one-minute in margins. This strategy samples all major stream habitats and is a well-calibrated method sufficient to detect differences between sites reliably (Weatherly & Ormerod, 1987; Bradley & Ormerod 2002b). Samples were preserved on-site by adding 100% IMS (Industrial Methylated Spirit) to the existing sample volume. In the

laboratory, samples were sorted initially by rinsing through a 500 µm sieve, immersing in fresh water and removing all macroinvertebrates by hand. The sorted samples were then preserved in 70% IMS and identified to species or genus for the orders Plecoptera, Ephemeroptera, Trichoptera and Coleoptera, and family for Simuliidae, Chironomidae and Tipulidae.

3.3.3. Chemical data analysis

Initially, streams were categorised into those with base-flow pH < 5.7 (= acidic), pH 5.7-7 (episodic) and pH > 7 (circumneutral). Upland British streams with base-flow pH ≤ 7 usually have minimum pH < 5.7, previously recognised as a threshold at which many acid sensitive taxa become scarce (Sutcliffe & Carrick 1973; Weatherley & Ormerod 1991). Mean values for major acid-base determinands at high and low flow were calculated for each of these stream categories.

I also assessed variations among regions or stream groups in the calculated loss of alkalinity due to base-cation dilution, and in the percentage contribution of major acid anions to any titration effects. For this I used Kruskal Wallis tests. These additional analyses were carried out using data derived from the ionic concentrations in µeq L⁻¹ of alkalinity (Alk), the cations NH₄⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, Alⁿ⁺ and the anions Cl⁻, NO₃⁻, SO₄²⁻ (including both marine and excess SO₄²⁻) and organic acids (OA⁻). For calculations, aluminium was given a charge of 2⁺ (Sullivan *et al.*, 1989) while OA⁻ were estimated using a value of 5 µeq mg⁻¹ of DOC, the approximate amount expected to act as strong organic acids (Munson & Gherini, 1993; Bishop, 1996).

The overall percentage loss of alkalinity attributable to dilution for the measured events at each site relative to base-flow was determined after (Kahl *et al.*, 1992):

$$\text{Alk dilution} = \frac{((\Sigma\text{BC}_{\text{low}} - \Sigma\text{BC}_{\text{high}}) / \Sigma\text{BC}_{\text{low}}) \text{Alk}_{\text{low}}}{(\text{Alk}_{\text{low}} - \text{Alk}_{\text{high}})}$$

(Equation 1)

Based on their common origin in weathering, this procedure assumes that total base cations should dilute in proportion to alkalinity (mostly bicarbonate) during storm events. While small discrepancies are possible due to exchange between base cations and protons in surface soils during events (Bishop *et al.* 2000), values for dilution substantially less than 100% indicate the addition of strong acids from deposition or catchment processes, and hence titration effects. The ratio between alkalinity and the sum of BC ($\text{alkalinity}/\sum\text{BC}$), set with a lower limit of zero, was used as a measure of the relative importance of titration (Kahl *et al.*, 1992).

The relative contributions of acid anions (NO_3^- , mSO_4^{2-} , xSO_4^{2-} and OA) to any titration effects during storm-flow were estimated from their proportionate contribution to anion loading at each site ($\text{anion}/\sum\text{AA}$). Since the sum of anions should equal the sum of cations (with minor errors due to missing contributions from minor ionic species), these relative contributions are usually calculated as $\text{anion}/\sum\text{BC}$ because cations can be determined analytically with more certainty (Kahl *et al.*, 1992; Lepori *et al.* 2003a).

As much of the work was carried out in the relatively maritime location of western Britain, it was expected that Cl^- would contribute substantially to anion loading at each site but to be balanced by Na^+ rather than base cations. This was assumption was verified from a biplot of ionic concentrations across sites (Fig. 2). Sites with markedly elevated chloride, and hence those possibly at risk of sea-salt episodes, were overwhelmingly in coastal locations and they were always circumneutral.

Once the chemistry of episodic acidification was known at each site, the proportionate contribution to anion loadings from non-marine sulphate ($\text{xSO}_4/\sum\text{AA}$) were plotted against the percentage of alkalinity loss at high flow due to dilution (from equation 1). This identified groups of sites where episodic acidification was driven mostly by elevated non-marine sulphate with minor base cation dilution (Group 1 sites) or mostly by dilution alone (Group 3 sites). Intermediate sites (Group 2) had episodic acidification driven by small amounts of base-cation dilution with elevated concentrations of anions other than sulphate (mostly organic acids). I compared acid-base determinands between these groups using oneway Analysis of Variance (ANOVA) after appropriate transformations to homogenise variances. These same

groups were also used in comparisons of invertebrate assemblages across sites (see below).

3.3.4. *Invertebrate data analysis*

I analysed data from the whole macroinvertebrate assemblages and from individual taxa summed from both marginal and riffle samples. Assemblages were first ordinated in CANOCO using detrended correspondence analysis (DCA), one of the most established of all ordination methods (ter Braak & Smilauer, 1998). DCA produces an objective ordination of samples along orthogonal axes that reflect similarity in taxonomic composition without being artificially constrained to fit any environmental variable. Differences between sites in scores on each axis thus reflect differences solely in assemblage composition. To test predictions 1 and 2 (see introduction), I assessed variations in DCA scores between streams characterised by contrasting episode chemistry (Groups 1-3: see above) using one-way ANOVA with Tukey-Kramer range tests. I also assessed differences from well-buffered circumneutral streams (= Group 4). I evaluated trends on the first two DCA axes with major acid-base determinands, including alkalinity loss due to dilution and $x\text{SO}_4/\sum\text{AA}$, using Pearson correlation coefficients.

Variations in abundance were appraised for a range of common species whose sensitivity to acidity is known from previous data (Weatherley & Ormerod 1987; Wade *et al.* 1989; Rutt *et al.* 1990). These were:

- i) relatively acid-tolerant species from the Plecoptera (*Chloroperla torrentium* (Pictet), *Leuctra hippopus* (Kempny), *L. inermis* Kempny, *L. nigra* (Olivier), *Nemurella picteti* Klapálek and *Amphinemura sulcicollis* (Stephens)) and the Trichoptera (*Plectrocnemia conspersa* (Curtis) and *Rhyacophila dorsalis* (Curtis));
- ii) acid-sensitive taxa from the Ephemeroptera (*Baetis muticus* (L.), *B. rhodani* (Pictet), *Ecdyonurus* spp. Eaton, *Rhithrogena semicolorata* (Curtis) and *Heptagenia lateralis* (Curtis)), the Plecoptera *Isoperla grammatica* (Poda), and four other acid-sensitive species (the trichoptera *Hydropsyche siltalaii* Döhler, the coleopterans

Hydraena gracilis Germar and *Elmis aenea* (Müller), and the crustacean *Gammarus pulex* (L.).

I assessed variations among stream groups (i.e. Groups 1-4) with contrasting episode chemistry using ANOVA as above. Abundance data were first $\log_{10}(x + 1)$ transformed to homogenise variances. All invertebrate analyses were made on data pooled across regions since parallel investigations discounted the possibility that regional and acid-base effects on invertebrates were confounded (R. A. Kowalik & S. J. Ormerod, unpubl. data).

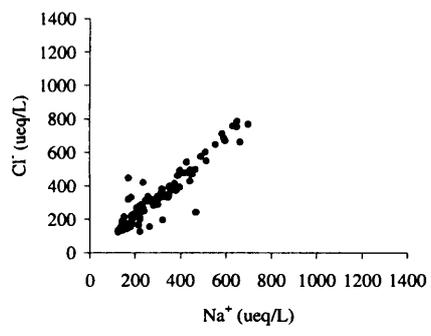
3.4. RESULTS

3.4.1. Causes and chemistry of episodes

Streams categorised initially as acidic or episodic comprised over 50% of the Scottish sample and almost 90% of the Welsh sample (where site selection was not random). In general, mean alkalinity and mean pH were similar within these groups across regions during both base-flow and storm-flow, when highly acid conditions were widespread (Table 1; Fig. 3). A small number of Scottish circumneutral streams with baseflow pH > 7 also had pH < 5.7 during storm-flow (n = 5).

The percentage loss of alkalinity during episodes that could be attributed to base-cation dilution was considerably less on average in Wales (median 7%) than in Scotland (median 43%; Kruskal Wallis test, $H_{1,88} = 17.21$, $P < 0.001$). Base cation dilution increased significantly in the order acidic streams < episodic < circumneutral in both Wales (medians: acidic = 2%; episodic = 8% and circumneutral = 70%; Kruskal Wallis test, $H_{2,38} = 11.79$, $P < 0.001$) and Scotland (medians: acidic = 0.6%; episodic = 31% and circumneutral = 63%; Kruskal Wallis test, $H_{2,47} = 17.81$, $P < 0.001$). Thus, episodic acidification in most episodic and acidic streams in all regions was dominated by titration.

a) Base-flow



b) High-flow

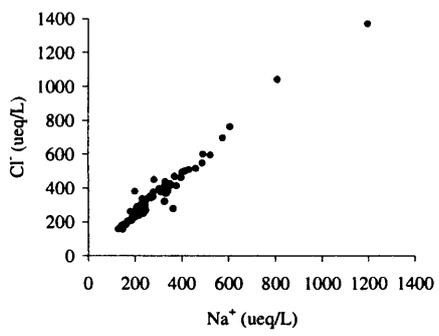


Figure 2. Ionic concentrations of chloride and sodium in streams in the Welsh and Scottish study streams during base-flow and storm-flow.

Table 1. Mean (\pm SD) pH, alkalinity ($\mu\text{eq L}^{-1}$), $\text{Al}^{\text{n}+}$ ($\mu\text{g L}^{-1}$) and DOC (mg L^{-1}) at base-flow and storm-flow of streams in Scotland and Wales grouped by base-flow pH (*acidic* <5.7 ; *episodic* >5.7 - <7.0 and *circumneutral* >7.0). Sample sizes in each group are given in Table 2.

Determinand	Stream-type	Wales		Scotland	
		Base-flow	Storm-flow	Base-flow	Storm-flow
pH	<i>Acidic</i>	5.3 (0.3)	4.7 (0.3)	5.4 (0.3)	4.5 (0.4)
	<i>Episodic</i>	6.3 (0.2)	5.1 (0.4)	6.7 (0.2)	5.2 (0.6)
	<i>Circumneutral</i>	7.4 (0.3)	6.7 (0.2)	7.5 (0.4)	6.7 (1.1)
Alkalinity	<i>Acidic</i>	27 (27)	-22 (15)	11 (16)	-25 (24)
	<i>Episodic</i>	109 (89)	4 (39)	153 (101)	3 (36)
	<i>Circumneutral</i>	902 (444)	308 (250)	908 (850)	428 (634)
$\text{Al}^{\text{n}+}$	<i>Acidic</i>	50 (51)	247 (195)	193 (171)	293 (340)
	<i>Episodic</i>	9 (3)	122 (114)	57 (52)	109 (102)
	<i>Circumneutral</i>	7 (3)	50 (37)	28 (26)	50 (47)
DOC	<i>Acidic</i>	7.4 (6.5)	4.2 (2.9)	7.4 (5.9)	9.1 (5.6)
	<i>Episodic</i>	5.3 (3.8)	3.3 (2.4)	5.9 (4.2)	7.0 (4.4)
	<i>Circumneutral</i>	3.6 (2.4)	5.0 (3.7)	4.9 (4.7)	6.1 (5.4)

As expected, most of the anion load was due to Cl^- during storm-flow in both regions (Wales 72% vs Scotland 78%), but was balanced by Na^+ rather than base cations (Figure 2). Otherwise, acid anions differed considerably between regions with sulphate ($H_{2,87} = 64.8$ $P < 0.001$), excess sulphate ($H_{2,87} = P < 0.001$) and nitrate ($H_{2,87} = 12.6$ $P < 0.001$) all contributing more to events in Wales than Scotland irrespective of stream types (Table 2; Figure 4). Probably as a consequence, $\text{Al}^{\text{n}+}$ concentrations in Wales increased between base-flow and storm-flow by 5-10X compared to a 2X increase in Scotland (Table 1). The reverse trend was true for DOC, which increased during storm flow in Scotland to concentrations around twice those in Wales ($F_{2,88} = 10.96$, $P < 0.001$). In keeping with this pattern, organic acids were at least as important as excess sulphate in the acid anion load of acidic and episodic Scottish streams during storm flow and varied significantly from circumneutral streams (Table 2; $H_{2,47} = 7.8$, $P < 0.05$).

Table 2. Percentage contributions (mean with range in parentheses) of acid anions to anion loading during storm-flow in Welsh and Scottish streams.

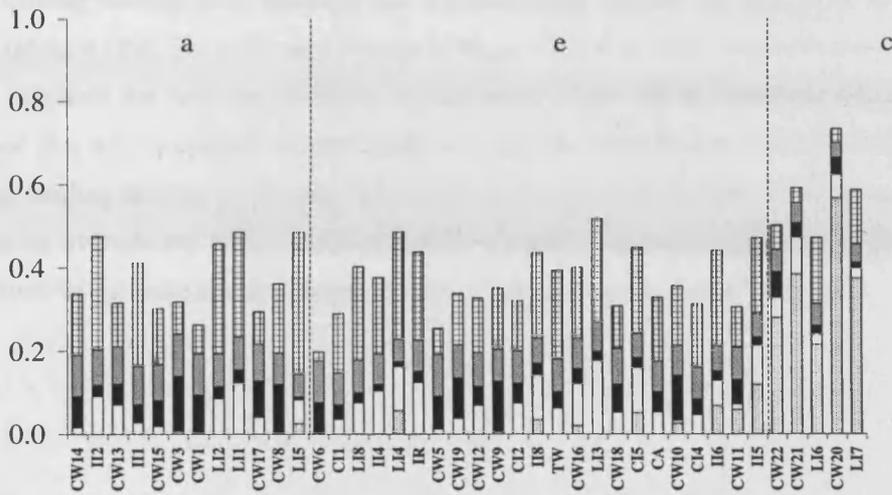
a) Wales

Anion fraction	All sites (n=40)	Acidic (n=12)	Episodic (n=23)	Circumneutral (n=5)
SO ₄ ²⁻	23% (7-39)	27% (17-39)	24% (12-32)	13% (7-21)
xSO ₄	16% (2-34)	18% (7-34)	16% (2-26)	8% (3-16)
NO ₃ ⁻	6% (0-18)	4% (0-11)	6% (0-16)	5% (3-9)
OA	5% (1-13)	6% (1-13)	5% (1-12)	3% (1-6)

b) Scotland

Anion fraction	All sites (n=49)	Acidic (n=6)	Episodic (n=19)	Circumneutral (n=24)
SO ₄ ²⁻	12% (4-30)	18% (9-30)	13% (9-23)	9% (4-18)
xSO ₄	5% (0-22)	9% (0-22)	5% (0-15)	4% (0-13)
NO ₃ ⁻	3% (0-17)	4% (0-15)	3% (0-13)	3% (0-17)
OA	8% (0-37)	9% (2-16)	9% (1-26)	6% (0-36)

a) Wales



b) Scotland

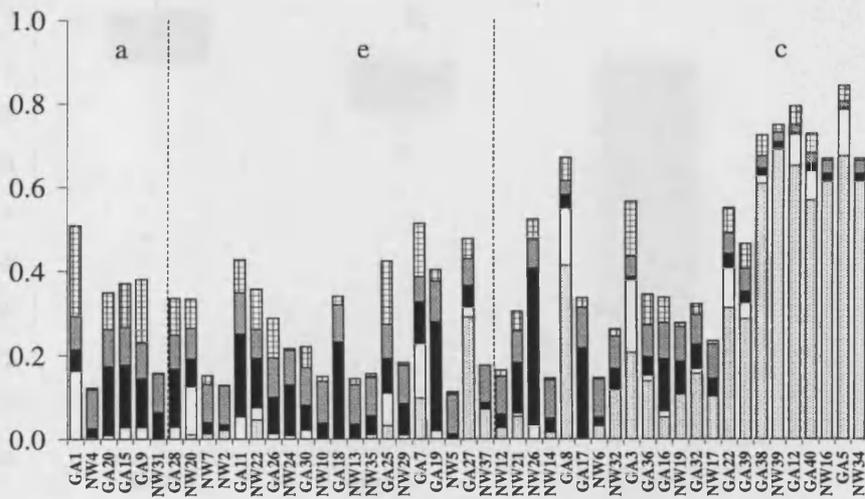


Figure 4. Contributions of base cations ($\text{Alk}/\Sigma\text{BC}$) and anion fractions ($\text{anion}/\Sigma\text{AA}$) to storm-flow chemistry in a) Wales ($n=40$) and b) Scotland ($n=49$). The y axis represents the proportions of BC (□), xSO_4 (▤), mSO_4^{2-} (▥), NO_3^- (□) and OA^- (■). Streams are ordered by increasing base-flow pH (a = acidic <5.7 , e = episodic $>5.7-7.0$ and c = circumneutral >7.0).

Other comparisons showed that total sulphate varied significantly in contribution to anion loading among acid, episodic and circumneutral streams at high flow in both Wales ($H_{2,38} = 12.8$, $P < 0.01$) and Scotland ($H_{2,47} = 7.2$, $P < 0.05$). Contributions from excess sulphate did not vary formally among these stream types, but there was other evidence of a role in episodic acidification: whereas the contribution of excess sulphate to anion loading fell due to dilution between base-flow and storm-flow in circumneutral streams on average by 10% (Scotland) to 15% (Wales), contributions to anion load at storm-flow in episodic and acid streams were maintained or increased (Figure 5).

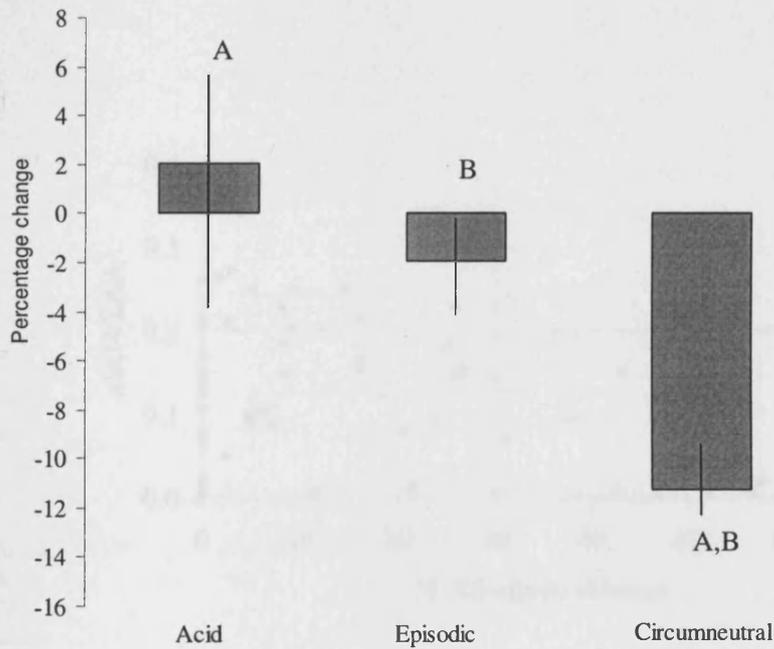


Figure 5. Mean percentage change (\pm SE) in the contribution of excess sulphate to anion loading in all acid, episodic and circumneutral streams between base-flow and storm-flow ($F_{2,87} = 5.04$, $P < 0.001$; A,B indicate significant pairwise differences according to Tukey-Kramer range tests).

As anticipated from the methods, a plot of $x\text{SO}_4/\Sigma\text{AA}$ against the percentage of alkalinity lost through dilution effectively separated sites at which episodes were driven by non-marine sulphate ($x\text{SO}_4^{2+}$ = Group 1) from those where episodes reflected other anions (e.g. mostly organic acids = Group 2) and those where episodic acidification reflected mostly dilution (Group 3; Figure 6). Acid-base chemistry varied progressively and significantly among these groups. For example, pH and ANC declined from Group 4 to Group 1 and, on average, ANC only fell below zero in the latter. Al^{n+} concentrations were significantly elevated in the acid-anion rich Groups 1 and 2 whereas organic acids, by contrast, were markedly elevated in Groups 2 and 3 (Table 3). Sulphate and nitrate were both significantly elevated in group 1 streams by comparison with other episodic streams.

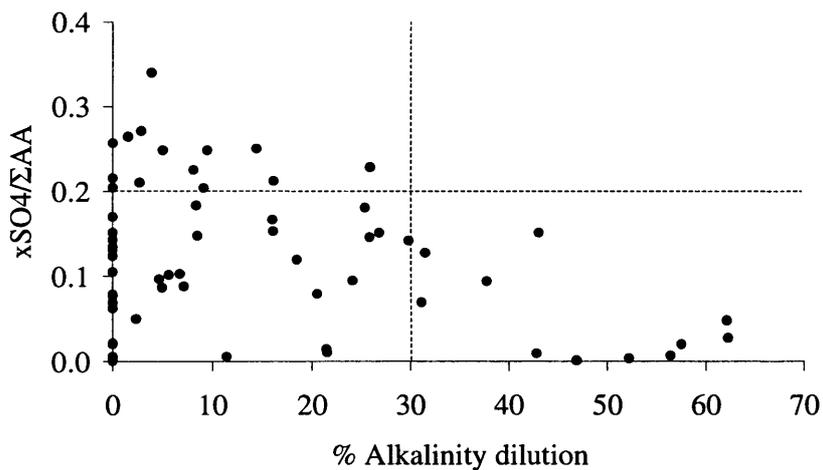


Figure 6. Contributions to anion loading due to sulphate during storm-flow in each stream plotted against percentage contributions by base cation dilution to alkalinity loss during episodes. Only episodic and acidic streams are plotted. (Group 1: $\text{SO}_4/\Sigma\text{AA} > 0.20$, percentage alkalinity loss through dilution $< 30\%$; Group 2: $\text{SO}_4/\Sigma\text{AA} < 0.20$, dilution $< 30\%$; Group 3: $\text{SO}_4/\Sigma\text{AA} < 0.20$, dilution $> 30\%$).

Wales

Scotland

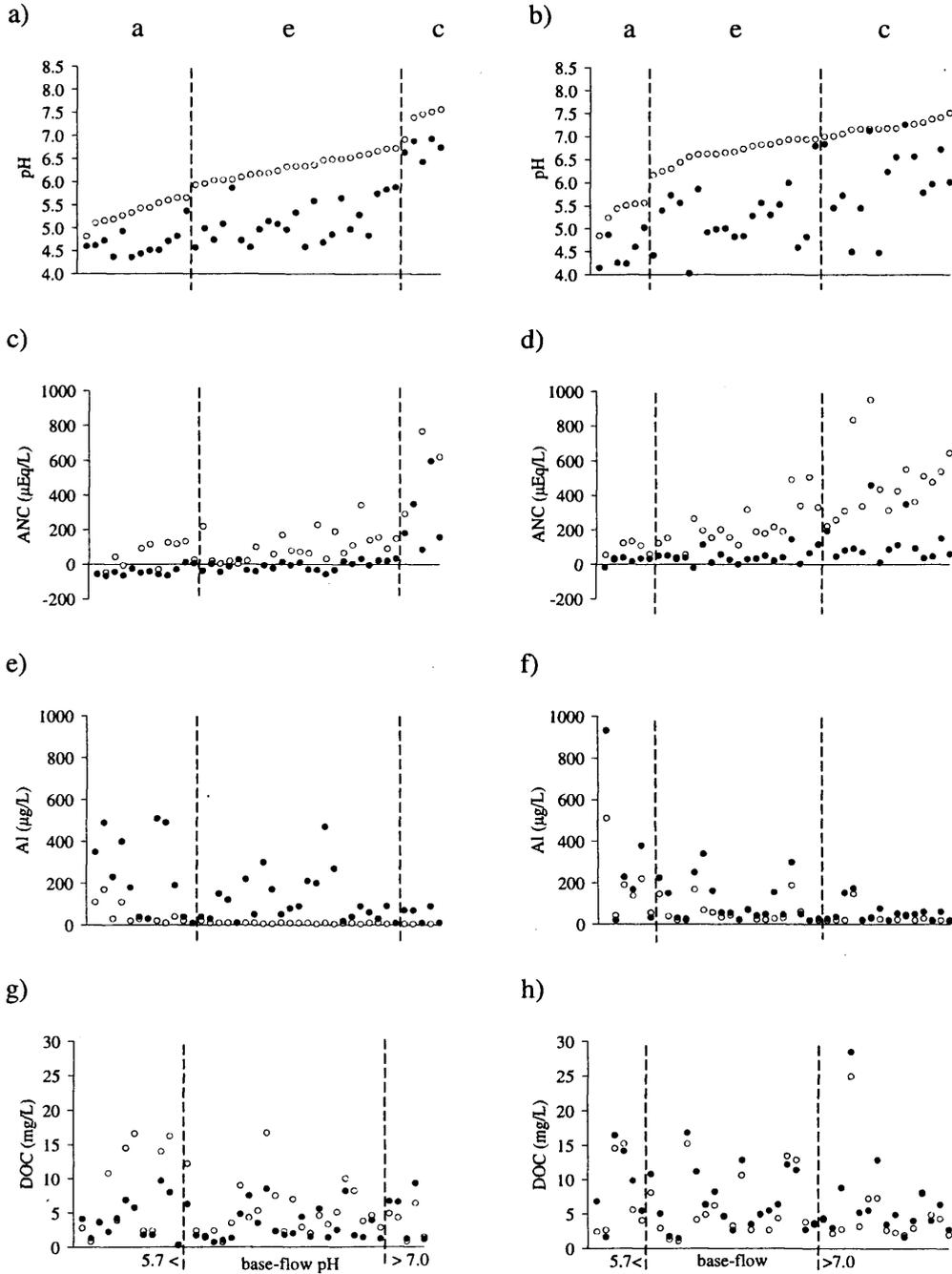


Figure 3. Key acid-base determinands for Wales and Scotland at storm-flow (●) and base-flow (○). Streams are arranged in increasing order of baseflow pH and acid (a), episodic (e) and circumneutral (c) sites are separated by dashed lines.

3.4.2. Variation among invertebrates

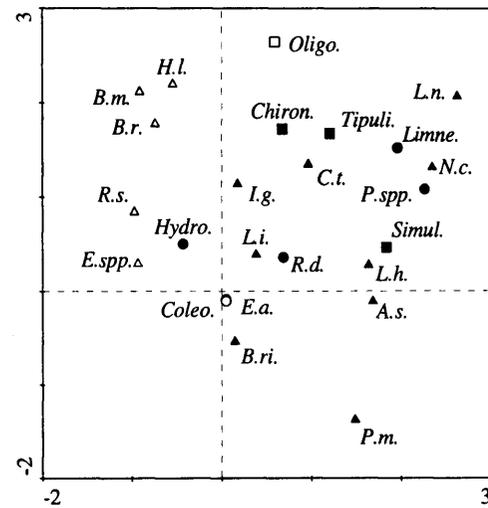
Most variation in invertebrate assemblages composition across all sites occurred along one DCA axis representing a turnover in species composition of c 50% (=2.2 standard deviations length; Fig. 7). Overwhelmingly, site distribution along this axis reflected acid-base status, with ordination scores highly correlated with storm-flow pH ($r = -0.75$, $P < 0.001$), ANC ($r = -0.61$, $P < 0.001$), aluminium ($r = 0.55$, $P < 0.001$) and the percentage of anion loading contributed by non-marine sulphate ($r = 0.56$, $P < 0.001$). Verifying the selection of acid-tolerant and acid-sensitive species, taxa associated with low-scoring (circumneutral) streams included a range of ephemeropterans (eg *Baetis rhodani*, *Baetis muticus*, *Rhithrogena semicolorata*) while species at acid sites included acid tolerant plecopterans (*Leuctra nigra*, *Nemoura cambrica*) and trichopterans (*Plectrocnemia* spp.).

Ordination scores (i.e. assemblage composition), differed highly significantly among stream groups defined from episode chemistry (Table 3). Although there was some overlap, mean DCA axis 1 scores declined progressively from circumneutral streams (Group 4) to those where episodes were driven predominantly by non-marine sulphate (Group 1; Table 3; Fig. 7). Individual species' abundances also followed episode chemistry closely. Among acid-tolerant taxa, *Plectrocnemia conspersa* was significantly more abundant at sites where episodes were driven by non-marine sulphate than by other effects or at circumneutral sites (Table 3). In contrast, all acid sensitive species were at significantly reduced abundance in the episodic groups by comparison with circumneutral sites. In particular, *Heptagenia lateralis*, *Rhithrogena semicolorata*, *Isoperla grammatica* and the two beetles *Elmis aenea* and *Hydraena gracilis* were significantly reduced at sites affected by strong acidity in Group 1. Most striking of all, only three of the nine acid-sensitive species analysed occurred anywhere where episodes were driven by non-marine sulphate (Group 1), but all but one occurred where episodes were driven mostly by dilution.

Table 3. Differences between streams grouped according to episode chemistry (see Figure 6) in the abundance of individual benthic invertebrate species; assemblage composition as shown by ordination scores; and the concentrations of key acid-base determinands. Variations were identified from one-way ANOVA after appropriate transformation ($P < 0.05^*$, $< 0.01^{**}$, $< 0.001^{***}$ and ns = not significant) with pair-wise differences shown by similar superscripts (Tukey-Kramer range tests). All values are means (\pm SD). Group 1 streams had episodes driven mostly by anthropogenic sulphate; Group 2 largely by organic acidity and Group 3 by base cation dilution. Group 4 streams were all circumneutral.

Groups	1 (n = 14)	2 (n = 35)	3 (n = 11)	4 (n=29)	F _{3,86}
Acid-tolerant species:					
<i>Chloroperla torrentium</i>	28(34)	23(30)	23(36)	21(21)	0.38ns
<i>Leuctra hippopus</i>	3(6)	7(23)	7(6)	5(6)	1.22ns
<i>L. inermis</i>	40(95)	25(34)	10(18)	65(87)	2.64ns
<i>Amphinemura sulcicollis</i>	146(162)	70(142)	22(34)	26(37)	1.83ns
<i>Nemoura cambrica</i>	15(47)	2(4)	3(6)	5(15)	1.09ns
<i>Plectrocnemia conspersa</i>	20(17) ^{AB}	7(8) ^A	13(19)	4(5) ^B	7.24**
<i>Rhyacophila dorsalis</i>	1(2)	1(2)	2(3)	2(4)	2.15ns
Acid-sensitive species:					
<i>Isoperla grammatica</i>	1(4) ^{AB}	8(12)	10(11) ^A	14(23) ^B	3.60*
<i>Hydropsyche</i> spp	2(4) ^A	1(1) ^{BC}	9(13) ^B	11(24) ^{AC}	7.78***
<i>Baetis muticus</i>	0(0) ^A	0(1) ^B	1(2) ^C	37(57) ^{ABC}	27.17***
<i>B. rhodani</i>	3(8) ^A	7(23) ^B	2(5) ^C	135(252) ^{ABC}	29.12***
<i>Heptagenia lateralis</i>	0(1) ^A	3(9)	1(2)	11(29) ^A	4.24**
<i>Rhithrogena</i> <i>semicolorata</i>	0(0) ^A	0(1)	7(21)	42(71) ^A	24.92***
<i>Hydraena gracilis</i>	0(0) ^A	0(1)	6(18)	5(11) ^A	7.78***
<i>Elmis aenea</i>	0(0) ^A	2(7)	7(12)	14(52) ^A	4.94**
<i>Gammarus pulex</i>	0(0) ^A	0(1) ^B	0(0) ^C	38(89) ^{ABC}	8.76***
DCA Axis 1 Score	1.61(0.38) ^A	1.21(0.53) ^B	1.06(0.51) ^C	0.40(0.25) ^{ABC}	30.64***
Determinand:					
Al ^{III} ($\mu\text{g L}^{-1}$)	288(271) ^A	123(105) ^B	116(110)	50(45) ^{AB}	6.23**
pH	5.0(0.6) ^A	5.0(0.4) ^B	5.4(0.6) ^C	6.7(1.0) ^{ABC}	39.46***
OA (mg L^{-1})	9(8) ^{ABC}	28(21) ^A	40(17) ^B	29(25) ^C	12.20***
ANC (ueq L^{-1})	-25(36) ^A	1(36) ^B	61(45) ^C	473(635) ^{ABC}	25.83***
$\cdot\text{NO}_3^-$ (mg L^{-1})	0.5(0.4) ^{AB}	0.2(0.2) ^A	0.2(0.4) ^B	0.7(1.3)	3.63*
SO_4^{2-} (mg L^{-1})	4.1(1.2) ^{ABC}	2.0(1.2) ^{AD}	0.9(0.4) ^{BDE}	1.7(1.1) ^{CE}	28.53***
xSO_4^{2-} (mg L^{-1})	3.8(1.2) ^{ABC}	1.7(1.0) ^A	0.5(1.3) ^B	1.4(2.1) ^C	5.07**

a) Species



b) Streams

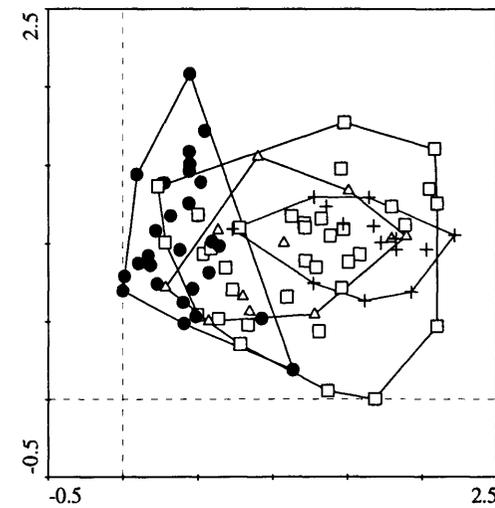


Figure 7. DCA ordination of a) major species (qualification > 20% weight) and b) streams across all regions. Streams are grouped 1-4 according to Figure 5 (Group 1 = +: sites with episodes driven mostly by sulphate; Group 2 = □: sites with episodes driven mostly by organic acids; Group 3 = Δ: sites with episodes driven mostly by dilution; Group 4 = ●: circumneutral sites).

Species key: Plecoptera (▲) A.s. *Amphinemura sulcicollis*, B.ri. *Brachyptera risi*, C.t. *Cholorperla torrentium*, I.g. *Isoperla grammatica*, L.h. *Leuctra hippopus*, L.i. *L. inermis*; L.n. *L. nigra* and N.c. *Nemoura cambrica*; Ephemeroptera (Δ) B.m. *Baetis muticus*, B.r. *Baetis rhodani*, E.spp. *Ecdyonurus* spp., H.l. *Heptagenia lateralis* and R.s. *Rithrogena semicolorata*; Trichoptera (●) Hydro. Hydroptychidae, Limne. Limnephilidae, P.spp. *Plectrocnemia* spp. and R.d. *Rhyocophila dorsalis*; Diptera (■) Chiron. Chironomidae, Simu. Simuliidae and Tipul. Tipulidae; Coleoptera (○) E.a. *Elmis aenea* and Coleop. Coleoptera larvae and Oligochaetae (□) Oligo.

3.5. DISCUSSION

3.5.1. *The chemistry of episodic acidification.*

By 2001/2002, contemporaneous with the collection of the data presented here, SO₂ emissions in the UK had declined to 15% of their peak in 1970. Sulphur deposition in some British locations had declined to 40-50% of 1980 values while oxidized nitrogen deposition had fallen by around 16%. However, trends have varied across sites and regions, with sulphur deposition over the wetter areas of western Britain (including Wales and Galloway) declining more slowly than eastern regions dominated by dry S deposition (Fowler *et al.* 2001). The most recent comprehensive survey of precipitation chemistry over Wales support these general results: by 1995, N and S deposition were lower by 20-25% than during the 1980s, but upland locations still received 20-25 kg N ha⁻¹ yr⁻¹ and 15-25 kg S ha⁻¹ yr⁻¹ with around 68-69% of sulphate coming from non-marine sources (a near identical value to the data from streams; Reynolds *et al.* 1999). This is generally similar to deposition in Galloway but more than in the NW Highlands, and these patterns contribute to the regional variations in critical load exceedance described in the Methods: only small areas of the Highlands receive more deposition than can be buffered, while less than half of Galloway and one third of Welsh freshwaters are now exceeded on the steady state criterion of ANC < 20 µeq L⁻¹ (<http://critloads.ceh.ac.uk>; Hall *et al.*, 2004). Sulphate, ANC and, to a lesser extent, pH data for streams in Wales and Galloway confirm that the chemical reversal of acidification is now well under way – at least for average conditions (Buckton *et al.* 1998; Evans *et al.* 2001; Harriman *et al.* 2001; Langan & Hirst 2004; Reynolds *et al.* 2004).

Despite these trends towards recovery in air and average water quality, however, the data illustrate that acid episodes still occur widely in what are some of the most acid-sensitive areas of Britain: acid-base status in acid-sensitive locations appears to be finely balanced hydrochemically – in keeping with the marginal conditions indicated by critical loads. Although the sample of streams was not random, it represented a large geographical area in which around 90% of the Welsh streams and 50% of the Scottish streams sampled were chronically or episodically acid. In some locations, episodic effects probably occurred naturally as a result of base-cation dilution and organic acid addition. However, at almost

30% of all the sites and 41% of the episodic sites, notably in Wales, titration effects were more important and consistent with continued anthropogenic acidification at high flow. Nitrate and non-marine sulphate, in particular, were the major driving anions and led to low pH and significantly elevated aluminium concentrations. Contrasts between Galloway and the Highlands, examined in more detail elsewhere, support this interpretation: while the frequency of episodic streams was no different between these areas of contrasting deposition (c 50% of streams in each), titration effects, and in particular the release of aluminium at high flow, were far more marked in Galloway (R. A. Kowalik and S. J. Ormerod unpubl.).

Data quantifying the regional frequency of streams currently characterised by acid episodes are uncommon. In the UK, this reflects more effort in recent acidification research on critical loads emphasising steady-state models and samples drawn largely from standing waters. Processes in streams, driven by dynamic hydrochemistry and variations with discharge, are less well represented. Episodic acidification nevertheless still occurs sufficiently widely across Europe (Moiseenko *et al.* 2001; Raddum *et al.* 2001; Armbruster *et al.* 2003; Lepori *et al.* 2003a) and North America (Clair *et al.* 2001; Campbell & Eagar 2002; Lawrence 2002) to be regarded as a potentially important feature in otherwise evidence of widespread recovery (Stoddard *et al.* 1999; Evans *et al.* 2001; Skjelkvale *et al.* 2003). As a result, acid episodes are still being widely implicated in biological effects (Lacroix & Korman 1996; Raddum *et al.* 2001; Gibbins *et al.* 2001; Bradley & Ormerod 2002). This data support the existence of such continued effects – and show also that they will be strongest where there are continuing additions of strong acids to base-poor run-off even in areas where recovery from acidification is otherwise clear. The data also show, however, that the causes, mechanisms, chemistry and trends in episodicity vary between locations so that careful appraisal is still required. Such variations elsewhere reflect contrasting hydrological drivers in rainfall and snowmelt, contrasts in the major anions involved, spatial variations in deposition, and climatic influences due to drought or sea-salt events (Davies *et al.* 1992; Tranter *et al.* 1994; Moldan *et al.* 2001; Lepori *et al.* 2003a; Laudon *et al.* 2004). Likely changes in episode character as recovery proceeds require further assessments (Laudon *et al.* 2002).

3.5.2. *Biological effects*

Invertebrate assemblages and the abundances of individual species varied significantly between circumneutral and episodic streams, and among streams characterised by natural and anthropogenic episodes. In the latter case, significant effects were apparent mostly from individual species that were absent or in reduced abundance only at locations where episodes were driven mostly by non-marine sulphate. While there was no evidence of direct toxic effects at these sites, previously accumulated data aid interpretation (see below). The assessment also depends critically on the assumption that single chemical samples at high and low flow were sufficient to characterise acidification mechanisms in individual streams, but this is supported by three arguments. Firstly, links between invertebrate and chemical data were highly replicated across sites. Second, high and low-flow chemistry is generally consistent in streams through time, at least where episodes are caused by the same hydrological mechanisms. Third, because of their prolonged larval stages, invertebrate assemblages integrate the effects of antecedent chemical conditions from months beforehand (Wade *et al.* 1989). Invertebrates and chemical data are therefore mutually corroborative in indicating episodic effects at individual sites.

Variations in aquatic invertebrate assemblages with acid-base status are among the most repeatable patterns in stream ecology (Sutcliffe & Carrick 1973). However, the exact causes are still being researched. Some explanations involve chemical effects mediated indirectly through biotic processes such as competitive exclusion along pH gradients, changes in food quantity or quality, or release from vertebrate predation at low pH (Hildrew *et al.* 1983; Schofield *et al.* 1988; Ledger & Hildrew in press). Increased abundance of *Plectrocnemia conspersa* at anthropogenically acidified sites in this study is consistent with biotically mediated effects because this trend cannot be explained by intolerance to high pH. Such biotic effects offer alternative explanations for impaired biological recovery at de-acidifying sites, for example because acid-sensitive organisms are required to re-build populations from small numbers of founders or to re-invade niches now occupied by acid-tolerant organisms (Hirst *et al.* 2004; Ledger & Hildrew in press). The need for previously extirpated organisms to recolonise recovering sites through inter-basin dispersal might also impose limits (Petersen *et al.* 2004).

Overwhelmingly, however, field and laboratory evidence shows that hydrogen-ion toxicity, exacerbated by metals such as aluminium, limits many acid-sensitive species directly at $\text{pH} < 5.7-6$ (Ormerod *et al.* 1987; Weatherley & Ormerod 1991). Acid episodes, in turn, provide the conditions under which hydrogen ions (at c $\text{pH} 4.5-5$) and metals reach their peak toxicity – as at the group 1 sites in this study. Recent field data not only show that such toxicological effects continue in upland Britain (Kowalik & Ormerod ms submitted), but they also show how species' response to episodes can determine population and community-level effects (Lepori & Ormerod, in press). Direct chemical effects on organisms in during episodes, even in recovering streams, are likely to subsume more subtle biotic processes: episodic acid toxicity may well be a sufficient explanation for weak biological recovery even where there are pronounced changes in mean pH (Bradley & Ormerod 2002). In this respect, the data are important since they were consistent with the hypothesis under test, and supported both of the required predictions.

The species apparently most affected by anthropogenic acid episodes – those either entirely absent from group 1 sites or present at significantly reduced densities only here by comparison with circumneutral sites – form a group whose acid-sensitivity is well known. For example, *Baetis muticus*, *Heptagenia lateralis* and *Rhithrogena semicolorata* are typically absent from sites with $\text{pH} < 5.7-6$, the plecopteran *Isoperla grammatica* often becomes scarce, while the absences of *Hydraena gracilis* and *Elmis aenea* from upland streams are amongst the clearest of all biological indications of acid conditions (Wade *et al.* 1989; Rutt *et al.* 1990). Some of these species (e.g. *R. semicolorata*) die following even brief exposures of c. $\text{pH} < 5.5$ with elevated aluminium (Merrett *et al.* 1991). In addition, dominantly univoltine life cycles among the above six species mean that individuals must overwinter as nymphs thereby increasing their risk of episodic acid exposure at this time (Kowalik & Ormerod ms submitted). By contrast, *B. rhodani*, despite marked sensitivity to low pH , can have rapid summer generations that lead to the colonisation of otherwise episodic sites during early spring and summer (Ormerod *et al.* 1987; Kowalik & Ormerod ms submitted). Its distribution during this study – with occurrence at a small number of episodic sites – is consistent with this pattern.

In contrast with individual species, whole assemblage composition changed more incrementally between circumneutral sites and episodic sites irrespective of the sources of acidification (i.e including those affected by base cation dilution and organic acids). While

the most impoverished assemblages still occurred in sites affected by anthropogenic acidification, the lack of pairwise statistical differences between site-groups meant that assemblages discriminated the causes of episodes less reliably than individual abundances. Assemblages clearly contained species affected by natural variations in water quality as well as anthropogenic effects. For example, the crustacean *Gammarus pulex* was absent from all the episodic site groups, including those affected by dilution alone. This species is naturally intolerant of low ionic strength waters where Ca^+ and K^+ are scarce due to the osmotic limits imposed and because of its requirement for calcium as a major body constituent (Sutcliffe & Carrick 1973). Similarly, *Hydropsyche* spp. are often scarce at low pH despite physiological tolerance perhaps because reduced rates of litter breakdown impair filter-feeding activity (Ormerod *et al.* 1987). The need to isolate progressive anthropogenic acidification effects on organisms from the natural limits of low ionic strength waters has been a long-standing need in acidification research. The data show that it applies also to the causes and effects of acid episodes, and to the interpretation of recovery: the biological effects of natural episodes, particularly through reduced base cation concentrations, might sometimes be confused with anthropogenic effects in the absence of careful assessments of the associated chemistry.

One area in which this study was equivocal concerned any specific biological effects related to elevated organic acidity. Increasing DOC concentrations from peat soils have been a recent characteristic of water quality trends over large upland areas, and in some locations this has offset the pH recovery that should have followed reduced sulphur deposition (Evans *et al.* 2001). Both the exact cause and biological consequences are still unclear, however. While the release of organic acids from peaty soils contributes to acidity in streams of low ionic strength, elevated DOC concentrations might ameliorate potential metal toxicity through complexation (Kullberg *et al.* 1993). In parts of Galloway, associated trends have resulted in a switch in forms of aluminium between toxic (labile) and less toxic forms that are organically complexed (McCartney *et al.* 2003). However, organic acidity can lead to substantial losses of ANC during episodes (Laudon *et al.* 2000; Wellington & Driscoll 2004) and metal toxicity can still result where deposition affects acid sensitive waters even with elevated DOC (Laudon *et al.* 2005). The data showed that increased DOC – and hence increased organic acidity – was a characteristic of storm-flow chemistry in episodic streams particularly in Scotland (Group 2 and 3 sites, Table 3). However, associated invertebrate assemblages were less distinct

from other episodic sites than from circumneutral sites so that acidification effects were still apparent. Similar effects elsewhere may confound the understanding of recovery processes and require fuller understanding (Skjelkvale *et al.* 2003).

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Appendix 1. Sampling locations in four regions of Scotland and Wales.

100

NW Highlands			Galloway			Central Wales			Conwy		
Stream	Name	OS Gridref	Stream	Name	OS Gridref	Stream	Name	OS Gridref	Stream	Name	OS Gridref
NW2	Allt an Tuill Bhain	NG920559	GA1	Glen Burn	NX888641	IR	Irfon	SN835555	CW1	Afon Ddu	SH769436
NW4	-	NH001642	GA3	Glen of Screel Burn	NX801545	II1	Nant y Cloddiaid	SN821564	CW3	Nant y Brwyn	SH791449
NW5	River Erradale	NG744713	GA5	-	NX785661	II2	Nant y Rhiw	SN832556	CW5	Afon y Foel 2	SH763458
NW6	-	NG845811	GA7	Auchenvey Burn	NX729762	II4	-	SN843547	CW6	Aber Las	SH779445
NW7	-	NH082883	GA8	-	NX651754	II5	Afon Aber	SN854525	CW8	Afon Nug	SH892541
NW10	-	NH205776	GA9	Mid Burn	NX614730	II6	next to Nant y Brain	SN849517	CW9	Afon Cadnant	SH862546
NW12	-	NC138068	GA11	-	NX563662	II8	Nant y Walch	SN856459	CW10	Nant y Foel	SH875560
NW13	-	NC108095	GA12	-	NX528528	CA	Camddwr	SN774560	CW11	Afon Bwlch y Groes	SH747516
NW14	Allt lochan Sgeirich	NC022115	GA15	Pultayan Burn	NX291680	CI1	Nant y Cwr	SN762576	CW12	Glasgwm 1	SH757498
NW16	Abhainn a Chnocain	NC212110	GA16	Drumpail Burn	NX225622	CI2	Nant Tyhelyg	SN763574	CW13	Glasgwm 2	SH755497
NW17	-	NC268108	GA17	Laganabeastie Burn	NX128727	CI4	Nant Gruffydd	SN770564	CW14	Trib of Hafod-Llilan	SH719504
NW19	Allt na Cailliche	NC317102	GA18	Davenholme Burn	NX162711	CI5	Nant y Coeleth	SN774556	CW15	Trib of Wybrnant	SH767520
NW20	Allt Srath Seasgaich	NC332047	GA19	-	NX184736	TW	Twyi	SN804572	CW16	Iwerddon stream	SH798539
NW21	-	NC397009	GA20	Water of Tig	NX185815	LI1	-	SN809530	CW17	Nant y Fflat Fawr	SH777506
NW22	-	NC470032	GA22	-	NX133845	LI2	Nant y Fannog	SN810515	CW18	Trib of Ceunant y Gamedd	SH699524
NW24	-	NC407238	GA25	Pulniskie Burn	NX366745	LI3	Nant y Craflwyn	SN814507	CW19	Trib of Oernant	SH796496
NW26	-	NC533219	GA26	Low Mill Burn	NX365813	LI4	Nant Cwm-bys	SN816503	CW20	Glasfryn stream	SH918497
NW29	-	NC568585	GA27	Loan Burn	NX372833	LI5	Nant Gwrach	SN821497	CW21	Pont Eidda stream 1	SH834504
NW31	-	NC503602	GA28	Butler Burn	NX362832	LI6	-	SN822496	CW22	Pont Eidda stream 2	SH835506
NW32	-	NC412552	GA30	-	NX437941	LI7	-	SN818493			
NW34	-	NC382646	GA32	-	NX475005	LI8	Trawsnant	SN805489			
NW35	-	NC258524	GA36	Benloch Burn	NX561951						
NW37	Allt an t-Srathain	NC179386	GA38	-	NX388031						
NW39	Allt nan Uamh	NC253179	GA39	-	NX314039						
			GA40	-	NX288062						

**CHAPTER 4. STREAM INVERTEBRATE COMMUNITIES AND
CONTINUED EPISODIC ACIDIFICATION IN REGIONS OF
CONTRASTING ACID DEPOSITION.**

4.1. SUMMARY

1. Surface-water acidification is reversing in Europe and N. America, but associated biological recovery in streams is patchy. A favoured hypothesis is that anthropogenic acid episodes continue to have biological effects despite increasing mean pH. Using data from 89 streams in the Scottish Highlands (low deposition), Galloway and Wales (high deposition), I tested three predictions required to support this hypothesis: i) that chemical variation between base- and storm-flow should be greater in regions receiving the most acid deposition; ii) that strong variations in invertebrate assemblages should result and iii) that they should reflect storm-flow (i.e. episode) chemistry more than base-flow.

2. pH fell during storm-flow by 0.5-1.8 units in acid and episodic sites everywhere. However, aluminium concentrations were significantly greater, or increased more between flows, in Galloway and Wales than in the NW Highlands. Loss of ANC at acid or episodic sites was greatest in Galloway whereas mean values became negative only in Wales.

3. Variations in invertebrate assemblages between acid, episodic and circumneutral streams were most marked in Wales and Galloway. Although these patterns reflected both base-flow and storm-flow chemistry, correlations with aluminium were strongest at storm-flow.

4. These data offer qualified support for all three predictions and are consistent with the hypothesis that acid episodes still affect stream organisms in areas receiving acid deposition. However, even base-flow chemistry at some locations is still sufficiently acid to retard recovery among sensitive taxa. I conclude that chemical recovery from acidification has not yet been sufficiently pronounced or sustained to allow biological recovery in many British locations. I predict that episodic effects will impose increasingly important controls on biological recovery as recovering sites reach the base-flow pH range 5.5-7.

4.2. INTRODUCTION

The chemical and biological effects of acidification on freshwaters have been researched intensively for over 30 years (Gorham & McFee, 1980; Stoner, 1984; Stoner *et al.*, 1984; Weatherley & Ormerod, 1987; Weatherley *et al.*, 1989; Donald & Gee, 1992; Reynolds *et al.*, 1999; Grant, 2003). Sulphur emissions, deposition and rainfall acidity in many impacted areas has declined over this period, and research interest is focussing increasingly on recovery (Fowler *et al.* 2001). Here, however, there is an apparent mismatch: while evidence is increasing for chemical reversal in acidification (Stoddard *et al.* 1999; Evans *et al.* 2001), associated biological recovery has been patchy or partial (Soulsby *et al.*, 1997; Collier & Quinn, 2003). Postulated hypotheses range from limits in the dispersal of lost organisms to recovering sites, resistance to recolonisation due to altered community composition and the development of new problems, for example climatic instability (Bradley & Ormerod 2001; Yan *et al.* 2003; Ledger & Hildrew 2005; Bowman *et al.* in press).

For European streams, a major candidate hypothesis is that anthropogenic acid episodes continue to affect organisms in spite of increasing mean pH (Bradley & Ormerod, 2001). I also showed how pH and aluminium concentrations in some upland streams still reach toxic concentrations for sensitive species during storm-flow (Lepori & Ormerod in press; Kowalik & Ormerod in press).

In this chapter, I test three further predictions required to support the 'episodic acidification' hypothesis. All follow from the postulated role of acid deposition in generating acid episodes. First, continued effects due to anthropogenic acid episodes require that variation between base- and storm-flow acidity in streams of otherwise comparable sensitivity should be greater in regions receiving the most acid deposition (*prediction 1*). Secondly, acid-related variations in invertebrate assemblages should result, and should be most marked in regions receiving the most acid deposition (*prediction 2*). Thirdly, variations in invertebrate assemblages should reflect storm-flow (i.e. episode) chemistry more than base-flow (*prediction 3*). In other words, storm-flow chemistry should be sufficiently acid to impact on organisms at sites that are not chronically acidified and hence explain failure in biological recovery. I focus on aquatic invertebrates due to their prominence in research into acidification, their well-

known variation in acid sensitivity (Wade *et al.* 1989), their value as bioindicators (Rutt *et al.* 1990) and their potential exposure to episodic conditions in winter or spring (Lepori *et al.* 2003a; Kowalik & Ormerod submitted).

4.2.1. Study areas

I tested these three predictions using data from 89 different upland streams in three regions with similar sensitivity to acidification but contrasting deposition: the northwest Scottish Highlands (n = 24); Galloway in south-west Scotland (n = 25); and Wales (n = 40; Figure 1). Both Galloway and Wales have been affected previously by acidification, while the relatively more pristine Scottish Highlands have not.

Sensitivity to acidification in these regions reflects variations in geology and soils. Rocks in the northwest Highlands are schists, gneisses, quartzites, granites and sandstones of limited buffering capacity inter-bedded with old red sandstone, limestone and shelly sands of medium buffering capacity. The overlying soils have little neutralising capacity except where brown forest soils overlay basic limestone that offer some neutralising capacity. In Galloway, most rocks are granite, greywacke and shale of limited buffering capacity but there are also areas with more base-rich basalt. Soils are dominantly podzols, non-calcareous gleys, basin and blanket peat, rankers, sub-alpine and brown forest.

The Welsh data came from the River Conwy catchment, and from the upper Irfon and Twyi adjacent to the Llyn Brianne experimental catchments (Stoner *et al.* 1984; Bradley & Ormerod 2002a). The Conwy is largely underlain by acid igneous rock, Ordovician and Silurian shale with low to medium buffering capacity and occasionally more basic lava. Soils are dominated by acid sensitive stagnopodzols (Hafren), stagnohumic gleys (Wilcocks) and brown podzols (Manod) with small areas of highly sensitive peat (Crowdy). In some areas stagnogleys (Cegin and BrE) offer some buffering. The Irfon/Twyi sites are largely on Ordovician and Silurian shale, mudstone and grit overlain by podzols, stagnopodzols and peats of low buffering capacity, though local calcite veins buffer some streams (Stoner, 1984; Weatherley & Ormerod, 1987).

Deposition in all three regions reflects dominantly wet, westerly airflow that brings relatively clean air from the Atlantic. However, high rainfall volumes under some circumstances increase the wet deposition of S and N compounds despite their moderate concentrations in rainfall. In Wales, for example, this effect leads to deposition of 20-25 kg N ha⁻¹ yr⁻¹ and 15-25 kg S ha⁻¹ yr⁻¹ in upland locations, with around 68-69% of sulphate coming from non-marine sources (Reynolds *et al.* 1999). Across all three regions, current estimates of deposition and sensitivity from representative locations (Wales = 67, Galloway = 55, and the NW Highlands = 98) show that critical loads for S deposition (>1.0 Keq ha⁻¹) are exceeded over a greater proportion of Wales (72% of sites) and Galloway (69%; > 15 kg S ha⁻¹ yr⁻¹) than the Highlands (40%; ≤ 6-15 kg S ha⁻¹ yr⁻¹). More pertinent for aquatic ecosystems, critical values for ANC (<20 µeq L⁻¹) in surface waters are now exceeded in very few catchments in the NW Highlands (6%), but around half or one third of those in either Wales (30%) or Galloway (42%; (Hall *et al.*, 2004).

4.2.2. Methods

Sampling sites were all second- or third-order streams. Overall selection was non-random since all were expected to be base-poor and acid-sensitive. The Welsh study areas were known from previous surveys of the effects of acid deposition (eg Wade *et al.* 1989; Reynolds *et al.* 1999), while streams in Galloway and the Scottish Highlands were drawn from a range of landscape types also known in outline from other research (e.g. Harriman *et al.*, 2001; Helliwell *et al.* 2001). Further logistical constraints required that all should be accessible by road.

4.2.3. Water chemistry

Water samples were taken from all the streams during field campaigns after heavy rainfall (= storm flow; Galloway, October 2001; northwest Highlands and mid Wales, February 2002; Conwy, January 2003) and at base-flow (northwest Highlands and Galloway, April 2002; Conwy and mid Wales August 2002). One assumption in this approach was that single samples at contrasting discharges are sufficient to characterise chemistry, and this is supported by four arguments. First, samples were highly replicated across many sites and three regions. Secondly, high and low-flow chemistry

is generally consistent in streams through time, at least where episodes are caused by the same hydrological mechanisms (Edwards, Stoner & Gee 1990). Third, because of their dominantly univoltine life-cycles, invertebrates integrate the effects of antecedent chemical conditions over long periods (Wade *et al.* 1989). Finally, invertebrates and chemical data at these sites were mutually corroborative in indicating episodic effects (Kowalik *et al.* ms submitted).

Samples were analysed for Na⁺, K⁺, Ca²⁺, Mg²⁺, NH₄⁺, Cl⁻, NO₃⁻, PO₄³⁻, SO₄²⁻, Si⁴⁺, Al and dissolved organic carbon (DOC), with anions determined by ion chromatography (Dionex), base cations and metals by Atomic Absorption Spectrophotometry (AAS) (Perkin Elmer) and Si by the molybdenum blue method. DOC was determined by continuous flow colorimetry with UV digestion (Skalar autoanalyser System) and Al colorimetrically using pyrocatechol violet (Dougan & Wilson, 1974). pH was measured using a hand-held combined pH/EC/TDS meter (Hanna Instruments HI 991300) and charge balance acid neutralising capacity (ANC) was calculated as the sum of base cations minus the sum of acid anions (Na⁺ + K⁺ + Ca²⁺ + Mg²⁺ - NO₃⁻ - SO₄²⁻ - Cl⁻). More detailed investigations of stream chemistry and the causes of episodes are described elsewhere (Kowalik & Ormerod ms submitted).

4.2.4. Macroinvertebrates

Macroinvertebrates were collected from all sites during April 2002 by standardised kick-sampling of two-minutes duration in riffles and one minute in the margins (standard net with 1 mm mesh size). This strategy aims to maximise as far as practically possible the collection of taxa across a range of stream habitats. It represents the fauna of upland streams effectively while reliably detecting differences between sites (Bradley & Ormerod, 2002a). Samples were preserved on site by adding 100% IMS (Industrial Methylated Spirit) to the existing sample volume. They were later sorted in the laboratory by initially rinsing through a 500 µm sieve, immersing in fresh water and removing the macroinvertebrates by hand. The sorted samples were preserved in a 70% IMS. Individual Ephemeroptera, Trichoptera, Plecoptera and Coleoptera were identified to species or genus. Immature individuals and more difficult groups such as Simuliidae, Chironomidae and Tipulidae were identified to family. The

abundance of each taxon was recorded for both the riffle and margin samples taken at each stream.

4.2.5. Data analysis

Prior to any analysis, all chemical variables except pH were \log_{10} transformed to homogenise variances. ANC values were $\log_{10}(x + 300)$ transformed to increase all the range of data to positive values. Abundances for each macroinvertebrate taxon were transformed as $\log_{10}(x + 1)$. To allow analysis and comparison across regions, streams were grouped on base-flow pH into 'acidic' (base-flow pH <5.7), 'episodic' (base-flow pH = 5.7-7) and 'circumneutral' categories (pH > 7). In Britain, streams with base-flow pH ≤ 7 usually have minimum pH < 5.7, previously recognised as a threshold at which many acid sensitive taxa become scarce (Sutcliffe & Carrick 1973; Weatherley & Ormerod 1991). The three predictions were next tested as follows:

To test *prediction 1*, I assessed variations in pH, ANC, Al^{n+} and DOC across regions and stream-types at both base-flow and storm-flow using two-way analysis of variance (ANOVA) based on the general linear model (GLM). I also assessed chemical change between flows. In each case, I tested for interactions between stream categories and regions to assess whether regions differed within some stream types more than others. In all cases, protocols for diagnostic checks in ANOVA followed Fry (1993).

To test *prediction 2*, trends in invertebrate assemblages across all sites were first ordinated by detrended correspondence analysis (DCA) using CANOCO (ter Braak & Smilauer, 1998). As one of the most widely used of all ordination methods, DCA orders sites objectively along orthogonal axes that reflect similarity in taxonomic composition without the constraint that trends should fit any environmental variable. Site scores on each axis thus reflect differences solely in assemblage composition. DCA scores (as quantitative expressions of trends in invertebrate composition) were then assessed for variations between stream-types, regions and interaction between stream-type and region using two-way ANOVA as for chemistry.

Finally, to test *prediction 3*, I assessed correlations between DCA site scores and major chemical determinands at base-flow and storm-flow using Pearson correlation coefficients.

4.3. RESULTS

4.3.1. Variations in acid-base chemistry

Acidic and episodic streams occurred in all three regions, though at a greater frequency in Wales (36/41 sites) than in Galloway (12/25) or in the NW Highlands (12/24; Figure 1). Sites in all three regions were characterised by episodic acidification at high-flow, with pH falling by an average 0.5-1.3 pH units to minima < pH 4.3-5.4 during the events recorded (Table 1). Variations in pH between flow were greatest at sites with intermediate base-flow pH (Figure 2). As a result, pH, Al^{n+} and ANC varied more strongly between stream types at both high- and low-flow than between regions (Table 2).

Some significant chemical variations between regions were nevertheless consistent with *prediction 1*, most notably in concentrations of aluminium (Table 2). Even at base-flow, Al^{n+} concentrations were particularly high in acid Galloway streams (region/stream-type interactions $F_{4,88} = 4.46$, $P < 0.01$). At storm-flow, values were greatest in episodic or chronically acid streams in Galloway and Wales, where considerable increase in Al^{n+} concentrations occurred between flows (region*stream-type interaction $F_{4,88} = 3.68$, $P < 0.01$). By contrast, aluminium concentrations at episodic sites in the Highlands increased during storm-flow on average by only $49 \mu\text{m L}^{-1}$ ($\pm 85 \mu\text{m L}^{-1}$), and actually fell at acidic sites probably due to dilution. Lower aluminium concentrations during events in Highland streams also reflected more moderate storm-flow pH in this region (Figure 2)

ANC values also varied regionally (Tables 1, 2). Reflecting base-cation dilution, reductions in ANC with increased discharge were largest in circumneutral streams. However, large reductions also occurred in episodic and acid streams in Galloway ($F_{2,88} = 4.91$, $P < 0.01$). Only in Wales did ANC values fall, on average, below zero, this

effect occurring in both acid and episodic streams. DOC concentrations varied regionally due to elevated concentrations in Galloway.

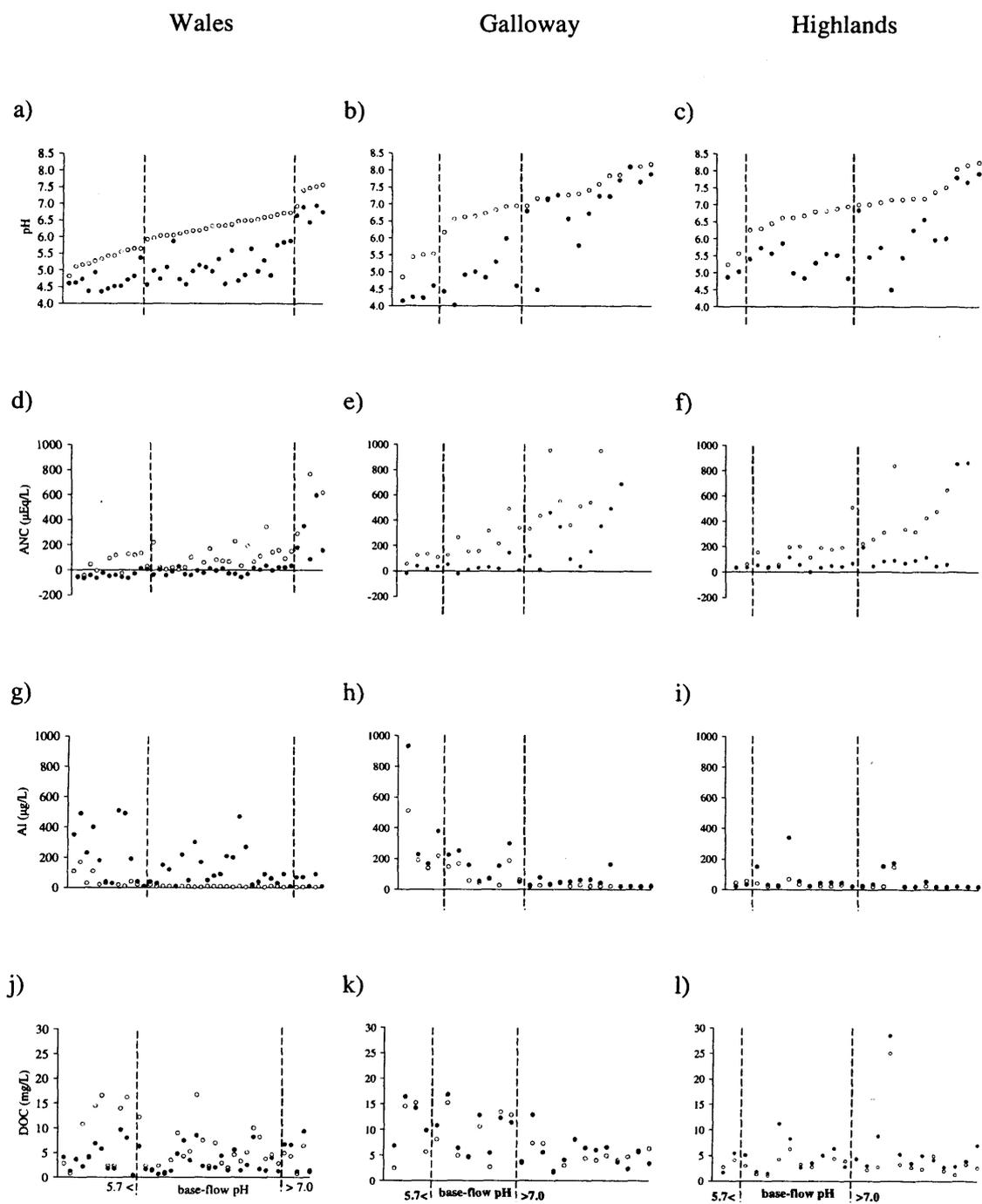


Figure 1. Relationships among changes in pH and aluminium between base-flow and storm-flow at 89 sites in Wales (triangles), Galloway (diamonds) and the NW Highlands (squares): a) storm-flow v. base-flow pH ($r_s = 0.78$, $P < 0.001$); b) pH change between base-flow and storm-flow in relation to base-flow pH; c) Aluminium at storm-flow v. pH at storm-flow ($r_s = -0.56$, $P < 0.001$).

Table 1. Stream chemistry (pH, Al³⁺, ANC and DOC) in the NW Highlands, Galloway and Wales at contrasting stream discharge. Mean values (\pm SD) are given for acidic (base-flow pH < 5.7), episodic (5.7-7.0) and circumneutral (> 7.0) streams, and the change between flows is also shown. The units are $\mu\text{eq L}^{-1}$ for ANC, mg L^{-1} for DOC and $\mu\text{g L}^{-1}$ for Al³⁺ (see Table 2 for statistical analysis).

Determinands	Stream-type	Region	Base-flow	Storm-flow	Change
pH	Acid	Wales	5.3 (0.3)	4.7 (0.3)	-0.7 (0.3)
		Galloway	5.3 (0.3)	4.3 (0.2)	-1.0 (0.3)
		Highlands	5.4 (0.2)	5.0 (0.1)	-0.5 (0.1)
	Episodic	Wales	6.3 (0.2)	5.1 (0.4)	-1.2 (0.4)
		Galloway	6.7 (0.3)	4.9 (0.6)	-1.8 (0.5)
		Highlands	6.6 (0.2)	5.4 (0.4)	-1.3 (0.5)
	Circumneutral	Wales	7.4 (0.3)	6.7 (0.2)	-0.6 (0.3)
		Galloway	7.6 (0.4)	7.0 (1.0)	-0.6 (0.8)
		Highlands	7.4 (0.5)	6.3 (1.1)	-1.1 (0.7)
Aln ³⁺	Acid	Wales	50 (51)	247 (195)	+197 (176)
		Galloway	265 (16)	427 (349)	+162 (183)
		Highlands	49 (9)	27 (9)	-22 (0)
	Episodic	Wales	9 (3)	122 (114)	+113 (115)
		Galloway	95 (62)	158 (95)	+63 (55)
		Highlands	30 (15)	78 (99)	+49 (85)
	Circumneutral	Wales	7 (2)	50 (37)	+43 (40)
		Galloway	24 (7)	49 (39)	+25 (40)
		Highlands	30 (37)	48 (55)	+18 (38)
ANC	Acid	Wales	23 (109)	-40 (27)	-63 (109)
		Galloway	106 (35)	18 (26)	-88 (20)
		Highlands	44 (21)	35 (1)	-10 (23)
	Episodic	Wales	91 (114)	-8 (27)	-98 (113)
		Galloway	258 (122)	33 (50)	-224 (102)
		Highlands	182 (128)	48 (30)	-134 (119)
	Circumneutral	Wales	845 (449)	274 (205)	-571 (296)
		Galloway	1199 (988)	658 (820)	-542 (331)
		Highlands	905 (971)	327 (455)	-578 (599)
DOC	Acid	Wales	7.4 (6.5)	4.2 (2.9)	-3.2 (4.4)
		Galloway	9.5 (6.4)	11.8 (4.3)	+2.4 (2.5)
		Highlands	3.4 (1.0)	3.6 (2.7)	+0.2 (1.7)
	Episodic	Wales	5.3 (3.8)	3.3 (2.4)	-2.1 (2.7)
		Galloway	9.1 (4.7)	10.1 (4.2)	+1.0 (1.7)
		Highlands	3.5 (1.6)	4.8 (3.1)	+1.3 (2.3)
	Circumneutral	Wales	3.6 (2.4)	5.0 (3.7)	+1.4 (1.3)
		Galloway	5.0 (1.8)	5.4 (2.9)	+0.4 (2.2)
		Highlands	4.7 (6.5)	6.6 (7.1)	+1.9 (2.0)

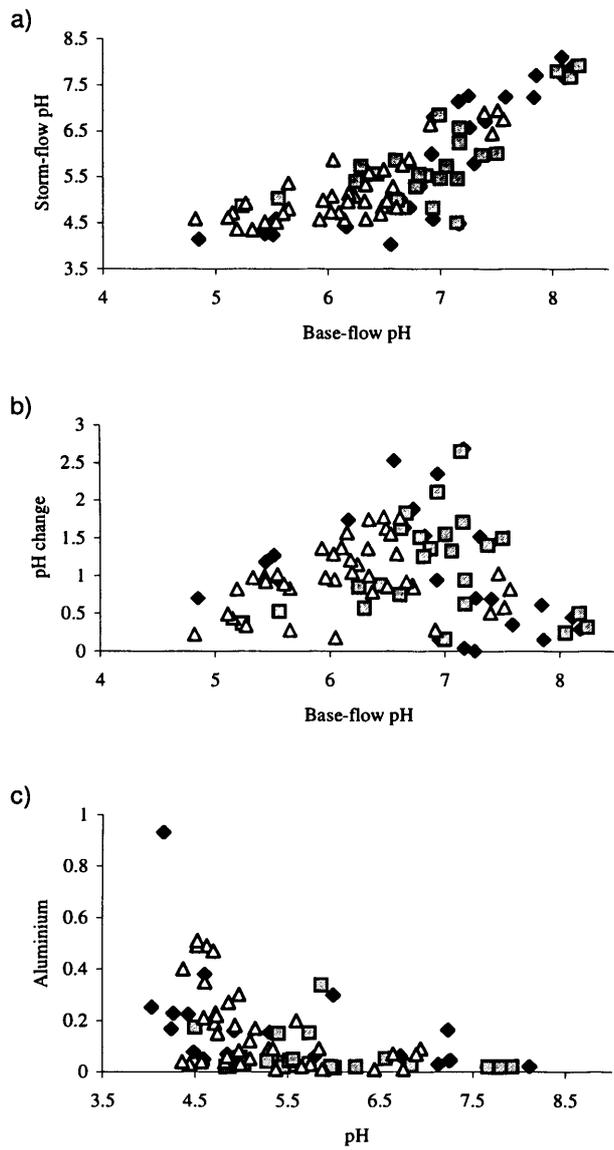


Figure 2. Key acid-base determinands recorded in Wales, Galloway and NW Highlands at storm-flow (●) and base-flow (○). Streams are arranged in increasing order of base-flow pH and acid (a), episodic (e) and circumneutral (c) sites are separated by dashed lines.

Table 2. Variations in pH, ANC, Alⁿ⁺ and DOC between stream-types (acidic, episodic and circumneutral), regions (Wales, Galloway and NW Highlands) and stream-type*region interactions according to two-way ANOVA. Significance levels: P <0.05*, P <0.001**, P <0.001*** and ns = not significant (See Table 1 for source data).

Determinands	Factor	Base-flow	Storm-flow	Change between flows
pH	<i>Stream-type</i> F _{2,88}	170.83***	51.46***	11.99***
	<i>Region</i> F _{2,88}	2.07ns	0.37ns	1.81ns
	<i>Interaction</i> F _{4,88}	1.03ns	2.97*	3.79**
ANC	<i>Stream-type</i> F _{2,88}	32.08***	23.25***	2.69ns
	<i>Region</i> F _{2,88}	2.17ns	1.99ns	4.91**
	<i>Interaction</i> F _{4,88}	0.27ns	0.94ns	1.20ns
Al ⁿ⁺	<i>Stream-type</i> F _{2,88}	30.68***	6.86**	0.72ns
	<i>Region</i> F _{2,88}	66.01***	6.73**	7.57**
	<i>Interaction</i> F _{4,88}	4.46**	1.84ns	3.68**
DOC	<i>Stream-type</i> F _{2,88}	1.30ns	0.04ns	23.33***
	<i>Region</i> F _{2,88}	4.26*	10.96***	0.82ns
	<i>Interaction</i> F _{4,88}	0.32ns	2.11ns	0.56ns

4.3.2. Macroinvertebrates and stream chemistry

In DCA, two major axes explained 23% of the variation in assemblage composition (Table 3). Both axes were around two standard deviations in length, reflecting species turnover of c. 50%. On axis one, taxa scoring negatively (weight > 20%) were dominantly acid-sensitive, including Ephemeroptera such as *Baetis muticus* (L.), *B. rhodani* (Pictet), *Heptagenia lateralis* (Curtis), *Rhithrogena semicolorata* (Curtis) and *Ecdyonurus* spp. Eaton; the plecopteran *Brachyptera risi* (Morton); hydropsychid trichopterans and the coleopteran *Elmis aenea* (Müller). Positively scoring taxa included the acid-tolerant plecopterans *Amphinemura sulcicollis* (Stephens),

Chloroperla torrentium (Pictet), *Isoperla grammatica* (Poda), *Leuctra inermis* Kempny, *L. nigra* (Olivier) and *L. hippopus* (Kempny), and the trichopterans *Rhyacophila dorsalis* (Curtis), *Plectrocnemia* spp. McLachlan and the Limnephilidae (Figure 4a).

Reflecting this array of species' sensitivities to acidification, invertebrate composition on DCA axis 1 varied highly significantly between acidic, episodic, and circumneutral streams across all regions (Table 4). However, there were also significant regional effects. Supporting *prediction 2*, assemblages were more clearly differentiated between acid, episodic and circumneutral streams in Wales and Galloway than in the NW Highlands (Figure 3).

As would be expected from all of these trends, DCA scores on axis 1 were highly significantly correlated ($P < 0.001$) with acid-base indicators such as pH, Ca^{2+} and ANC at both low- and high-flow (Table 5). Consistent with *prediction 3*, highly significant correlations between axis 1 scores and Al^{3+} were only apparent at high-flow. No other major ions were so highly correlated with this first axis. Axis 2 also indicated some acidification effects due to correlations with aluminium at base-flow, and less strongly with pH and calcium at storm-flow. All these correlations remained highly significant at near-identical levels when chronically acid sites were removed suggesting a large contribution to the overall pattern from episodic sites.

Table 3. Detrended Correspondence Analysis (DCA) of stream invertebrates at sites across Wales, Galloway and the NW Highlands showing eigenvalues, gradient length (standard deviation units of species turnover) and species variance (cumulative %) for the first four axes. See Fig. 4 for plots.

Axis	1	2	3	4
Eigenvalues	0.277	0.113	0.081	0.061
Gradient length	2.217	2.081	1.641	1.827
Species variance	16.3	22.9	27.6	31.2

Table 4. Mean site scores (\pm SD) on DCA axis 1 for each regions/stream category combination (see Table 1 for definitions). Significant variations are shown according to two-way ANOVA (GLM) (* = $P < 0.05$; *** = $P < 0.001$ ***. There were no significant region/stream type interactions ($F_{4,88} = 0.75$, N. S.).

	Galloway	NW Highlands	Wales	Variation between regions $F_{2,88}$
Acidic	1.64(0.29)	1.15(0.51)	1.74(0.33)	
Episodic	1.07(0.53)	0.89(0.55)	1.25(0.46)	3.54*
Circumneutral	0.35(0.26)	0.43(0.27)	0.54(0.09)	
Variation between stream types $F_{2,88}$		32.37***		

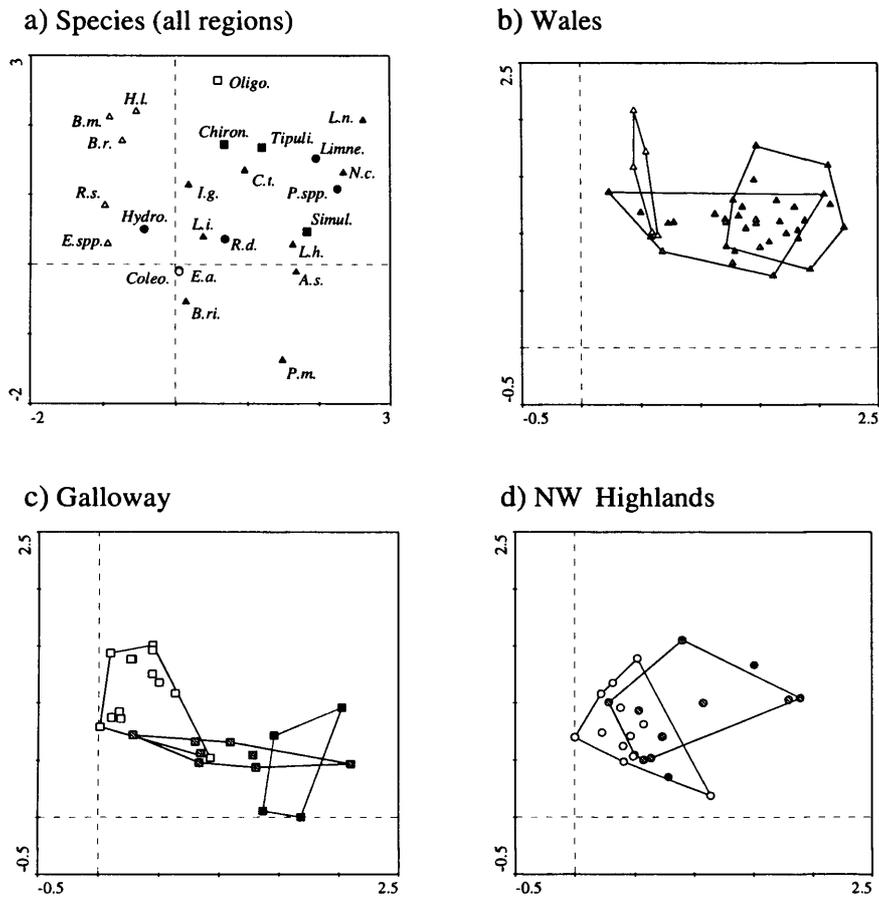


Figure 3. DCA ordination scores for a) major species (qualification > 20% weight) and streams in b) Wales (b), Galloway (c) and the NW Highlands (d) with sites grouped by stream-type (chronically acidic: black; episodic: hatched; circumneutral: open). See Table 3 for descriptive data on the ordinations, and Table 4 for analysis of significant effects.

Key: Plecoptera (▲) *A.s.* *Amphinemura sulcicollis*, *B.ri.* *Brachyptera risi*, *C.t.* *Cholorperla torrentium*, *I.g.* *Isoperla grammatica*, *L.h.* *Leuctra hippopus*, *L.i.* *L. inermis*; *L.n.* *L. nigra* and *N.c.* *Nemoura cambrica*; Ephemeroptera (△) *B.m.* *Baetis muticus*, *B.r.* *Baetis rhodani*, *E.spp.* *Ecdyonurus* spp., *H.l.* *Heptagenia lateralis* and *R.s.* *Rithrogena semicolorata*; Trichoptera (●) *Hydro.* Hydropsychidae, *Limne.* Limnephilidae, *P.spp.* *Plectrocnemia* spp. and *R.d.* *Rhyocophila dorsalis*; Diptera (■) *Chiron.* Chironomidae, *Simu.* Simuliidae and *Tipul.* Tipulidae; Coleoptera (○) *E.a.* *Elmis aenea* and *Coleop.* Coleoptera larvae and Oligochaetae (□) *Oligo.*

Table 5. Pearson correlations between DCA site scores and stream chemistry at base-flow and storm-flow (* = P < 0.05; ** = P < 0.01; *** = P < 0.001).

	Base-flow		Storm-flow	
	Axis 1	Axis 2	Axis 1	Axis 2
pH	-0.803***	0.094ns	-0.750***	0.342**
Ca ²⁺	-0.602***	0.205ns	-0.508***	0.358**
ANC	-0.635***	0.092ns	-0.606***	0.231*
Mg ²⁺	-0.630***	0.019ns	-0.512***	0.221*
K ⁺	-0.560***	-0.013ns	-0.495***	0.147ns
Na ⁺	-0.368***	-0.203ns	-0.327**	-0.082ns
Al ⁿ⁺	0.273*	-0.475***	0.547***	-0.179ns
SO ₄ ²⁻	0.243*	0.380***	0.376***	0.439***
Cl ⁻	-0.275**	-0.191ns	-0.314**	-0.034ns
NO ₃ ⁻	0.058ns	0.342**	0.142ns	0.267*
NH ₄ ⁺	-0.038ns	-0.182ns	0.029ns	-0.238*
DOC	0.163ns	-0.055ns	-0.031ns	-0.192ns
Si	-0.017ns	-0.058ns	-0.065ns	0.208ns
PO ₄ ²⁺	0.129ns	0.078ns	0.033ns	-0.130ns

4.4. DISCUSSION

The central aim of this study was to assess three predictions required to support the hypothesis that anthropogenic acid episodes still affect stream organisms in Britain and might therefore impede biological recovery from acidification. This is an important aim for three principal reasons. First, appraising any continued episodic effects is a pre-requisite to testing other hypotheses that could explain patchy or partial biological recovery. These hypotheses centre mostly on ecological processes such as dispersal or

competitive exclusion (e.g. Ledger & Hildrew 2005), but would be unlikely if acidity alone explained the scarcity of acid-sensitive in recovering streams. Second, differentiating the effects of acid episodes and chronic acidity on stream fauna has in the past presented some challenges (Weatherley & Ormerod 1991). This problem is now being revisited with respect to biological recovery because model projections show how continued acid episodes could cause serious delays despite increasing mean pH (Kowalik & Ormerod ms submitted). Finally, there has been a long-standing requirement to differentiate between anthropogenic and natural sources of acidification and this applies also to the cause or impact of episodes (Kahl *et al.* 1992 Wiggington *et al.* 1992; Laudon & Bishop 1999; Dangles *et al.* 2004). For example, the crustacean *Gammarus pulex* is absent from base-poor sites where episodic acidification results naturally from base-cation dilution, while other species are absent where episodes are driven by organic acids (Kowalik *et al.* ms submitted). Such effects could clearly confound the interpretation of recovery both now and in future.

Each of the three predictions was supported, but only with some qualification. The first prediction (*prediction 1*) was that variation between base- and storm-flow acidity should be greater in regions receiving more acid deposition than elsewhere, in other words in Wales and Galloway rather than the NW Highlands. The data show unequivocally that chronically or episodically acid streams are, in fact, present in all three regions, with pH falling on average by 0.5-1.8 units to pH 4.3-5.4 during the storm events measured. While site selection was not random, the geographical extent of the sampling programme and of the soil types covered suggest that sites affected by episodic acidification might still be widespread. However, chemical variations between flows were not identical in all regions. In particular, ANC fell in acidic and episodic streams during events most substantially in Galloway, while average values only fell below zero in Wales. More important, aluminium concentrations increased markedly during storm flow only in these two regions. Given the likely effects of non-marine sulphate in mobilising this metal, particularly over the soil pH range 4.2-5 (Reuss & Johnson 1986; Bredemeier *et al.* 1998; Moldan *et al.* 2004), this trend is entirely consistent with *prediction 1*. In Wales, for example, non-marine sulphate currently accounts for almost 70% of total sulphate in deposition

(Reynolds *et al.* 1999) with contributions in runoff near identical (64-66%). In turn, British streams with episodes driven dominantly by non-marine sulphate have lower pH (5.0 ± 0.6 vs pH 5.4 ± 0.6) and significantly more dissolved aluminium ($288 \mu\text{g L}^{-1} \pm 271$ vs $116 \mu\text{g Al L}^{-1} \pm 110$) than streams where episodes are caused by base-cation dilution (Kowalik *et al.* ms submitted). Similar effects are well established in other locations (Wiggington *et al.* 1992) and are of particular significance given the role of aluminium in acid toxicity (see below). These regional contrasts in chemical behaviour during episodes underline the importance of determining not only varying episode chemistry, but also the major contributing processes: it cannot be assumed that falling pH during storm events always increases concentrations of toxic metals, nor that acid deposition is always responsible.

Prediction 2 was that acid-related variations in invertebrate assemblages should be most marked in regions receiving the most acid deposition, and once again the data gave qualified support. Consistent with a large body of information, acid-base status was a major correlate with invertebrate composition (Sutcliffe & Carrick 1973). These effects occur through direct physiological impacts on organisms at acid or episodic sites coupled with indirect biological processes such as altered predation or prey availability (Steinberg & Wright 1994). In this case, the most highly significant variations in assemblage composition occurred among acid, episodic and circumneutral streams irrespective of region. This would be expected because this categorisation of streams was based on pH in the first instance. However, consistent with *prediction 2*, differences between stream groups were more clearly demarcated in the acid-impacted regions of Wales and Galloway than in the NW Highlands. Although qualitative, this result is a consequence of both the smaller number of chronically acid streams in the Highlands and also the greater overlap here between the assemblages of episodic and circumneutral streams (see Figure 4). In turn, episodic acidification in the Highlands is less likely to result in low aluminium concentrations and more likely to be driven by base-cation dilution than elsewhere (see Table 1).

The third and final prediction (*prediction 3*) required to support the hypothesis under test was that variations in invertebrate assemblages should reflect storm-flow (i.e. episode)

chemistry more than base-flow. This test follows previous attempts by other authors to assess similar distinctions between chronic and episodic effects on stream organisms elsewhere. In Wales, Weatherley & Ormerod (1991) showed that invertebrate assemblages varied more with episode pH or aluminium concentration than average chemistry, but only marginally. However, measures of episode and mean chemistry in the latter study were not independent since extreme values contributed to the overall means. Consequently, Lepori *et al.* (2003b) sampled baseflow and episodes separately in Swiss Alpine streams and detected marked episodic effects on invertebrates. Similarly, Bradley & Ormerod (2002a) showed that the richness and abundance of acid sensitive taxa in the Llyn Brianne experimental catchments varied from year-to-year according to episode chemistry. Elsewhere, a range of field experiments on whole stream systems have demonstrated clear impacts of acid, metal-rich episodes on benthic invertebrates (Ormerod *et al.* 1987; Hall 1994; Kratz *et al.* 1994) In this case, there was again qualified support for the prediction being tested, since assemblages varied with aluminium concentration only during storm-flow. This metal is likely to be a major source of toxicity to sensitive invertebrates at pH < 5.5 at the concentrations recorded (> 100 ug L⁻¹; Merrett *et al.* 1991). Quantitative invertebrate sampling coupled with field toxicological data confirm that aluminium-rich episodes in autumn and winter are still sufficient to eliminate sensitive organisms that colonise episodic streams during periods of summer base-flow (Kowalik & Ormerod in press). Additionally, the majority of acid-impacted sites in the data set were episodic rather than chronically acid: 23/40 of all sites in Wales, 8/25 in Galloway and 10/24 in the Highlands had base-flow pH>5.7, but pH 4.9-5.4 during events. However, invertebrates varied strongly also with other indicators of acidity at both storm-flow and base-flow – notably pH. One explanation is that the remaining acid-impacted sites in the data set (12/40 in Wales, 4/25 in Galloway and 2/24 in the Highlands) have base-flow pH sufficiently acid (< 5.7) to limit some sensitive organisms thereby giving rise to strong correlations between invertebrate assemblages and base-flow chemistry. In addition, storm-flow and base-flow pH values were significantly correlated across sites ($r_s = 0.78$ $P < 0.001$; Fig. 2) thereby confounding clear assessment of any effects solely due episodic pH change alone.

In combination and with appropriate qualification, these data are consistent with the hypothesis that anthropogenic acid episodes in some British streams still affect stream organisms. All three predictions were supported in some form, mostly due to variations linked to concentrations of aluminium. This outcome is in agreement with other tests of this hypothesis at the same suite of sites (Kowalik & Ormerod, in press; Kowalik *et al.* ms submitted) and at other European locations (Lepori *et al.* 2003a, 2003b, Lepori & Ormerod, in press). At the same time, however, a significant number of upland streams in Britain still have sufficiently low pH (< 5.3-5.4) to prevent the recovery of sensitive species even during base-flow. This result is important in indicating the retarded biological recovery from stream acidification in Britain most likely reflects continued chronic acidification in some locations as well episodic effects. This is despite clear evidence of long-term recovery in air quality and average water quality (Fowler *et al.* 2001; Evans *et al.* 2001). One inference would be that further time is required both for biological recovery and for the chemical processes that will engender it. This includes the time required for further emission controls, for these to affect deposition quality, and for catchment soils to re-equilibrate to lower anion throughputs (Fowler *et al.* 2001). Biological recovery in streams cannot be assumed without ongoing monitoring, or without a fuller assessment of the factors that either promote or retard it. On the evidence in this paper and associated work, the impact of episodic acidification in otherwise recovering sites is potentially important and may well be a major control on biota in the base-flow pH range 5.5-7 (Figure 2b).

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**CHAPTER 5. MODELLING AND PROJECTING THE EFFECTS OF EPISODIC
ACIDIFICATION ON STREAM MACROINVERTEBRATES**

5.1. SUMMARY

1. Acid episodes might affect biological recovery from acidification despite increasing average pH but these effects are seldom modelled. Using data from 89 independent British streams, I used regression to model the effects of base-flow or storm-flow pH on invertebrates. Models were evaluated at calibration sites ($n = 67$) and independent test sites ($n = 22$). I then projected future biological change in a model stream based on trends identified from the UK Acid Waters Monitoring Network (UKAWMN).

2. Assemblage composition during calibration was best predicted by pH alone ($R^2 = 0.65$) or combined with calcium, aluminium or nitrate ($R^2 = 0.66-0.71$). ANC performed poorly ($R^2 = 0.45-0.46$). Aluminium had significant effects at storm-flow, when chemistry explained marginally more variance in assemblages than at base-flow. Abundance in acid-sensitive *Baetis* spp was more effectively predicted by storm-flow pH ($R^2 = 0.67$) than base-flow pH ($R^2 = 0.52$).

3. In projected trends from the UKAWN (+0.11-0.45 pH units per decade), simulated biological recovery reflected variations in the rate of pH increase. However, for any given scenario, episodes retarded recovery by 10-20 years by comparison with trends in base-flow pH, and in the worst-case by up to 50 years. Release from episodic effects ($< \text{pH } 5.5$) at base-flow pH > 6.5 considerably augmented final recovery.

4. I concluded that episode chemistry provides the most realistic and accurate predictions of acidification effects on stream invertebrates. However, model projections were affected by critical assumptions about future recovery process. The strongest recommendation is for better understanding and monitoring of the hydrochemistry and biological effects of acid episodes as acid deposition abates.

5.2. INTRODUCTION

The post-industrial acidification of soft-water lakes and rivers has altered communities of aquatic organisms over large areas of Europe and North America. Many taxonomic groups at all trophic levels have been affected including primary producers, benthic invertebrates, fish and aquatic birds (Muniz 1990). Local species extirpation, changes in ecosystem function and economic damage have resulted. Although much research interest in the past involved understanding how acidification initially impacted acid-sensitive communities, emphasis has now switched to the assessment of recovery. With major emission controls now in place in many countries, there is unequivocal evidence that acid deposition is declining (Fowler *et al.* 2001). As a result, surface waters on both sides of the Atlantic have increasing mean pH, declining sulphate and increasing acid neutralising capacity (ANC; Soulsby *et al.* 1997; Stoddard *et al.* 1999; Evans *et al.* 2001). By contrast, associated biological recovery is still patchy or partial (Soulsby *et al.* 1995 Tipping *et al.* 2002) and a range of hypotheses are emerging to explain the apparent mis-match (Yan *et al.* 2003). For streams, that most supported by available data is that acid episodes – transient periods of low pH during rainstorms or snowmelt (Wigington *et al.*, 1996) – still have widespread effects on organisms and might prevent biological recovery during short-term periods of increased toxicity (Bradley & Ormerod 2002a; Lepori *et al.* 2003a; Kowalik & Ormerod ms submitted; Kowalik *et al.* ms submitted). Episodes were considered major drivers of the initial impacts of acidification (Ormerod *et al.*, 1987; Giberson & Hall, 1988; Hopkins *et al.*, 1989; Weatherley & Ormerod, 1991) and, if they continue, could offset any biological gains despite increasing mean pH.

While interactions between acid episodes and recovery are of fundamental interest, they also have a major bearing on management options. In particular, there is a need to predict the longer-term effects on freshwaters of abatement strategies now being initiated and to inform expectations of the timescales required for full recovery (Jenkins *et al.* 2003a). Prominent among such modelling approaches are hydrochemical tools such as MAGIC (Model of Acidification of Groundwaters in Catchments)(Cosby *et al.*, 1985a, b) that

simulate long-term trends in surface water chemistry in response to deposition. However, such hydrochemical models give a complete and realistic indication of recovery only when they predict effects on biological targets. Although such biological prediction is possible from empirical data (Ormerod *et al.* 1988), episodes have seldom been incorporated into models due to the inherent difficulties of modelling the processes and their impacts (e.g. Weatherley & Ormerod 1991; Gerritsen *et al.* 1996; Van Sickle *et al.* 1996). With episodes increasingly a central feature of biological recovery from acidification in streams, the ability to model episodic impacts is becoming an important need.

In this chapter, I developed statistical models to simulate the effects of acid episodes on macroinvertebrates in 89 upland streams spread across acid-sensitive areas of Wales and Scotland. I compared the effectiveness of models drawn from different acid-base determinands in predicting assemblage composition or taxon-specific abundance, and I compared models drawn from base-flow and storm-flow chemistry at these sites. Finally, I illustrated the application of the resulting models to average trends apparent currently from the UK Acid Waters Monitoring Network (Evans & Monteith 2001) to project likely future changes among invertebrates.

5.3. METHODS

5.3.1. *Study area*

Models were calibrated using purpose-collected data from 89 independent streams in regions that are mostly sensitive to acidification but receive contrasting acid deposition. All were second- and third-order streams in the northwest Scottish Highlands ($n = 24$), Galloway in south-west Scotland ($n = 25$), north Wales ($n = 19$) and locations in central Wales centred on the Llyn Brianne experimental catchments ($n = 21$; Appendix 1). The exact site selection reflected a combination of logistics, access and previous knowledge. In Wales and Galloway, sites were dominantly from areas known to acid-impacted, while sites in the Highlands were drawn more randomly but stratified crudely by land-use.

Sensitivity in these areas reflects soils and geology. In the northwest Highlands, many catchments are underlain by schists, gneisses, quartzites, granites and sandstones of limited buffering capacity interbedded with old red sandstone, limestones and shelly sands of medium buffering capacity. In Galloway, most rocks are granites, greywackes and shales but there are also areas with more base-rich basalt. In Wales, sites in the upper Conwy are underlain by acid igneous rocks, Ordovician and Silurian shales with low to medium buffering capacity but occasionally more basic lavas. The central Welsh sites were on Ordovician and Silurian shales, mudstones and grits (Stoner, 1984; Weatherley & Ormerod, 1987)

Ameliorations in S and N deposition over western Britain, and hence rainfall pH, have roughly tracked emissions since their peak in the late 1970s (Fowler *et al.*, 2001). Critical loads for mostly standing waters at adjacent representative locations (Conwy = 41, mid Wales = 26, Galloway = 55, and the NW Highlands = 98) are now exceeded on the steady-state criterion of ANC ($<20 \mu\text{eq L}^{-1}$) for a small part of the NW Highlands (6%), around 30% of Wales and 42% of Galloway (Hall *et al.*, 2004). Concentrations of sulphate in previously acidified streams are declining, while mean pH values have increased by around 0.2-0.4 units over the last decade (Evans *et al.* 2001).

5.3.2. Sampling

Water samples were collected from each site during storm events (Galloway, October 2001; northwest Highlands, Conway and mid Wales, January/February 2002) and at base-flow (northwest Highlands and Galloway, April 2002; Conwy and mid-Wales August 2002). This method effectively separates event chemistry from base-flow (Lepori *et al.* 2003b) and avoids any confounds or errors, for example where episodic values are not sufficiently differentiated from means calculated at the same sites (Weatherley & Ormerod 1991; Brewin *et al.* 1996). Samples were analysed for a wide range of determinands using methods reported fully elsewhere. Both episodic variations and major anions driving episodes were corroborated strongly to variations among invertebrates (Kowalik *et al.* ms

submitted). pH was recorded on-site using a hand-held combined pH/EC/TDS meter with an electrode specially designed for low ionic-strengths (Hanna Instruments HI 991300). Charge-balance acid neutralising capacity (ANC) calculated as the sum of base cations minus the sum of acid anions ($\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+} - \text{NO}_3^- - \text{SO}_4^{2-} - \text{Cl}^-$).

Invertebrates were collected from each stream using semi-quantitative kick-samples (standard net with 1 mm mesh size) during April 2002. Two-minute riffle and one-minute margin samples aimed to represent taxa from all stream habitats. The method is well-calibrated and detects differences reliably between sites (Weatherly & Ormerod, 1987; Bradley & Ormerod 2002b). Samples were preserved on-site by adding 100% IMS (Industrial Methylated Spirit) to the existing sample volume. In the laboratory, sorting and identification followed procedures described elsewhere (Kowalik *et al.* unpubl.). Species or genera were identified in the orders Plecoptera, Ephemeroptera, Trichoptera and Coleoptera, while dipterans were identified to family. These data allowed an assessment of either overall community composition or taxon-specific abundance.

5.3.3. Data analysis

Macroinvertebrate abundances and all chemical variables except pH were \log_{10} or \log_{+n} transformed to homogenise variances as far as possible. Stepwise regression or multiple regression was then used to relate the abundance or community composition of invertebrates to stream chemistry. The representation of invertebrate composition for this purpose required reduction to a synoptic, continuous variable that represented major variations. I used Principal Components Analysis (PCA) on the correlation matrices between taxon abundances (usually species) across all sites. Detrended correspondence analysis was explored, but axis lengths were small (< 50% species turnover) and the data sufficiently rectilinear for PCA to represent a preferred option (ter Braak, 1995). Contributions by each taxon to the PCs were interpreted from their loading values. Changes in their probabilities of occurrence along each PC were determined for the sites in each segment of each major component thus indicating how assemblages would be

expected to change as pH varied. To derive the actual chemical models, PC scores for each site were regressed against acid-base determinands at either storm-flow, base-flow or a combination of the two. These analyses involved a stepwise procedure in which all possible explanatory chemical variables were available for selection provided that each individually explained a significant fraction of overall variation based on *t*-ratios ($P < 0.05$). Comparisons were also made with models in which some potentially important acid-base indicators (eg ANC) had not been automatically selected. Final regressions were assessed from the coefficient of determination (R^2 , adjusted for degrees of freedom) coupled with a lack-of-fit test ($P < 0.05$). Models were also checked for the effects of multicollinearity by assessing variance inflation factors (VIF), with values > 10 indicating multicollinearity (Iles, 1993).

In all cases, models were calibrated from 75% of the survey sites ($n = 67$) and then tested independently against the remaining 25% ($n = 22$) by correlating the actual invertebrate PC scores against those predicted.

To illustrate models for individual invertebrate taxa, I used an ephemeropteran genus known to be acid-sensitive (*Baetis* spp.) and a plecopteran genus known to be acid tolerant (*Chloroperla* spp.). For each, abundances were regressed against low and high-flow chemical data as above, with model calibration ($n = 67$) and test sites ($n = 22$) again separated. All formal analytical procedures were identical.

5.3.4. Scenario modelling

To illustrate a potential model application, I developed four possible water quality scenarios for a model acidified stream – typical of the experimental catchments at Llyn Brianne (Weatherley & Ormerod 1987). I assumed a starting base-flow pH of 5.2 in 2000, and thereafter progressively increased pH in each decade at rates equivalent to the median (*Scenario 1*: 0.21 pH units/decade), lower quartile (*Scenario 2*: 0.11 pH units/decade) and upper quartile (*Scenario 3*: 0.45 pH units/decade) range of significant trends apparent

across the United Kingdom Acid Waters Monitoring Network (Evans & Monteith 2001). For each of these scenarios, the pH minimum for each base-flow pH was estimated from a relationship in extensive Welsh survey data described by Weatherley & Ormerod (1991):

$$\text{Minimum pH} = 3.82 + 0.00217e^{(\text{base-flow pH})}$$

In a fourth scenario (*Scenario 4*), base-flow pH trends were identical to *Scenario 1* except I simulated worst-case conditions by substituting the lowest minimum pH for any given base-flow pH recorded across Weatherley & Ormerod's (1991) survey. In other words, *Scenario 4* represented conditions under which severe acid episodes continued despite amelioration in average conditions. In all these model applications, the assumptions were that current pH trends at the UKAWMN would continue at a linear rate into the future; that average trend data from all lake/stream sites in the UKAWMN could represent trends in streams; that mean pH described by Weatherley & Ormerod (1991) dominantly reflected base-flow conditions; and, for Scenarios 1-3, that the relationship used to link minimum to base-flow pH would persist over decades into the future. *Scenario 4* did not make this latter assumption, but instead assumed that episodic pH could continue to fall substantially as base-flow pH rose.

In each model year (2000, 2010, 2020...), I used base-flow and storm flow-pH separately in each scenario to predict i) changes in invertebrate PCA scores which were then converted into probability of occurrence of the acid sensitive *Baetis muticus*; ii) changes in the abundance of all *Baetis* spp.

5.4. RESULTS

5.4.1. Invertebrate assemblage composition

In PCA, one major component (PC1) explained 22% of all variation in invertebrate assemblage composition. Correlating significantly with most measures of acid-base status (see below) it represented a progression from ephemeropterans and other acid-sensitive

taxa at negatively scoring sites to acid-tolerant taxa such as nemourid and leuctrid stoneflies at positive sites (Table 1). Overall scores differed significantly between regions (Kruskall-Wallis $H = 17.72$, $df = 2,87$ $P < 0.001$) due to the greater frequency of acid streams in Wales (median PC1 score = -0.53) than in Galloway (median = -2.26) or the NW Highlands (median = -1.92), but the overall trends across sites were similar (Fig. 1).

Individual taxa varied in probability of occurrence across different segments of the PC. For example, ephemeropterans such as *Rhithrogena semicolorata* and the acid sensitive beetle *Hydraena gracilis* declined in probability of occurrence at sites with positive PC scores where acid tolerant *Leuctra nigra* and *Nemurella pictetii* increased (Fig. 2). Similar responses were clear in many organisms although a small number had apparently wide tolerance (see Appendix 1).

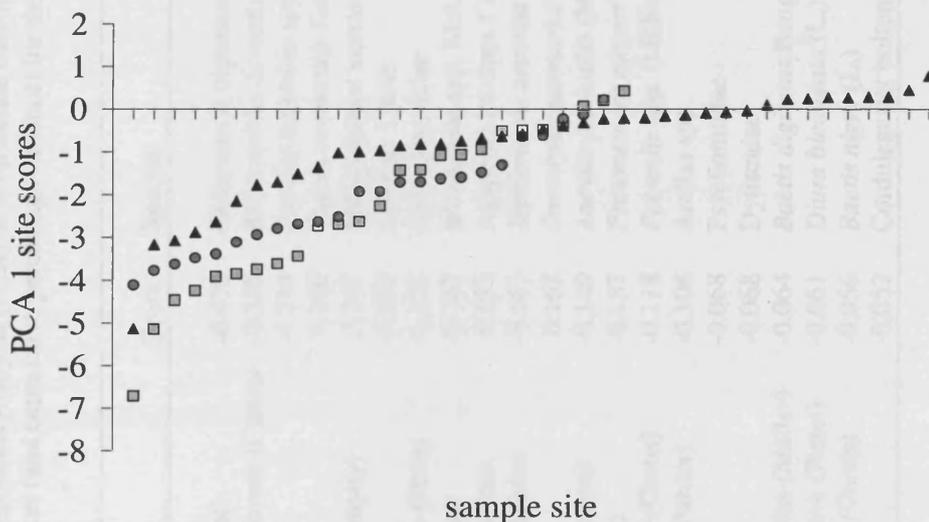


Figure 1. Regional variation in scores along a principle component reflecting the effects of acid-base status on invertebrates (see Table 1). Site scores for Wales (▲), Galloway (■) and NW Highlands (●) are arranged in increasing order.

Table 1. Principle Components Analysis (PCA) of invertebrate community composition across 89 sites in Wales and Scotland showing species scores along PC 1. Eigenvalues (and cumulative percentage variance) for the first three PCA axes were respectively 2.27 (22.6), 1.36 (35.1) and 0.92 (43.5).

Species	Score	Species	Score	Species	Score
<i>Baetis rhodani</i> (Pictet)	-0.478	<i>Chloroperla tripunctata</i> (Scopoli)	-0.050	<i>Helophorus</i> spp.	0.004
<i>Rhithrogena semicolorata</i> (Curtis)	-0.358	<i>Rhyacophila dorsalis</i> (Curtis)	-0.049	<i>Pisidium</i> spp.	0.004
<i>Baetis muticus</i> (L.)	-0.314	<i>Paraleptophlebia</i> spp. Lestage	-0.038	Hydracarina	0.006
Coleopteran larvae	-0.300	<i>Caenis rivulorum</i> Eaton	-0.033	<i>Phagocata</i> spp.	0.007
<i>Leuctra inermis</i> (Kempny)	-0.247	<i>Philopotamus montanus</i> (Donovan)	-0.033	<i>Leuctra hippopus</i> (Kempny)	0.010
Hydropsychidae	-0.224	<i>Silo</i> spp. Curtis	-0.031	<i>Nemoura</i> spp.	0.010
<i>Isoperla grammatica</i> (Poda)	-0.222	Glossiphoniidae	-0.027	<i>Sialis fuliginosa</i> Pictet	0.010
<i>Ecdyonurus</i> spp. Eaton	-0.187	<i>Wormaldia</i> spp. McLachlan	-0.025	Limnephilidae	0.012
<i>Hydraena gracilis</i> Germar.	-0.175	<i>Agapetus fuscipes</i> Curtis	-0.022	Pyralidae	0.012
<i>Gammarus pulex</i> Fabricius	-0.163	<i>Siphonurus armatus</i> (Eaton)	-0.020	<i>Anacaena globulus</i>	0.014
Chironomidae	-0.162	<i>Oreodytes sanmarkii</i> (Sahlberg)	-0.019	<i>Siphonurus lacustris</i> Eaton	0.014
<i>Brachyptera risi</i> (Morton)	-0.149	<i>Ancylus fluviatilis</i> (Müller)	-0.019	<i>Nemoura cambrica</i> (Stephens)	0.020
<i>Elmis aenea</i> (Müller)	-0.137	<i>Protonemura meyeri</i> (Pictet)	-0.017	<i>Agabus didymus</i>	0.024
<i>Heptagenia lateralis</i> (Curtis)	-0.118	<i>Polycelis</i> spp. (Müller)	-0.017	<i>Amphinemura sulcicollis</i> (Stephens)	0.03
<i>Limnius volckmari</i> (Panzer)	-0.108	<i>Asellus</i> spp.	-0.015	<i>Leptophlebia</i> spp. Westwood	0.035
Tipulidae	-0.068	Psychomiidae	-0.011	Simuliidae	0.052
Oligochaeta	-0.068	Dytiscidae	-0.004	<i>Leuctra nigra</i> (Olivier)	0.092
<i>Esolus parallelepipetus</i> (Müller)	-0.064	<i>Baetis digitatus</i> Bengtsson	-0.003	<i>Plectrocnemia</i> spp. McLachlan	0.130
<i>Chloroperla torrentium</i> (Pictet)	-0.061	<i>Diura bicaudata</i> (L.)	0.000	<i>Nemoura cinerea</i> (Retzius)	0.134
<i>Dinocras cephalotes</i> (Curtis)	-0.056	<i>Baetis niger</i> (L.)	0.000	<i>Nemurella picteti</i> Klapálek	0.143
<i>Oulimnius</i> spp.	-0.052	<i>Cordulegaster boltonii</i>	0.001		

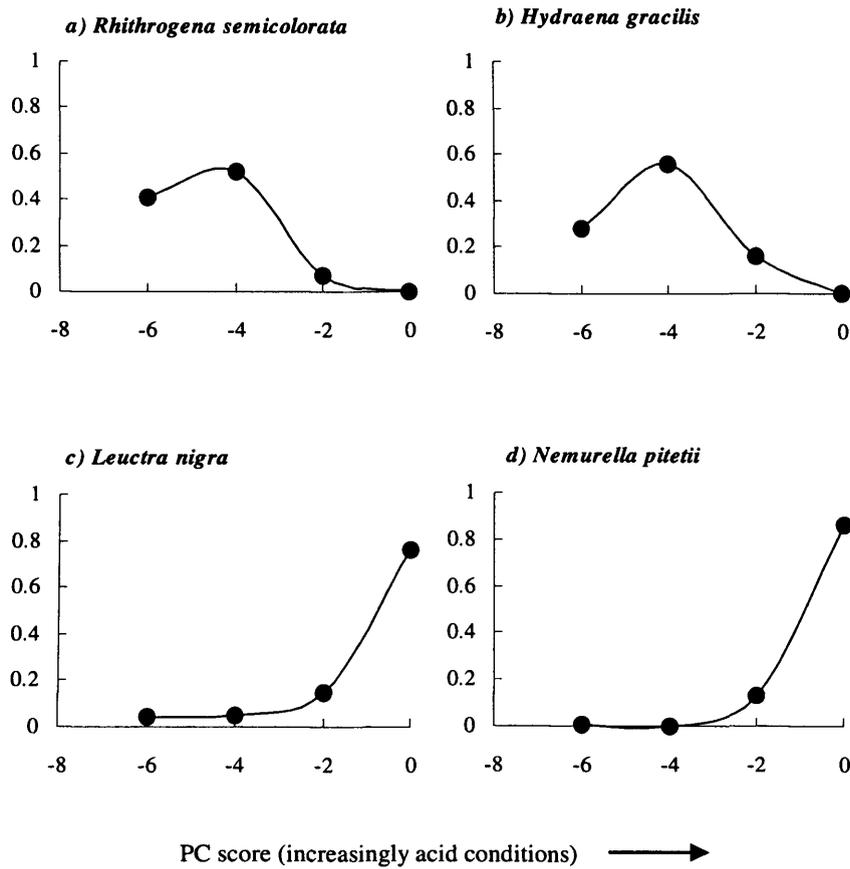


Figure 2 Changes in the probability of occurrence of selected invertebrate taxa between segments of a principle component reflecting the effects of acid-base status on invertebrates (See Table 1). Similar probability values are given for other taxa in Appendix 2 and allow the probabilistic recreation of invertebrate assemblages for a given set of acid-base conditions.

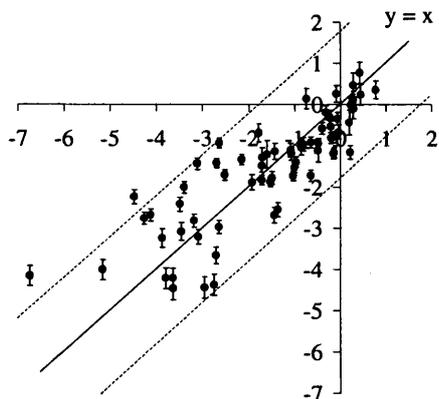
5.4.2. Model calibration and testing: invertebrate communities

ANC alone was a poor predictor of invertebrate community composition, explaining c45% of the variance in PC scores at both storm-flow and base-flow (Table 2). ANC models also gave poor overall fit to the data. ANC in combination with pH did not significantly improve prediction based on pH alone at either low- or high-flow ($R^2_{\text{adj}} = 0.62$ and 0.65 respectively).

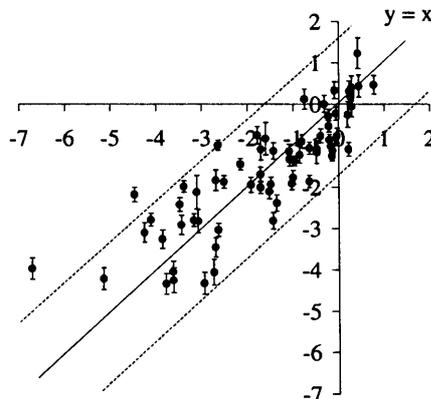
Regression models using other combinations of pH, DOC, Ca^{2+} , $\text{Al}^{\text{n}+}$ and NO_3^- always explained around 60-70% of the variance in invertebrate PC scores. The single best predictor of invertebrate assemblages was pH, and this variable was always selected first in any stepwise regression model (Table 2).

Storm-flow chemistry on average explained more variance (64-69%) than base-flow (62-64%). However, differences were seldom significant and combined pH measurements from both base- and storm-flow ($R^2_{\text{adj}} = 0.69$), or in conjunction with Ca^{2+} ($R^2_{\text{adj}} = 0.71$), were often effective predictors of invertebrate assemblages based on explained variance (Figure 3a and b). Predicted invertebrate scores at the 22 test sites followed observed scores closely ($r = 0.65$ - 0.75 ; $P < 0.01$). There was some minor under-prediction where sites held more acid-sensitive species than the models indicated but this effect was confined to circumneutral sites, (Figure 3c and d).

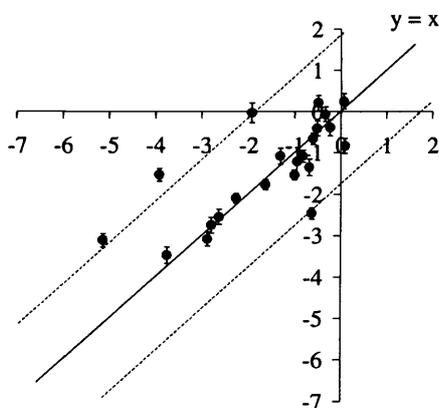
a) $\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}}$ (calibration)



b) $\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}} + \text{Ca}^{2+}_{\text{l-f}}$ (calibration)



c) $\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}}$ (test)



d) $\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}} + \text{Ca}^{2+}_{\text{l-f}}$ (test)

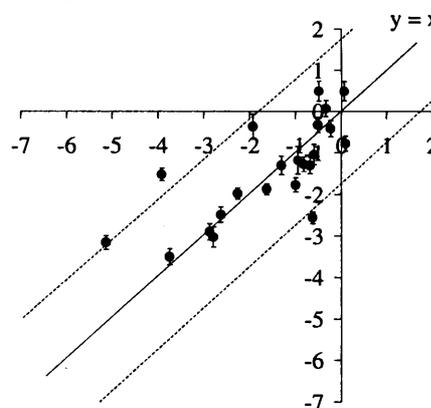


Figure 3. Predicted (y-axis) and observed (x-axis) PC scores describing invertebrate assemblages at sites across Wales and Scotland (\pm estimated SD of the fit). The models were calibrated at 67 sites using low-flow (l-f) and/or high-flow (h-f) chemistry and plots a) ($\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}}$; $R^2_{\text{adj}} = 0.69$) and b) ($\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}} + \text{Ca}^{2+}_{\text{l-f}}$; $R^2_{\text{adj}} = 0.70$) indicate performance at the calibration sites. Models were then tested at 22 independent sites (c and d). Also shown are the upper and lower prediction intervals (at 95% level of significance) around the $y = x$ line.

Table 2. Results of regression analyses predicting invertebrate species composition (as PC 1 site scores) from chemical data collected at base-flow (l-f), storm-flow (h-f) and both flows combined. Regression parameters are indicated as intercept and slope (a_1 , a_2 , a_3 and a_4) with standard errors (\pm) for each in parentheses. All R^2 values are adjusted for d.f. while results from ANOVA (F-statistic, df = 65, 64, 63 or 62 for one, two, three or four predictor variables) and lack-of-fit test are also shown (significance of the regression model not fitting the data, $P < 0.1^*$). All chemical variables were \log_{10} transformed ($\log_{10} + 300$ for ANC to accommodate negative values) with the exception of pH. Original concentrations were in mg L^{-1} except for $\mu\text{eq L}^{-1}$ for ANC.

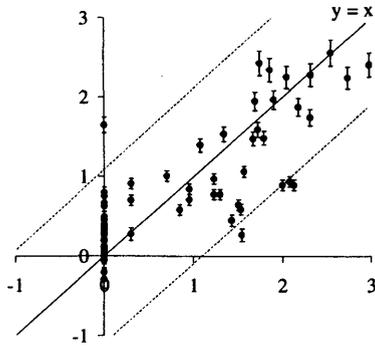
Flow	Intercept	a_1	a_2	a_3	R^2	F	Fit
Base	7.276 (1.20)	-3.225 (0.44) ANC	-	-	0.45	54.92***	0.005*
	8.270 (0.94)	-1.491 (0.14) pH	-	-	0.62	110.60***	>0.1ns
	8.622 (1.03)	-1.322 (0.24) pH	-0.535 (0.60) ANC	-	0.62	55.51***	0.018*
	7.834 (0.95)	-1.488 (0.14) pH	0.667 (0.34) DOC	-	0.64	59.76***	>0.1ns
Storm	10.18 (1.57)	-4.540 (0.60) ANC	-	-	0.46	56.58***	<0.001*
	5.130 (0.62)	-1.199 (0.11) pH	-	-	0.65	120.91***	>0.1ns
	5.490 (0.66)	-1.208 (0.11) pH	-0.527 (0.35) DOC	-	0.65	62.62***	>0.1ns
	4.985 (0.61)	-1.040 (0.13) pH	0.641 (0.30) Aln+	-	0.66	66.10***	>0.1ns
	7.480 (1.06)	-1.665 (0.20) pH	1.257 (0.47) Ca2+	-	0.68	69.71***	>0.1ns
	6.011 (0.64)	-1.278 (0.10) pH	0.444 (0.13) NO3-	-	0.69	75.37***	>0.1ns
Both	7.335 (0.89)	-0.706 (0.18) pHh-f	-0.751 (0.23) pHl-f	-	0.69	74.79***	>0.1ns
	9.217 (1.15)	-1.155 (0.25) pHh-f	-0.690 (0.22) pHl-f	1.104 (0.45) Ca2+h-f	0.71	55.91***	>0.1ns

One other consideration in selecting storm-flow or base-flow data in modelling was that some differences were apparent in the predictive effects of some determinands. For example, increased DOC at base-flow significantly increased invertebrate scores thereby moving sites towards acid assemblages. By contrast, increased DOC at storm-flow had significant negative effects and apparently moved sites away from acid assemblages. Aluminium and nitrate at storm flow had apparently positive effects on invertebrate scores indicating increased concentrations associated with acid conditions (Table 2). This was also true of calcium implying that, after the effects of pH had been taken into account, increased concentrations tended to be associated with more acid-type assemblages. This would result, for example, from increase base-cation release at acid-impacted locations.

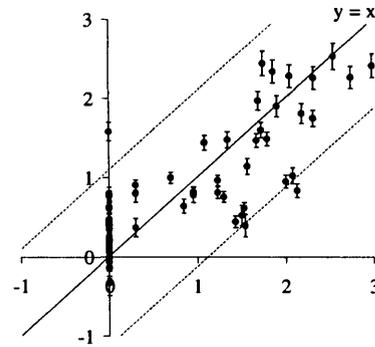
5.4.3. Model calibration and testing: genus-level abundance

Stream chemistry predicted only 26-35% of the overall variance in the abundance of the acid-tolerant plecopteran *Chloroperla* spp and predictor variables were unrelated to acid-base status (e.g. K^+ , NH_4^+ , Cl^- , PO_4^{3-} and Si^{4+}). Predictability was relatively poor at test sites ($r = 0.20-0.38$) (Table 3). By contrast, the abundance of the acid-sensitive ephemeropteran *Baetis* spp. was predicted well by acid-base variables in regressions with R^2_{adj} 52-67% (Fig. 4). High-flow pH performed considerably better than base-flow (Table 3). Predicted abundances for *Baetis* spp. followed real abundances at the 22 test sites closely, with performance again better using storm-flow data ($r = 0.73-0.86$) (Figure 4c,d).

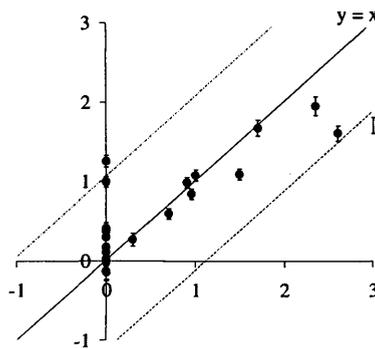
a) $\text{pH}_{\text{h-f}}$ (calibration)



b) $\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}}$ (calibration)



c) $\text{pH}_{\text{h-f}}$ (test)



d) $\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}}$ (test)

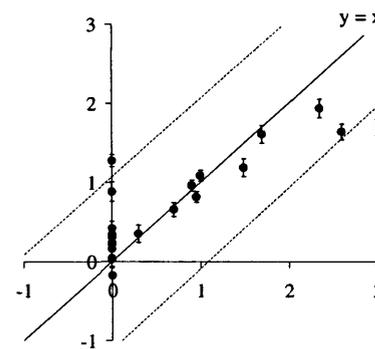


Figure 4. Predicted (y-axis) and observed (x-axis) \log_{10} abundances of *Baetis* spp. at sites across Wales and Scotland (\pm estimated SD of the fit). The models were calibrated at 67 sites using low-flow (l-f) and/or high-flow (h-f) chemistry. Plots a) ($\text{pH}_{\text{h-f}}$; $R^2_{\text{adj}} = 0.67$) and b) ($\text{pH}_{\text{h-f}} + \text{pH}_{\text{l-f}}$; $R^2_{\text{adj}} = 0.67$) indicate performance at calibration sites and b) and c) show performance at test sites. Also shown are the upper and lower prediction intervals (at 95% level of significance) around the $y = x$ line.

Table 3. Results of regression analysis predicting the abundances ($\log_{10} + 1$) of *Chloroperla* spp. and *Baetis* spp. All conventions are as in Table 2.

	Flow	Intercept	a_1	a_2	a_3	a_4	R^2	F	Fit
<i>Chloroperla</i>	Base	0.661(0.70)	1.085(0.31)Cl ⁻	0.724(0.31)PO ₄ ³⁻	-0.378(0.17)Al ⁿ⁺	-	0.26	8.76***	>0.1ns
	Storm	6.155(0.92)	-0.575(0.18)Al ⁿ⁺	0.782(0.25)Si	0.375(0.14)pH	-2.926(0.56)ANC	0.35	9.90***	>0.1ns
	Both	5.075(0.91)	0.500(0.13)pH _{h-f}	-2.168(0.61)ANC _{h-f}	-0.937(0.32)Cl _{1-f} ⁻	-	0.32	11.17***	>0.1ns
<i>Baetis</i>	Base	-4.351(0.61)	0.780(0.09)pH	-	-	-	0.52	71.82***	0.005*
	Storm	-3.113(0.34)	0.699(0.06)pH	-	-	-	0.67	134.44***	>0.1ns
	Both	-3.555(0.53)	0.151(0.14)pH _{1-f}	0.600(0.11)pH _{h-f}	-	-	0.67	68.07***	>0.1ns

5.4.4. Scenario modelling.

The four scenarios varied considerably in rates of base-flow pH increase, and consequently also in storm-flow pH. Thus, if pH in the model stream increased at the fastest rates apparent in the UKAWMN, and if pH minima tracked this trend (Weatherley & Ormerod 1991), storm-flow would reach pH 6 shortly before 2040. Biological recovery would be well underway as illustrated by the abundance and probability of occurrence of *Baetis* spp. Rates of biological recovery would be considerably slower if pH increased at the slowest rates in the UKAWMN (Figure 5).

A range of effects due to simulated episodes were apparent. For each of Scenarios 1-3, models based on storm-flow pH indicated that recovery among sensitive invertebrates would be retarded by 10-20 years by comparison with trends suggested by base-flow pH (Figure 5). Simulations using storm-flow pH also indicated more pronounced eventual recovery once invertebrates were released from the negative effects of acid episodes (pH <5.5) at base-flow pH >6.5 (e.g. after 2080 in *Scenario 1* and after 2040 in *Scenario 2*). Most interesting of all, the worst-case scenario, with continuing severe episodes despite increasing base-flow pH (*Scenario 4*), effectively offset recovery among acid-sensitive taxa until 2080. Final abundances among acid sensitive *Baetis* spp. in this scenario were modest even by 2090.

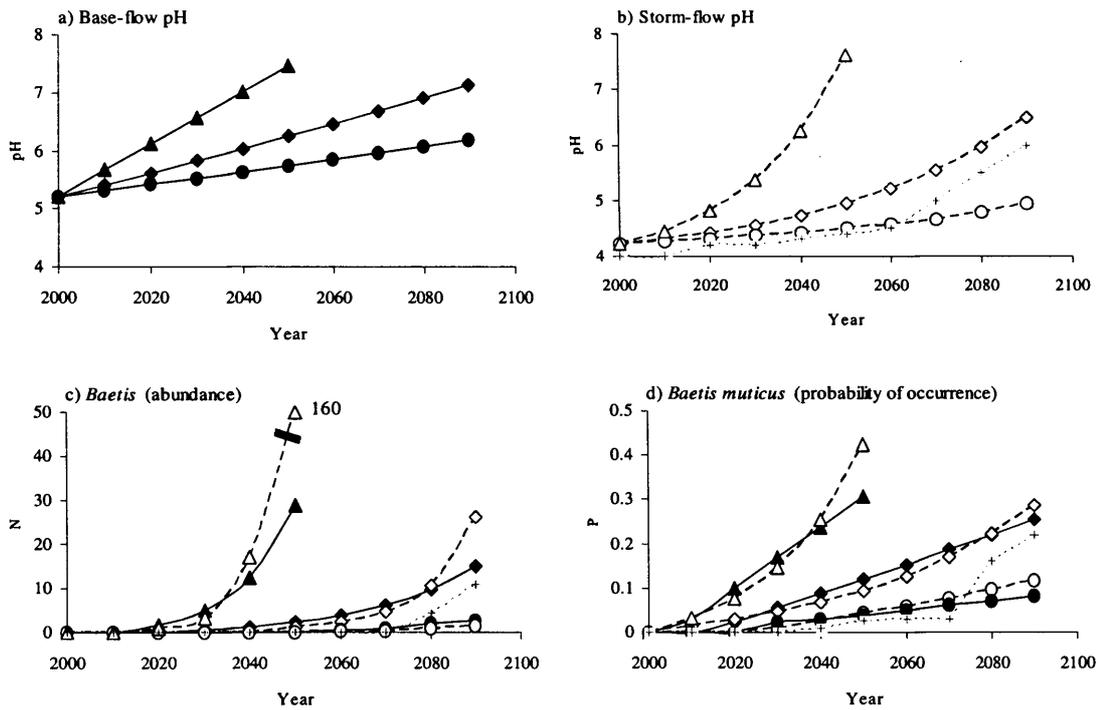


Figure 5 Outputs simulating change in base-flow pH (a), storm-flow pH (b), the abundance of *Baetis* spp. (c; from models using pH alone) and changes in the probability of occurrence of *Baetis muticus* (d; from PCA and models using pH alone) under 4 scenarios based on current trends at the UK Acid Waters Monitoring Network. *Scenario 1* (◆ = median rate of pH increase of 0.21 pH units per decade); *Scenario 2* (● = lower quartile rate of increase of 0.11 pH units/decade); *Scenario 3* (▲ = upper quartile rate of pH increase of 0.45 pH units per decade); *Scenario 4* (+ = as for *Scenario 1* but with worst-case episodic pH for any given base-flow pH). Open symbols and broken lines are values modelled on storm-flow pH; closed symbols and solid lines are modelled on base-flow pH.

5.5. DISCUSSION

Attempts to model the potential chemical recovery of freshwaters from acidification are now well established from applications in both Europe and North America (Cosby *et al.* 1985; Wright *et al.* 1991; Jenkins *et al.* 2003b). However, models that reflect hydrochemistry alone have limited management relevance given the major impacts of acidification on organisms, ecological processes and their attendant resource value (Muniz 1990). The need to link acidification modelling with biological responses is now emphasised particularly in Europe by the drive to (re-)establish good ecological status for all freshwaters under the EU Water Framework Directive (2000/60/EC; Jenkins *et al.* 2003b). So far, models simulating biological recovery have been few, so that the current illustration of a potentially successful approach is encouraging. As with previous biological models of acidification, the approach requires that observed relationships between invertebrates and chemistry across sites can represent likely trends through time. The strengths, weaknesses and assumptions of this general method have been explored or tested previously (Ormerod *et al.* 1988). They include, for example, the need for extirpated organisms to colonize and persist in recovering streams in ways that are represented by empirical variations across sites. Confidence in the models comes from the repeatability of relationships between invertebrates and acid-base status across sites (eg Ormerod & Weatherly 1987; Rutt *et al.* 1990; Lepori *et al.* 2003a; this study). Important validations and indications of limitations have also arisen from large-scale experiments (Bradley & Ormerod 2002a; see below).

In general, invertebrate distributions were effectively modelled from acid-base variables: pH alone or in combination with other predictors always explained over 60% of the variance in assemblage scores and over 50-67% of the variance in the abundance of *Baetis* spp. Both at test sites and calibration sites, patterns closely followed those predicted with standard errors around regression parameters in pH models usually <10-12% of the estimated mean. Two exceptions were the abundance of acid-tolerant plecopterans, whose distribution is often unrelated to acidity, and models using ANC as the sole explanatory

variable. ANC performed particularly poorly in model calibration and produced assemblage scores which departed significantly from those observed at calibration or test sites. These patterns are of some concern given that ANC is now a central feature in the modelling or estimation of critical loads throughout Europe. One possibility is that ANC indicates thresholds or average chemical conditions under which some organisms become scarce at acidified sites (Henriksen *et al.* 1999), but does not represent accurately the progressive and dynamic ecological effects of hydrogen ions or metals along acid-base gradients (Holt *et al.* 2003). By contrast, field and laboratory evidence accumulated over three decades has revealed how acidity or aluminium affect many species in acidified locations either through direct toxicity or consequent ecological process (Sutcliffe & Carrick 1973; Ormerod *et al.* 1987; Ledger & Hildrew *in press*). Such effects still occur in upland British streams with the consequences most marked at sites affected by strong acids of anthropogenic origin (Kowalik & Ormerod *ms submitted*; Kowalik *et al.* *ms submitted*). Models using pH and/or aluminium are therefore most likely to reflect real processes in addition to being the most accurate biological predictors examined.

While effort aimed so far at modelling the biological effects of acidification and recovery has been small, the processes represented have also been limited. Most important in the present context, acid episodes have seldom been incorporated (Gerritsen *et al.* 1996; Lacroix & Korman 1996; Van Sickle *et al.* 1996; Marmorek *et al.* 1998). This is a potentially serious omission given increasing evidence that a mismatch between hydrochemical and biological recovery from acidification might reflect episodic effects (Bradley & Ormerod 2002a; Kowalik *et al.* *ms submitted*). As far as I know, only Weatherley & Ormerod (1991) and Lepori & Ormerod (*in press*) have modelled the effects of episodes on aquatic invertebrates. As in this work, Weatherley & Ormerod (1991) showed how measures of episodicity moderately increased precision when predicting invertebrate assemblages. However, they suggested that biological models based on mean pH or aluminium were reasonable since they avoided model complexity, and were justified because means and minima were in any case inter-correlated. I believe that this suggestion is no longer tenable for four reasons. First, overwhelming evidence now indicates that episodes are central to the effects of acidity on stream organisms so that models excluding

episodes lack realism (Merret *et al.* 1991; Lepori *et al.* 2003a; Hirst *et al.* 2004; Kowalik *et al.* ms submitted). This evidence includes direct experimental tests revealing a shortfall in previous models based on mean chemistry (Bradley & Ormerod 2002a). Further evidence in this study arose because episodes improved abundance models for *Baetis* – among the most sensitive of all organisms to brief pH exposure (Ormerod *et al.* 1987; Kratz *et al.* 1994; Lepori & Ormerod in press; Kowalik & Ormerod ms submitted). Moreover, some variables – notably aluminium - only had significant effects on invertebrates only at storm-flow where toxicity would be greatest (Muniz 1990). Secondly, acidification models are now largely focussed on recovery and therefore need to capture possible limits imposed by episodes (Raddum *et al.* 2001; Bradley & Ormerod 2002a). Accounting for such effects will become important if severe episodes (pH < 5.5) persist at otherwise recovering sites (eg *Scenario 4*). This situation could arise for a range of reasons including changing climate, depleted soil base saturation, mobilisation of stored acid anions, oxidation of organic soil sulphate following drought or increasing nitrogen break-through from soils (Stoddard *et al.* 1999; Alewell *et al.* 2001; Campbell & Eager 2002; Lawrence 2002). Thirdly, the capability to isolate (and hence model) episodic effects on organisms is now known to be enhanced by selectively sampling storm-flow and base-flow rather than relying on random sampling to calculate mean chemistry (Weatherely & Ormerod 1991; Brewin *et al.* 1996; Lepori *et al.* 2003b). Fourthly, as the simulations show, model outcomes based on episodes are different from base-flow and could have an important bearing on indicating expected trends (see Fig. 5). In this respect, recent indications that models of episodic effects on *Baetis alpinus* not only reflect observed acid toxicity, but also responses in whole invertebrate assemblages, gives some confidence to episodic modelling approaches (Lepori & Ormerod in press)

One interesting aspect of the models was in the apparently contrasting effects of DOC between flows. Regression parameters hinted that DOC apparently increased acidification effects at base-flow but moderated effects at storm-flow when organic acidity represented around 5-9% of anion loading (Kowalik *et al.* ms submitted). Although experimental data to support these apparently dual effects are still scarce, patterns were consistent with the theoretical view that organics can contribute acidity but also ameliorate metal toxicity

through complexation (Kullberg *et al.* 1993). In parts of Galloway, chemical recovery from acidification has led to a decline in toxic forms of aluminium in favour of less toxic organic forms (McCartney *et al.* 2003). However, effects in the study were small and variable (SE > 40%), and also insufficient to wholly protect acid-sensitive assemblages at sites characterised by organic acidity (Kowalik *et al.* ms submitted). Moreover, metal toxicity can still occur in acid sensitive waters even with elevated DOC (Laudon *et al.* 2005).

In the final analysis, the trends simulated by projections of recovery were most critically affected by the choice of model structure, and by the rates selected of underlying chemical change. Thus, differences in pH increase between scenarios that had the greatest effect on simulated recovery providing that episodes abated in step with base-flow pH. However, within scenarios, the choice of whether or not to incorporate episodes affected both rates and endpoints of biological recovery. More substantially of all, delays in biological recovery of up to 50 years occurred where severe episodes of pH < 5.5 were allowed to continue when base-flow pH increased. All of these contrasting outcomes might be equally plausible and reveal that predictions about recovery from acidification depend fundamentally on assumptions about processes or trends that are not yet well quantified. For example, the extrapolations about hydrochemical trends from the UK Acid Waters Monitoring Network require current rates of base-flow pH increase to continue linearly into the future – at least until some upper limit at which sites are once more well-buffered by weathering as predicted by MAGIC (eg Jenkins *et al.* 2003b). Validation of this assumption clearly requires the collection of further monitoring data. More importantly, there are few quantitative data illustrating how the severity of pH episodes will track increasing base-flow pH in future. In Scandinavia, Laudon & Hemmond (2002) have shown how the anthropogenic contribution to acid episodes has declined with declining sulphur deposition. However, as indicated above, a range of natural and anthropogenic processes could continue to cause episodic acidification for decades to come in recovering streams either locally or generally. Key among these processes will be future interactions with the flood-drought effects of changing climate and the potential future effects of nitrogen (Skelvare *et al.* 2003). Any such effects might make *Scenario 4* a real possibility,

while impacts on invertebrates or other organisms could occur irrespective of acid sources (Kowalik *et al.* ms submitted). The strongest recommendations from this work are therefore that there should be better understanding and monitoring of the hydrochemical processes driving acid episodes and their likely biological effects as acid deposition abates.

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Appendix 1. Probability of occurrence of each species in sites located in different segments of a Principal Component describing site trends along a gradient of acid-base status (see Figure 1, Table 1 and Table 2).

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Species	1.1 to -1.0	-1.1 to -3.0	-3.1 to -5.0	-5.1 to -7.0	Species	1.1 to -1.0	-1.1 to -3.0	-3.1 to -5.0	-5.1 to -7.0
<i>Baetis rhodani</i>	0.00	0.56	0.25	0.19	<i>Oreodytes sanmarkii</i>	0.32	0.29	0.39	0.00
<i>Rhithrogena semicolorata</i>	0.00	0.07	0.52	0.41	<i>Ancylus fluviatilis</i>	0.00	0.40	0.60	0.00
<i>Baetis muticus</i>	0.00	0.24	0.41	0.35	<i>Protonemura meyeri</i>	0.57	0.22	0.13	0.08
Coleopteran larvae	0.07	0.32	0.38	0.24	<i>Polycelis</i> spp.	0.00	0.21	0.79	0.00
<i>Leuctra inermis</i>	0.23	0.35	0.32	0.09	<i>Asellus</i> spp.	0.07	0.00	0.93	0.00
Hydropsychidae	0.06	0.32	0.48	0.14	Psychomyiidae	0.27	0.07	0.67	0.00
<i>Isoperla grammatica</i>	0.22	0.30	0.28	0.21	Dytiscidae	0.46	0.31	0.12	0.12
<i>Ecdyonurus</i> spp.	0.00	0.36	0.43	0.22	<i>Baetis digitatus</i>	0.00	1.00	0.00	0.00
<i>Hydraena gracilis</i>	0.00	0.16	0.56	0.28	<i>Diura bicaudata</i>	0.25	0.58	0.17	0.00
<i>Gammarus pulex</i>	0.00	0.50	0.21	0.30	<i>Baetis niger</i>	0.00	1.00	0.00	0.00
Chironomidae	0.26	0.43	0.29	0.03	<i>Cordulegaster boltonii</i>	0.38	0.50	0.13	0.00
Brachyptera risi	0.15	0.50	0.30	0.05	<i>Helophorus</i> spp.	0.80	0.20	0.00	0.00
<i>Elmis aenea</i>	0.21	0.14	0.10	0.55	<i>Pisidium</i> spp.	0.50	0.50	0.00	0.00
<i>Heptagenia lateralis</i>	0.08	0.53	0.22	0.17	Hydracarina	0.53	0.41	0.06	0.00
<i>Limnius volckmari</i>	0.11	0.15	0.33	0.41	<i>Phagocata</i> spp.	0.62	0.12	0.02	0.24
Tipulidae	0.41	0.20	0.22	0.17	<i>Leuctra hippopus</i>	0.68	0.20	0.10	0.02
Oligochaeta	0.28	0.42	0.25	0.05	<i>Nemoura</i> spp.	0.28	0.71	0.01	0.00
<i>Esolus parallelepipedus</i>	0.03	0.02	0.40	0.55	<i>Sialis fuliginosa</i>	0.64	0.36	0.00	0.00
<i>Chloroperla torrentium</i>	0.43	0.30	0.22	0.05	Limnephilidae	0.60	0.16	0.16	0.08
<i>Dinocras cephalotes</i>	0.00	0.43	0.08	0.49	Pyralidae	0.83	0.17	0.00	0.00
<i>Oulimnius</i> spp.	0.52	0.42	0.06	0.01	<i>Anacaena globulus</i>	0.73	0.10	0.13	0.05
<i>Chloroperla tripunctata</i>	0.02	0.03	0.88	0.07	<i>Siphonurus lacustris</i>	1.00	0.00	0.00	0.00
<i>Rhyacophila dorsalis</i>	0.32	0.38	0.22	0.08	<i>Nemoura cambrica</i>	0.63	0.15	0.07	0.15

Continued on next page.

Appendix 1 continued.

Species	1.1 to -1.0	-1.1 to -3.0	-3.1 to -5.0	-5.1 to -7.0	Species	1.1 to -1.0	-1.1 to -3.0	-3.1 to -5.0	-5.1 to -7.0
<i>Paraleptophlebia</i> spp.	0.24	0.45	0.25	0.06	<i>Agabus didymus</i>	0.94	0.06	0.00	0.00
<i>Caenis rivulorum</i>	0.00	0.15	0.85	0.00	<i>Amphinemura sulcicollis</i>	0.72	0.17	0.07	0.03
<i>Philopotamus montanus</i>	0.00	0.65	0.22	0.13	<i>Leptophlebia</i> spp.	0.96	0.03	0.01	0.00
<i>Silo</i> spp.	0.00	0.00	0.45	0.55	Simuliidae	0.67	0.14	0.17	0.02
Glossiphoniidae	0.00	0.63	0.38	0.00	<i>Leuctra nigra</i>	0.76	0.15	0.05	0.04
<i>Wormaldia</i> sp.	0.26	0.46	0.25	0.03	<i>Plectrocnemia</i> spp.	0.75	0.15	0.07	0.03
<i>Agapetus fuscipes</i>	0.06	0.35	0.58	0.00	<i>Nemoura cinerea</i>	0.99	0.00	0.00	0.00
<i>Siphonurus armatus</i>	0.02	0.68	0.00	0.30	<i>Nemurella picteti</i>	0.86	0.13	0.00	0.01

**CHAPTER 6. DISCUSSION: ECOLOGICAL SIGNIFICANCE AND
CONSEQUENCES OF ACID EPISODES**

6.1. INTRODUCTION

Despite emerging evidence that reductions in acid deposition is leading to chemical recovery in streams across large areas of North America and Europe (Fowler *et al.*, 2001; Laudon & Bishop, 2002b), a major need has been to explain the patchy and partial nature of associated biological recovery. It was hypothesised that while biological recovery from acidification is expected to follow chemical recovery, continued episodic acidification might still be affecting stream organisms (Svensson & Herrman, 1998; Bradley & Ormerod, 2002b; Masters, 2002).

In this thesis, I identified those areas that need to be addressed by reviewing past and present work on episodic acidification. I then assessed empirically episodic effects on an acid-sensitive indicator species using a combination of intra-annual invertebrate abundances and survival data from experimental transplantations. This was followed by an assessment using surveys of variations in the chemical signature of episodic acidification to identify factors influencing the character of episodes and their risk to the biota. I then assessed regional differences in acid deposition, the effects on episode chemistry and associated variations in invertebrate assemblage. Finally, I developed models to compare which variables at both high- and low-flow better explain invertebrate community composition.

6.2. CHEMISTRY OF 'ACID EPISODES'

Variations in the chemistry of acid episodes are influenced by regional and temporal differences in deposition, geology and land-use (Björnborg, 1983; Kahl *et al.*, 1992). However, although there is now strong evidence for continued chemical recovery from acidification these variations are still being influenced by anthropogenic emissions of SO_x and NO_x (Chapter 3). Critical loads is a method used to assess the risks of ecological damage as a result of acidification due to sulphur and nitrogen emissions and subsequent deposition (Kernan *et al.*, 2001; Hall *et al.*, 2004), and with increased awareness of the importance of acid episodes, critical loads are now being considered

alongside episode chemistry (Bridcut *et al.*, 2004). The causes, mechanisms, chemistry and trends in episodicity that vary between locations also reflect contrasting hydrological drivers in rainfall and snowmelt, contrasts in the major anions involved, spatial variations in deposition, and climatic influences due to drought or sea-salt events. A useful method of determining the ecological influences of episode chemistry, such as effects on biological communities, is by evaluating the relative contribution of acid anions and dilution during episodes (Kahl *et al.*, 1992). This method has helped to determine the continued influence of aluminium in addition to continued effects from sulphate and the influence of organic acidity ((Kowalik *et al.*, ms submitted), Chapter 3). The effects of episodes are strongest and continue to have an effect where there are continuing additions of strong acids to base-poor run-off even in areas where recovery from acidification is otherwise clear.

Freshwaters can be well buffered for most of the year until seasonal patterns such as snowmelt in spring or orographic rainfall during autumn and winter can produce episodes of contrasting duration, chemistry and severity (Davies *et al.*, 1992; Gibbins *et al.*, 2001; Lepori *et al.*, 2003a). In some streams, such as found in high mountain areas, the sensitivity to acidity caused by increased flow can be far greater than when only considering the surrounding geology and soils (Björnberg, 1983). Continued inputs of acidifying compounds derived from anthropogenic sources, natural acidity caused by dilution and organic acidity is not unusual in many acid-sensitive areas such as the uplands of Wales and Galloway in Scotland. In addition, organic acidity (DOC) can often be highly variable during episodes but is usually associated with seasonal increases in rainfall during the autumn period (Hall *et al.*, 1990). Discharge due to increased flow and episodes are usually highly correlated and therefore the risk of exposure during these periods can have a significant biological effect (Foster *et al.*, 2001) see Chapter 2 and 5). Finally, with the proposition that climate change is a phenomenon we will all have to accept (Serreze *et al.*, 2000) the link between climate variability and variability in freshwaters, such as changes in temperature and flow, is firmly connected (Tunberg & Nelson, 1998; Lake, 2000). The consequences of climate change include reduced flows, as well as major increases in flow, that may result in unusual drought conditions that have

a profound effect on freshwater chemistry (Stoddard *et al.*, 1999; Laudon & Bishop, 2002a). In addition, increased incidence of precipitation whether related to climate change or not, can enhance the effect of sea salts on both episode chemistry and chronic acidification (Markich & Brown, 1998; Hutchins *et al.*, 1999).

6.3. BIOLOGICAL CONSEQUENCES OF EPISODIC ACIDIFICATION

The life-cycles of many freshwater species reflect patterns related to seasonal effects such as increased rainfall and discharge, drought conditions, increased temperatures and changes in chemistry. It is thought, and much evidence points to this, that the life-cycles of many acid-sensitive benthic macroinvertebrates are highly associated with exposure to episodic acidification over the spring and winter periods (Chapter 2, (Gibbins *et al.*, 2001).

Field and laboratory evidence accumulated over three decades has revealed how acidity or aluminium affect many species in acidified locations either through direct toxicity or consequent ecological process (Sutcliffe & Carrick 1973; Ormerod *et al.* 1987; Ledger & Hildrew in press). Such effects still occur in upland British streams with the consequences most marked at sites affected by strong acids of anthropogenic origin (see Chapter 2 and Chapter 3, (Kowalik *et al.*, ms submitted; Kowalik & Ormerod, ms submitted).

Models using episode chemistry have provided the most realistic and accurate predictions of acidification effects on stream invertebrates and models using pH and/or aluminium are most likely to reflect real processes in addition to being the most accurate biological predictors examined in Chapter 5 (Weatherley & Ormerod, 1991; Lepori & Ormerod, in press). In addition, there is now strong evidence which indicates that episodes are fundamental to the effects of acidity on stream organisms so that models excluding episodes lack realism.

6.4. EXPLANATIONS FOR RETARDED BIOLOGICAL RECOVERY

Colonisation by acid-sensitive species appears to be highly influenced by life-cycle patterns which could be regulated by seasonal changes in pH (Chapter 2). These patterns raise interesting possibilities about the capacity of species to colonise episodic streams. Assuming eggs could be laid by dispersing adults into acid or episodic streams (Masters, 2002; Peterson *et al.*, 2004), any acid-sensitive species with overwintering nymphs would be at risk of low winter pH. Species such as *Baetis vernus* and *Ephemerella ignita*, by contrast, have life cycles by which episodes would be less likely to limit distribution unless conditions were also toxic to eggs (Kowalik & Ormerod, ms submitted). However, there is often a time lag from between chemical recovery to reestablishment and subsequent dispersal of acid-sensitive mayflies, which can exceed tens of years (Snucins, 2003).

More recently, biological recovery is expected to be dependant on biotic and abiotic interactions, such as habitat quality and the ability to recolonise, and not necessarily on factors affecting species dispersal (Keller & Yan, 1998; Keller *et al.*, 2002). Hydrochemistry was a sufficient explanation of the pattern of occurrence of acid-sensitive mayflies in Chapter 2, but other ecological effects may restrict recolonisation. It is possible that, in streams affected by anthropogenic acidification, the grazing niches formerly occupied by, for example, acid-sensitive mayflies, could now be filled with acid tolerant species such as stoneflies (Ledger & Hildrew, 2000; Ledger, 2000). As a result, recolonisation by, for example, mayflies may be more prone to failure as a result of competition from acid-tolerant species (Ledger & Hildrew, in press). In addition, (re)colonisation requires more than dispersal, the limits on persistence may be a result of toxicity at any stage of the life-cycle, as research has shown that the eggs of acid-sensitive species can hatch in acidified waters (Masters, 2002).

6.5. IMPLICATIONS FOR MANAGEMENT

As evidence is suggesting, slow biological recovery may need, in some cases, effective management. It is important to understand the response to chemical recovery and advance effective management strategies that understand the ecological steps towards biological recovery and where and how to intervene if the process of recovery should stall (Yan *et al.*, 2003).

Land management processes have long been known to impact on freshwaters, causing variations in sulphate, nitrate, calcium and pH in addition to natural variations in sea-salt episodes that impact inland as well as in coastal areas (Langan & Hirst, 2004). Identifying restoration priorities and reintroducing traditional management techniques can help to rehabilitate the semi-natural and cultural landscapes, in order that freshwaters can recover the abiotic habitat conditions that are a requirement for the return of target species (Nienhuis *et al.*, 2002; Petty & Thorne, 2005). However, any management option will have limited success if it does not remove or control the severity of acid episodes. This has been one of the shortcomings of the remediation strategy of catchment and direct liming (Bradley & Ormerod, 2002b). It was noted early on that the frequent persistence of acid episodes continued in limed waters and there was evidence to suggest they continued to adversely affect some freshwater species (Weatherley, 1988; Rundle *et al.*, 1995; Laudon, 2000).

6.6. CONCLUSION

This thesis has contributed to an area of research that is multi-faceted in its approach to the problem of acidification of freshwaters. Episodic acidification is a more significant component than once thought and this study has considered it through several approaches. The hypothesis presented in this thesis, that while biological recovery from acidification is expected to follow chemical recovery, continued episodic acidification might still be affecting stream organisms, has failed to be falsified. Therefore, acid

episodes and chronic acidification, and hence incomplete chemical recovery, are sufficient to explain weak biological recovery from acidification.

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