THE IMPACT OF CATCHMENT LIMING TO MITIGATE ACIDIFICATION ON WATER QUALITY AND MACROINVERTEBRATES IN THE WYE RIVER SYSTEM

Thesis submitted for the degree of Doctor of Philosophy

BETHAN RHIANNON LEWIS

Cardiff School of Biosciences,
Cardiff University, March 2006.

To my parents

Acknowledgements

Firstly I would like to thank my supervisor, Professor Steve Ormerod, for invaluable advice, guidance, and support throughout my Ph.D, particularly during the writing stage. I also thank Dr. Ingrid Jüttner for advice, assistance and company in the field and for providing diatom data for chapter 3. Funding for this research was gratefully received from The Wye and Usk Foundation. Staff at the Centre for Ecology and Hydrology have been extremely helpful, especially Dr. Brain Reynolds with advice on the chemistry data, and Annie Britton in analysis of chemical samples.

On a personal note I would like to thank members of the catchment research group past and present, particularly Ian, Ester and Liz. Also thanks to Renata, Daire, Alison and Kristina for their friendship and the good times we have shared in Cardiff.

Thank you to Mark for his encouragement, support and patience, particularly over recent months.

Final and special thanks to my family, in particular my parents, for their constant support throughout my studies. Thanks to Dad for his help in the field and for always showing a keen interest in my work, and to mum for her encouragement and emotional support!

Contents

N.B: each chapter is presented as an individual paper and thus includes its own self contained reference list.

Summary

i) Acid deposition has declined across Europe and North America but chemical and biological recovery in streams is slow. Mitigation techniques such as liming are still being considered, but they have seldom been evaluated across whole catchments.

ii) At 42 stream sites in the upper catchment of the River Wye, macroinvertebrates and diatoms were evaluated as bio-indicators of water quality. Both groups indicated continued acidification in headwaters.

iii) Chemical and biological responses to catchment liming in the upper Wye were assessed through a Before-After-Control-Impact (BACI) experiment across multiple limed, acid control and circumneutral reference sites. Liming did not change pH, alkalinity, calcium or aluminium at limed sites relative to acid controls because effects were small or masked by marked inter-annual variations in discharge.

iv) There was no significant change in the abundance or richness of macroinvertebrate assemblages at treated sites in the first two post-liming years. Acid-sensitive species (e.g. *Baetis rhodani*) colonised some limed sites, but not at a significantly increased frequency.

v) *In-situ* survival experiments revealed increased mortality in *Baetis rhodani* in limed and acid streams relative to circumneutral reference streams even following brief exposures.

vi) These data illustrate that recent liming of streams in the Wye catchment has not yet changed stream chemistry sufficiently to support acid-sensitive macroinvertebrate assemblages similar to those found at circumneutral sites. Further lime applications and continued evaluation is recommended.

Chapter 1

General Introduction

1.1 Introduction

Although much of the Welsh uplands are dominated by soils that are acidified naturally by leaching, by dilution of ANC (Acid Neutralising Capacity), and by organic acidity and $S0_4^2$ derived from decomposition processes, lake sediments reveal a post-industrial increase in acidity (Fritz *et al.*, 1990) to which acidic atmospheric deposition, high rainfall, and base-poor soils contribute (Gee, 1989). More generally, evidence linking the deposition of oxides of sulphur and nitrogen, and the resulting acidification, to aquatic biota has accrued since the 1920s. Impacts on valuable fish species such as Atlantic salmon (*Salmo salar* Linnaeus) were among the first documented examples (Dahl, 1927; Rosseland and Skogheim, 1984) and effects were subsequently reported for other sensitive species across much of Europe and North America.

Successful emission controls, use of lower sulphate fuels, application of desulphurisation methods, catalysts and NO_x removal technology have led to declines in S and N deposition across Europe since peaks in the late 1970's (Pawlowski, 1997; Fowler *et al.*, 2001). Data from the UK suggest that, following a 71% decrease in S emissions, dry and wet deposition of non-marine sulphur decreased by 61% from 525 ktonnes year $^{-1}$ to 205 ktonnes year $^{-1}$ between 1986 and 2001 (Fowler *et al.*, 2005). Trends in the deposition of nitrogen oxides are less clear, although a 10% reduction in emission seems likely to give current estimates for deposition at 206 ktonnes year $^{-1}$ (Fowler *et al.*, 2005). Data collected over 15 years by the UK Acid Waters Monitoring Network also indicate chemical recovery from acidification at individual monitoring sites (Davies *et al.*, 2005). Nevertheless, 12,000 km of Welsh rivers are estimated to be impacted by acidification (Rimes *et al.*, 1994; Stevens *et al.*, 1997).

Biological recovery has been slow and modest in many cases (Soulsby *et al.*, 1997; Monteith *et al*, 2005) with acid-sensitive species often failing to return or persist at recovering sites, and little change in biodiversity observed.

In order to accelerate the recovery process in acid waters, possible management strategies include the addition of basic substances – for example calcium carbonate. The aim is to detoxify acid water and raise the pH in order to allow re-colonisation by acid-sensitive species. The predominant restorative methods involve lime addition to the catchment, to surface waters or to the benthos. So far, however, the biological effects of liming schemes are surprisingly poorly described, particularly where they involve large areas. In this study, the effects on the stream chemistry and biology of the catchment of the Welsh River Wye following limestone treatment were assessed.

1.2 Experimental Design

This large-scale experiment, involving a range of indicators, builds on existing information from smaller liming studies at the adjacent Llyn Brianne catchment (O.S. grid reference SN790480) in mid-Wales (Jenkins *et al.*, 1991; Waters *et al.*, 1991; Donald and Gee, 1992; Rundle *et al.*, 1995; Bradley and Ormerod, 2002; Kowalik and Ormerod, 2006). This study focuses on 42 sites in the upper tributaries and main stem of the Wye (Figure 1). An advantage of selecting the Wye as a study catchment is the range of land-uses, physical attributes and hydrological characteristics represented across the catchment. Initial focus was on the acid-base status of the catchments, and this was assessed using diatoms and macroinvertebrates as bio-indicators. The latter group was then used, along with water chemistry data, to detect changes before and after liming, at treatment, control and reference sites. These data were analysed using a repeated measures ANOVA (Analysis of Variance) model (SPSS, version 12), which was constructed to include the effects of Treatment (limed vs acid; limed vs circumneutral), Time (Before/After), and Time-Treatment interactions, with discharge included as a covariate. Such a design allows comparison of temporal trends in treated streams with un-manipulated control sites, and avoids pseudo-replication (Stewart-Oaten *et al.*, 1986). Further, inclusion of multiple treatment, control and reference sites, and multiple sampling times, allowed an MBACI (Multiple-Before-After-Control-Impact) to be performed using repeated measures ANOVA (Underwood, 1993; Paine, 1996; Niemi *et al.*, 1999). Although the chemical response to liming has been monitored in other catchments at individual stream sites, the extent to which any such responses can scale-up to effects over large upland watersheds is poorly understood, and experiments involving multiple impact and control locations, and multiple before and after sampling events, using an array of indicators, is lacking. Further, biological changes resulting from liming and the potential for liming methods to restore biodiversity in impacted upland catchments require elucidation.

Although there is a substantial body of literature describing the response of single taxa (e.g macroinvertebrates, diatoms, fish) at individual locations (Lacroix, 1992; Cameron, 1995; Clayton and Menendez, 1996; LeFevre and Sharpe, 2002), a large scale study over multiple indicators, and at multiple treatment, control and reference sites, is lacking. Inclusion of multiple indicators can allow immediate changes to be compared to integrated responses over different time-scales, whilst inclusion of multiple locations avoids pseudoreplication, gives statistic robustness and allows changes at treated sites to be assessed across the catchment. As a study catchment, the

Wye provides an opportunity for this, and allows acid-base status and changes following liming, to be assessed across sites with differing physical characteristics.

1.3 Study area

More than 20 years of research has been focused on acidified streams in the Llyn Brianne catchment (Stoner *et al.*, 1984; Weatherley and Ormerod, 1987, 1992; Bradley and Ormerod, 2002; Kowalik and Ormerod, 2006). However, less is known about the current status of the upper reaches of the River Wye despite much ecological research during the 1970s and 1980s (Brooker and Morris, 1980; Ormerod and Edwards 1987). Although data on epilithic algal, macroinvertebrates and riverine birds (Ormerod and Tyler, 1986; Antoine and Bensonevans, 1987; Ormerod, 1987) for example, have been assessed, recent investigation into the ecological status of the catchment has been scarce, and little attention given to low order tributaries or small catchments in the upper reaches (e.g. Reynolds *et al.*, 1992). The Wye was historically considered one of the most productive salmonid fishing rivers in Europe but recent declines in populations of Atlantic salmon and Brown trout (*Salmo trutta* Linnaeus) have been, in-part, linked to acidification (Fowler *et al.*, 2001; Monteith and Evans, 2001). Present European policies promote chemical monitoring and the use of biological indicators for the protection and conservation of freshwater habitats (Council of the European Union, 1979; Council of the European Union, 2000). Thus the pre and post-treatment conditions at streams targeted for liming, acid controls and circumneutral reference sites are assessed with respect to a range of physio-chemical and biological parameters.

1.4 Aims

In a hypothesis driven approach, this research aims to assess the recovery from acidification by testing the following predictions:

i) Acidification continues to affect upland Welsh streams despite recent reductions in the deposition of acidifying compounds.

ii) As a consequence, stream organisms (e.g abundance, assemblage composition) are affected – notably diatoms and invertebrates.

iii) Liming increases pH, alkalinity and calcium concentration, while decreasing concentrations of aluminium.

iv) Liming, in turn, will cause changes in biota, including the gain of previously excluded acid-sensitive species and increases in macroinvertebrate abundance and richness, by providing conditions suitable for their survival.

1.5 References

Antoine, S.E. and Bensonevans, K. 1987. Epilithic algal population-dynamics of the river Wye system, Wales, UK. *British Phycological Journal*, **22**, 299-299.

Bradley, D.C. and Ormerod, S.J. 2002. Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater Biology*, **47**, 161-171.

Brooker, M.P and Morris, D.L. 1980. A survey of the macroinvertebrate riffle fauna of the river Wye. *Freshwater Biology*, **10**, 437-458.

Cameron, N.G. 1995. The representation of diatom communities by fossil assemblages in a small acid lake. *Journal of Paleolimnology*, **14**, 185-223.

Clayton, J.L. and Menendez, R. 1996. Macroinvertebrate responses to mitigative liming of Dogway Fork, West Virginia. *Restoration Ecology*, **4**, 234-246.

Council of the European Union. 1979. Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Europa, Brussels.

Council of the European Communities. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy, *Official Journal of the European Communities*, L327, 1-72.

Dahl, K. 1927. The effects of acid water on trout fry. *Salmon and Trout Magazine,* **46**, 35-43.

Davies, J.J.L., Jenkins, A., Monteith, D.T., Evans, C.D. and Cooper, D.M. 2005. Trends in surface water chemistry of acidified UK Freshwaters, 1988-2002. *Environmental Pollution*, **137**, 27-39.

Donald, A. P. and Gee, A. S. 1992. Acid waters in upland Wales: causes, effects and remedies. *Environmental Pollution*, **78**, 141-148.

Fowler, D., Coyle, M., ApSimon, H.M., Ashmore, M.R., Bareham, S.A., Battarbee, R.W., Derwent, R.G., Erisman, J-W., Goodwin, J., Grennfelt, P., Hornung, M., Irwin, J., Jenkins, A., Metcalfe, S.E., Ormerod, S.J., Reynolds, B. and Woodin, S. 2001. Transboundary Air Pollution: acidification, eutrophication and ground-level ozone in the UK, Report. No. EPG 1/3/153. DEFRA, Edinburgh.

Fowler, D., Smith, R.I., Muller, J.B.A., Hayman, G. and Vincent, K.J. 2005. Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, **137**, 15-25.

Fritz, S.C., Kreiser, A.M., Appleby, P.G. and Battarbee, R.W. 1990. Recent acidification of upland lakes in North Wales. In *Acid Waters in Wales* (Eds R. W. Edwards, J. H. Stoner and A. S. Gee). Kluwer Academic Publishers, Dordrecht

Gee, A.S. 1989. A review of the causes and effects of acidification of surface waters in Wales and potential mitigation techniques. *Archives of Environmental Contamination and Toxicology*, **18**, 121-130.

Jenkins, A., Waters, D. and Donald, A. 1991. An assessment of terrestrial liming strategies in upland Wales. *Journal of Hydrology*, **124**, 243-261.

Kowalik, R.A. and Ormerod, S.J. 2006. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology*, **51**, 180-191.

Lacroix, G. L. 1992. Mitigation of low stream pH and its effects on salmonids. *Environmental Pollution*, **78**, 157-164.

LeFevre, S.R. and Sharpe, W.E. 2002. Acid stream water remediation using limestone sand on Bear Run in southwestern Pennsylvania. *Restoration Ecology*, **10**, 223-236

Monteith, D.T. and Evans, C.D. 2001. United Kingdom Acid Waters Monitoring Network (UKAWMN): Summary Report. Department of the Environment, Transport, and the Regions.

Monteith, D.T., Hildrew, A.G., Flower, R.J., Raven, P.J., Beaumont, W.R.B, Collen, P., Kreiser, A.M., Shilland, E.M. and Winterbottom, J.H. 2005. Biological responses to the chemical recovery of acidified fresh waters in the UK. *Environmental Pollution*, **137**, 83-101.

Niemi, G.J., Hershey, A.R., Shannon, L., Hanowski, J.M., Lima, A., Axler, R.P. and Regal, R.R. 1999. Ecological effects of mosquito control on zooplankton, insects and birds. *Environmental Toxicology and Chemistry*, **18**, 549-559.

Ormerod, S.J. and Tyler, S.J. 1986. The diet of dippers *Cinclus-cinclus* wintering in the catchment of the river Wye, Wales. *Bird Study*, **33**, 36-45.

Ormerod, S.J. 1987. The influences of habitat and seasonal sampling regimes on the ordination and classification of macroinvertebrate assemblages in the catchment of the river Wye, Wales. *Hydrobiologia*, **150**, 143-151.

Ormerod, S.J. and Edwards, R.W. 1987. The ordination and classification of macroinvertebrate assemblages in the catchment of the River Wye in relation to environmental factors. *Freshwater Biology*, **17**, 533-546.

Paine, M.D. 1996. Repeated measures designs. *Environmental Toxicology and Chemistry*, **15**, 1439-1441.

Pawlowski, L. 1997. Acidification: its impact on the environment and mitigation strategies. *Ecological Engineering*, **8**, 271-288.

Rimes, C.A., Farmer, A.M. and Howell, D. 1994. A survey of the threat of surface-water acidification to the nature conservation interest of fresh-waters on sites of special scientific interest in Britain. *Aquatic conservation: marine and freshwater ecosystems*, **4**, 31-44.

Reynolds, B., Emmett, B.A. and Woods, C. 1992. Variations in streamwater nitrate concentrations and nitrogen budgets over 10 years in a headwater catchment in mid-Wales. *Journal of Hydrology*, **136**, 155-175.

Rosseland, B.O. and Skogheim, O.K. 1984. Attempts to reduce effects of acidification on fishes in Norway by different mitigation techniques. *Fisheries*, **9**, 10-16.

Rundle, S. D., Weatherley, N. S. and Ormerod, S.J. 1995. The effects of catchment liming on the chemistry and ecology of upland Welsh streams: testing model predictions. *Freshwater Biology,* **34**, 165-175.

Soulsby, C., Turnbull, D., Hirst, D., Langan, S.J. and Owen, R. 1997. Reversibility of stream acidification in the Cairngorm region of Scotland. *Journal of Hydrology*, **195**, 291-311.

Stevens, P.A., Ormerod, S.J. and Reynolds, B. 1997. *Final Report on the Acid Waters Survey for Wales.* CEH Bangor, Bangor.

Stewart-Oaten, A., Murdoch, W.W. and Parker, K.R. 1986. Environmental impact assessment: 'pseudoreplication in time'? *Ecology*, **67**, 929-940.

Stoner, J.H., Gee, A.S. and Wade, K.R. 1984. The effects of acidification on the ecology of streams in the upper-Tywi catchment in west Wales. *Environmental Pollution*, **35**, 125-157.

Waters, D., Jenkins, A., Staples, T. and Donald, A.P. 1991. The importance of hydrological source areas in terrestrial liming. *Journal of the Institution of Water and Environmental Management*, **5**, 336-341.

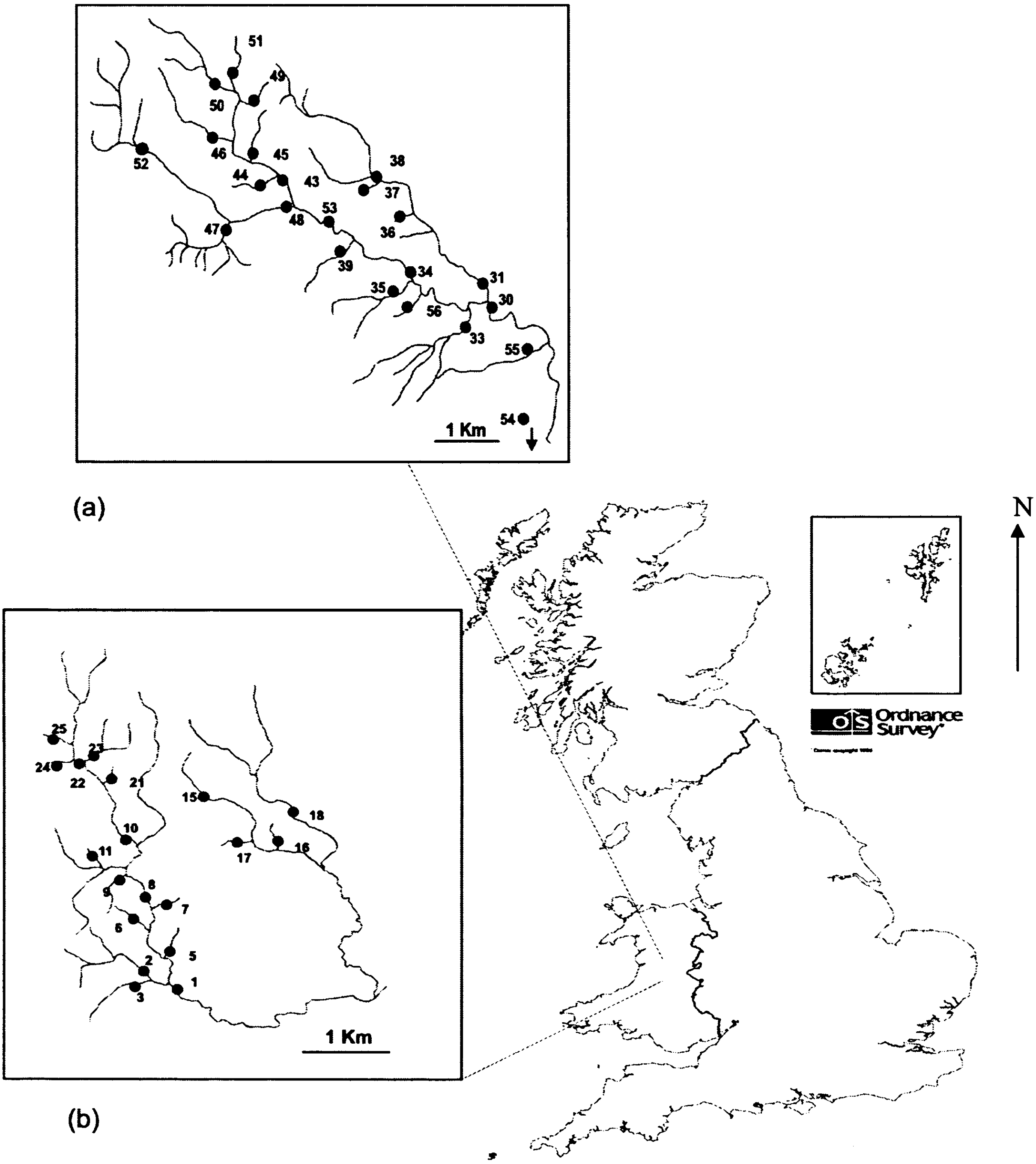
Weatherley, N.S. and Ormerod, S. J. 1987. The impact of acidification on macroinvertebrate assemblages in welsh streams: Towards an empirical. *Environmental Pollution,* **46,** 223-240.

Weatherley, N.S. and Ormerod, S.J. 1992. The biological response of acidic streams to catchment liming compared to the changes predicted from stream chemistry. *Journal of Environmental Management*, **34**, 105-115.

Underwood, A.J. 1993. The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. *Australian Journal of Ecology*, **18**, 99-116.

1.6 List of figures

Figure 1. Map illustrating 42 survey sites on (a) the upper reaches of the River Wye and (b) the River Irfon (a major tributary of the Wye), mid-Wales.



Chapter 2

The methods, scope and effectiveness of liming to restore acidified rivers: a review of published literature

2.1 Summary

1. Requirements for accelerated chemical and biological recovery from acidification and the need to mitigate damage have led to the widespread application of calcium carbonate to affected areas using various methods. However, there have been few reviews of the effectiveness of these techniques particularly i) for biota; ii) with respect to responses over larger spatio-temporal scales and iii) for rivers, where problems associated with flow variability might limit effects. Here, the efficacy of three major stream liming methods (streambed liming, direct dosing and catchment liming) is reviewed with respect to effects on water quality and aquatic biota.
2. Experimental streambed liming and dosing have increased pH and calcium concentration and reduced metal concentrations in some cases. In addition, direct stream dosing can also be targeted to high flows and hence acid episodes. Potential drawbacks include the formation of toxic precipitates downstream following streambed treatment during episodes of high run-off, while dosers cannot readily be deployed on sufficient small tributaries to restore all impacted headwaters. Catchment liming can change average pH, calcium, and aluminium concentrations for years to decades following single applications, but there are logistical challenges to widespread application. Moreover, effects at high flow have been less durable than at baseflow.
3. Across all treatments effects on biota have been mixed. Liming has sometimes increased fish density but results are inconsistent between programmes. Studies involving macroinvertebrates as indicator species suggest that responses are sometimes patchy, partial and potentially short-lived for reasons that are still unclear. Negative effects on non-target species, especially following catchment

liming, are possible but can be avoided by careful site-selection. There are few data on the effects of liming on ecosystem processes.

4. Catchment application has several advantages as a liming technique (penetration into headwaters; proven durability from single applications; low management costs following treatment; opportunity for synergy with land management and agri-environment schemes). However, discrepancies remain over whether liming methods result in pre-acidification chemistry, and the extent to which available approaches can scale-up across larger areas of acidified catchments needs further evaluation. Furthermore, the factors which limit biological recovery in cases where chemical recovery has been achieved require additional investigation

Key words: *conservation, liming, pH, recovery, restoration, upland streams.*

2.2 Introduction

Between the Industrial Revolution and the late twentieth century, the deposition of sulphur and nitrogen oxides from fossil fuel combustion has acidified base-poor aquatic ecosystems over extensive areas of North America and Europe (Hildrew and Ormerod, 1995; Fowler *et al.*, 2001). Consequences for aquatic organism have been equally widespread, with a wide range of organisms affected (Dahl, 1927; Rosseland *et al.,* 1986; Ormerod *et al.*, 1987; Sutcliffe and Hildrew, 1989; Jüttner *et al.,* 1997; Orendt, 1998). For macroinvertebrates, low pH and associated increases in metal concentrations not only led to direct toxic effects, but also altered food resources, predator-prey interactions and habitat quality, with major consequences for community composition (Sutcliffe and Carrick 1973; Fiance, 1978; Hall *et al.*, 1980; Herrmann and Andersson 1986; Ormerod *et al.*, 1987; Sutcliffe and Hildrew, 1989; Ledger and Hildrew, 2001).

Since the 1970s, the emissions and deposition of acidifying compounds have declined across much of Europe and North America (Stoddard *et al.*, 1999; Fowler *et al.*, 2005; Monteith and Evans, 2005). For example, across the UK, sulphur emissions have decreased by 71% with an associated decrease of 61% in dry and wet deposition from 525 ktonnes year $^{-1}$ to 205 ktonnes year $^{-1}$ between 1986 and 2001, whilst a 10% reduction in emission has been observed for oxides of nitrogen with current deposition estimated at 206 ktonnes year $^{-1}$ (Fowler *et al.*, 2005). As a result, there is unequivocal evidence that the chemistry of surface waters is changing, with increases in pH and acid neutralizing capacity (ANC) now widespread (Stoddard *et al.*, 1999; Evans *et al.*, 2001; Davies *et al.*, 2005; Fowler *et al.*, 2005; Monteith and Evans, 2005; Skjelkvåle *et al.*, 2005). However, with rates of pH increase typically by < 0.1-

0.3 pH units per decade (Fölster and Wilander, 2002; Fowler *et al.*, 2005; Skjelkvåle, *et al.*, 2005) full recovery to pre-acidification conditions is likely to require decades more, and some ecosystems will never recover (Dupont *et al.*, 2005). Moreover, in rivers, continued episodic acidification is still likely at high flow (Lepori *et al.*, 2003; Kowalik *et al.*, in press). Finally, biological responses to reduced deposition, although now apparent, appear to be slow, partial and patchy across locations and between taxa (Soulsby *et al.*, 1997; Tipping *et al.*, 2002). Under these circumstances, liming is advocated as a symptomatic treatment to offset acidification effects.

Several methods are available for liming, and include direct dosing of rivers, catchment liming, and stream-bed liming using lime sand (Gunn and Keller, 1984; Jenkins *et al.*, 1991; Donald and Gee, 1992; Hall *et al.*, 1994; Clayton *et al.*, 1998; Donnelly *et al.*, 2003). There are still, however, questions over whether any or all of these methods (i) replaces pre-acidification chemistry; ii) is durable over prolonged time periods and at high flow; iii) is defensible on conservation criteria; iv) promotes biological recovery at rates that can be differentiated from background; and v) can be scaled-up across the large areas that are now recovering from acidification. Previous reviews addressed some of these questions (Weatherley, 1988; Weatherley *et al.*, 1995; Donnelly *et al.*, 2003) but there are still outstanding issues, for example with respect to biological recovery, and whether treatment is effective across all discharges. This review summarises the development and testing of these palliative liming methods, and evaluates their restorative potential and ecological impacts, with particular focus on rivers and streams.

2.3 Methods of application and effects on chemistry

2.3.1 *Streambed liming*

Streambed liming, involving placement of lime material on the stream benthos has been used extensively in the treatment of acidified streams, including in the U.S.A. and Scandinavia (Gunn and Keller, 1984; Rosseland and Skogheim, 1984; Downey, 1994; Keener and Sharpe, 2005). Investigations are increasing also in the UK. The intention is that lime sand (see below for definition) deposited on the stream bed dissolves while being carried downstream to neutralise acidity (Downey *et al.*, 1994). An added benefit may be increased interstitial pH, important for example to pre-emergent salmonids during their development. This method is generally inexpensive and easy to implement (Keener and Sharpe, 2005). Experimentally this method has been tested with varying results (Rosseland and Skogheim, 1984; Keener and Sharpe, 2005). Typical experimental evaluations have involved placing limestone on the stream floor to neutralise the acid load in fish spawning areas, where habitats may be suitable for spawning but pH may be low (Lacroix, 1992).

Gunn and Keller (1984) used 320 tonnes of limestone (2 cm diameter particles) along 100m of the stream bed with an additional 25 tonnes of larger material (15 cm diameter) placed downstream to keep the former material in place. Average increases of 0.5 pH units (average from 5.9 to 6.4) and an increase in calcium (Ca) concentration (4.7 mg L^{-1} to 11.1 mg L^{-1}) were recorded and mostly sustained during the three-year study. Lacroix (1992) used 200 tonnes of crushed limestone, spread in bars on the streambed, to restore water quality and thus support fish stocks. This led to an increase in pH by an average 0.4 units while calcium and Magnesium (Mg) were increased by 0.5 mg L^{-1}, sustained over three years, although significant changes in aluminium (Al) were not recorded. These difference between treated and control sections were reduced during autumn and winter when discharge was higher.

More recently, Keener and Sharpe (2005) investigated the effects of limestone sand on water chemistry and macroinvertebrates at two chronically and episodically acidified streams in Pennsylvania, U.S.A. Although significant increases in pH and ANC were recorded, total dissolved aluminium increased while changes in macroinvertebrate assemblages were limited (see below). In an earlier experiment in the same catchment, LeFevre and Sharpe (2002) recorded significant reductions in concentrations of total dissolved aluminium and hydrogen ions (H^+), and increased ANC in the first year after intervention, relative to upstream controls. Elsewhere, Downey *et al.* (1994) detected decreased total and monomeric aluminium (by up to 50%), and restoration of 60% of lost ANC following a similar treatment method.

Despite these apparently positive results, some authors suggest that the effectiveness of bed-liming is limited by the contact time between the lime and runoff at high flow when pH is often lowest (Downey *et al.*, 1994). Further, the coating of lime particles with precipitate can also lead to deactivation (Rosseland and Skogheim, 1984; Zurbuch, 1984), and the remobilization and flushing of aluminium and iron precipitates has been observed downstream, particularly during episodes of high run-off (Gunn and Keller, 1984; Downey, 1994; LeFevre and Sharpe, 2002) (Table 1).

2.3.2 *Direct stream dosing*

Methods of dosing stream waters directly with calcium carbonate, with either dry powder or slurry, have been developed (Gunn and Keller, 1984; Zurbuch, 1984; Hall *et al.*, 1994). These methods are based on the proposition that the release of neutralising material can be regulated by flow, thus potentially targeting periods of high flow and associated low pH. Zurbuch (1984) tested a self-feeding rotary drum system, powered by stream flow which allowed production and release of up to 57 kg ha^{-1} of limestone, at a flow rate of 0.184 $m^3 s^{-1}$ and near continuous treatment over 4 years (Zurbuch *et al.*, 1996). Downstream of the treatment site pH and alkalinity were raised to 8.4 and 680 μeq L^{-1}, respectively (relative to pH 4.7 and alkalinity 30 μeq L^{-1} upstream of application point). Following single point applications to Virginian streams, Menendez *et al.* (1996) reported an increase in pH from 5.0 to 6.5, sustained for the three quarters of the post-treatment study time. Additional advantages included low financial costs. Dosing of streams in a similar way in Massachusetts using 56 tonnes of limestone, resulted in a significant increase in pH from 5.9 to 6.5, an average change in ANC from 20 μeq L^{-1} to 50 μeq L^{-1}, and a decrease in monomeric aluminium, despite higher flows during the treatment period (Simmons and Cieslewicz, 1996).

In the 1990s in the Llyn Brianne experimental catchment in mid-Wales, two purpose-built lime dosing units were installed in the headwaters of the rivers Tywi and Camddwr, upstream of the Llyn Brianne reservoir, at a cost of over £90,000 (http://www.carmarthenshire.org.uk/). This followed earlier experiments into the effects of one-off dosing which resulted in a pH increase to 7.2, relative to an untreated reference pH of 5.0, and a reduction in filterable aluminium of 0.37 mg L^{-1} to 0.14 mg L^{-1} (Weatherley *et al.*, 1989). In a similar study, filterable (0.22 μm) aluminium fell from 580 μg L^{-1} to 120-230 μg L^{-1} (Weatherley *et al.*, 1991).

In addition to the targeting of high flow and apparent cost effectiveness (Weatherley *et al.*, 1995), direct liming avoids some of the logistical difficulties of catchment liming (see below), although installation and supply difficulties may arise in remote areas (Weatherley, 1988). In addition, this method avoids any negative impacts on wetland areas that may arise from liming of the catchment (see below; Weatherley *et al.*, 1991). Dosing, however, only allows treatment of the tributaries where equipment is installed and the main river downstream. Low-order tributaries (Weatherley *et al.*, 1995), which may contribute to the acid load of the main river, cannot easily be treated without the increased costs associated with increasing doser numbers across all affected streams (Table 1).

2.3.3 *Catchment liming*

Catchment liming involves introducing weatherable carbonates into a given watershed either to the whole catchment or through application to wetland areas, in order to increase the base-cation concentration of run-off. Many applications have involved European catchments, where there was previously a tradition of liming in agricultural practice (Ormerod and Edwards, 1985). Sources of calcium carbonate, expertise and many basic techniques were therefore all available.

In a southern Norwegian catchment, spreading 3 tonnes $CaCO_3$ ha^{-1} over terrestrial areas raised lake pH from 4.5 to 7.0, calcium from 40 μeq L^{-1} to 200 μeq L^{-1}, and ANC from -30 μeq L^{-1} to 70 μeq L^{-1}, whilst reactive aluminium decreased from 10 μeq L^{-1} to 3 μmol L^{-1} (Traaen, 1997). In the 11 years following this treatment, the water chemistry changed back towards the pre-treatment situation, although was still adequate for brown trout (*Salmo trutta* Linnaeus) and brook trout (*Salvelinus* *fontinalis* Mitchell) survival. In another Norwegian catchment, 4 % of the catchment of Lake Røynelandsvatn was limed by helicopter with 20 tones ha^{-1} limestone. Three years after intervention calcium remained twice as high as pre-treatment concentrations and monomeric aluminium was 50% lower than pre-liming values (Hindar, 1996). More recently, Hindar (2005) investigated the effects of 2 tonnes ha^{-1} of dolomite spread over a catchment in Western Norway. The resulting average increases in pH (of up to 0.6 pH units) in the study catchments were stable for the three and a half years of monitoring. Liming of Adirondack catchments in New York was followed by an immediate increase in ANC by 200 $\mu eq\ L^{-1}$, to 1000 $\mu eq\ L^{-1}$ (Newton *et al.*, 1996), a stabilising of seasonal pH fluctuations, and increased spawning habitat and reproductive success for brook trout in the downstream lake (Schofield and Keleher, 1996).

The effects of catchment liming methods on streams in mid-Wales were described by Donald and Gee (1992) as part of the Llyn Brianne Acid Waters Project. 'Agricultural improvement of moorland' involved ploughing and harrowing, followed by addition of magnesian limestone (a blend of $CaCO_3$ and $MgCO_3$) at a rate of 10 tonnes ha^{-1}, with the further addition of NPK fertiliser (15 tonnes) and phosphate (7.5 tonnes). There was no amelioration of acid stream chemistry following the treatment and the conditions remained 'unsatisfactory' for salmonids. 'Source-area moorland liming' involved the distribution of 80 tonnes of powdered limestone over 4 ha, by helicopter and by hand. Changes in stream chemistry following treatment included a sustained rise in pH from 5.0 to 6.7 and an increase in calcium concentration from 0.8 mg L^{-1} to 4-10 mg L^{-1} during the first year after treatment. Liming of 'forested source areas' was also investigated with 20 tonnes of limestone powder ($CaCO_3$) applied following a more extensive treatment of headwater areas. Stream pH rose from 5.1 to 6.5, although the post-liming water chemistry was variable. In another treatment, the whole catchment area was limed with fine powdered limestone by tractor or by hand at a rate of approximately 9 tonnes ha^{-1}. pH and calcium concentration rose (by up to 1.6 units and 3 mg L^{-1} respectively), and pre-treatment peaks in aluminium concentration were avoided. Liming (60 tonnes of $MgCO_3$) in combination with bank-side clearance also produced an increase in pH (by up to 1.7 units) and decrease in aluminium concentration (to < 0.1 mg L^{-1}), but neither effect was prolonged. The authors concluded that methods involving the use of finely ground limestone, where the treated area includes hydrological sources, are most effective in producing positive and prolonged results.

In the same catchment, Ormerod *et al.* (1990) compared the chemical and biological effects of catchment liming to modelled reductions in sulphate deposition. Following liming, mean pH and total hardness increased (from 5.0-5.2 to 6.4-6.9 and from 3.9-7.1 mg L^{-1} to 11.3-22.6 mg L^{-1} respectively) whilst aluminium decreased (from 0.15-0.17 mg L^{-1} to 0.06-0.07 mg L^{-1}). Jenkins *et al.* (1991) found that catchment liming of all moorland areas (300 tonnes over 33.4 hectares) and liming of source areas (80 tonnes over 53.5 hectares) were both effective strategies for raising the pH in the long-term, including during storm events.

Whilst these studies in Wales continued, assessment of liming was also undertaken by the Loch Fleet project. Loch Fleet is a small acid loch in southwest Scotland and was a popular trout fishery several decades ago (Brown *et al.*, 1988). Fish populations have since declined in association with increases in lake acidity in the 1970s and

1980s (Brown *et al.*, 1988; Howells *et al.*, 1992). Following extensive investigation into the pre-treatment status and the suitability of different liming methods in the early stages of the Loch Fleet Project (Howells and Brown, 1988), calcium carbonate was applied to wetland areas, to areas under forest canopy (lime slurry) and to moorland areas (dry powder) at dosages of 10-30 tonnes ha^{-1} (Howells *et al.*, 1992). Application to the catchment was deemed more appropriate than directly to water courses due to the low retention times of the local lakes (Brown, 1988). Mean H^+ concentration dropped from 29.0 μeq L^{-1} to 0.08 μeq L^{-1} whilst calcium rose from 58 μeq L^{-1} to 437 μeq L^{-1}. In another Scottish catchment (Miller *et al.*, 1995), 5% of the watershed area was limed at a dosage of 10 tonnes ha^{-1}. Although calcium initially showed a positive response (increase from 40 μeq L^{-1} to 150-200 μeq L^{-1}), this was not sustained over the four years of post-liming study. In contrast to the above results, during an experiment on the river Esk in England involving liming of 10% of a catchment, there was no significant effect of liming on the water chemistry (pH, Ca, Al, total humic substances) (Diamond *et al.*, 1992).

There is evidence supporting liming targeted at forest floors (Raulund-Rasmussen, 1989; Huettl and Zoettl, 1993) but logistical difficulties may limit its suitability for upland streams, and evidence for treatment targeted at hydrological source areas is compelling (Jenkins *et al.*, 1991; Waters *et al.*, 1991; Donald and Gee, 1992). In comparison to other methods, application of lime to the watershed rather than directly to the stream can avoid the accumulation of fine calcium carbonate on the benthos, avoids the need for maintenance of permanent dosing equipment and does not act as a barrier to fish migration, as some other methods may (Table 1). Moreover, this method can allow penetration of headwaters, which not only act as valuable spawning habitats for fish but may also contribute largely to acid inputs into the main river. Moreover, chemical changes can result from a single treatment and can be effective over years to decades (Bradley and Ormerod, 2002). This method can thus be an important means of achieving long-lasting results in upland catchments (Jenkins *et al.*, 1991; Donald and Gee, 1992) and allows the opportunity for cooperative efforts between conservation bodies, agri-environment schemes, and business and government land management groups. However, this approach may be expensive in some catchments (Weatherley *et al.*, 1995) and conservation implications need to be evaluated before implementation. Potential effects of catchment liming on non-target terrestrial and wetland species have been examined to some extent. For example, Buckton and Ormerod (1997) investigated the effects of liming of hydrological source areas on terrestrial invertebrates in upland mires. Four families were more abundant at limed sites (Veliidae, Hemiptera; Hydrophilidae, Coleoptera; Linyphiidae, Araneae; Tetragnathidae, Araneae), whereas two others (Carabidae, Coleoptera; Lycosida, Araneae) were more abundant at untreated sites, although there was no significant difference in diversity (Buckton and Ormerod, 1997). Ormerod and Rundle (1998) compared the effects of acidification and liming of three catchments in upland Wales on the abundance and chemical composition of terrestrial invertebrate prey. Following liming (9 tonnes ha^{-1}), lower numbers of most invertebrate groups were recorded, although the only significant reduction in prey was a reduction in the abundance of spiders and Hemiptera. Shore and Mackenzie (1993) predicted that any adverse affects of catchment liming on terrestrial invertebrates would in turn impact upon shrew species, which consume invertebrates. In moorland areas where limestone was applied, the authors reported that the surface activity and abundance of pygmy shrews (*Sorex minutes* Linnaeus) were reduced (the latter by 30–55%) with effects lasting up to and over three years (Shore and Mackenzie, 1993). For macro-floral species a move from *Calluna* to *Molinia* and death of *Sphagnum* spp. and other acidophilus bryophyte species (Brown *et al.*, 1988; Duliere *et al.*, 2000) have been recorded, whereas during a liming experiment in the Adirondack region mentioned above, Mackun *et al.* (1994) found limited effects of liming on wetland vegetation, with the frequency or cover of only six of 64 species altered after treatment. Liming may lead to higher levels of inorganic carbon than those present under natural conditions (Brandrud, 2002) and in peaty soils, liming may increase ammonia through mineralization of organic nitrogen and increased nitrification (Raulund-Rasmussen, 1989, cited in Donelly *et al.*, 2002).

2.4 Limestone: size, amount and material

Many factors relating to the application of lime may determine the impact on water chemistry and biota. These include particle size, dosage rates, amount, flow, soil and hydrological characteristics, topography, and the presence of other elements in the treatment material (Gunn and Keller, 1984; Hasselrot and Hultberg, 1984; Jenkins *et al.*, 1991; Kreutzer, 1994; Clayton *et al.*, 1998). For streambed liming, Gunn and Keller (1984) used 2 cm diameter particles which had to be supported by larger material (15 cm diameter) downstream, whereas smaller particles used experimentally have ranged between 150 μm to 4 mm (Downey *et al.*, 1994) with significant changes in water chemistry (increased pH, ANC and Ca) resulting downstream. Lacroix (1992) investigated changes resulting from the addition of 200 tonnes of 4 cm particles, and found significant increases in pH, calcium, Mg and ANC downstream, and increased survival, abundance and density of salmon fry. Clayton *et al.* (1998) suggested that sand-size particles (approx 0.06 mm – 2 mm) may be most effective in direct stream dosing as these have a large surface area to mass ratio and are easily transported by flowing water whereas extremely small particles (<0.05 mm; 'crusher-run particles') may be rapidly dissolved or washed downstream during the first high-flow event after treatment (Clayton *et al.*, 1998). However, sand sized particles and 'crusher-run' limestone were compared by Clayton *et al.* (1998) for direct dosing, and in most years following addition there was no significant difference between the results from these two particle sizes. With regard amount, Clayton *et al.* (1998) used a calculation based on the stream watershed area, annual mean pH and acid load. For lakes 10 grams per m^3 is suggested for a rise in pH from 4.5 to 6.5 (Swedish Ministry of Agriculture, 1982) whilst for streams and rivers recommendations for watershed treatment vary from 50 kg ha^{-1} to 80 tonnes ha^{-1} (Hasselrot and Hultberg, 1984; Donald and Gee, 1992). In a particular example at least 7.5 tonnes ha^{-1} was suggested for sustained results (pH increase of 1.8 units, Ca increase by a factor of 10) in moorland liming (Brown *et al.*, 1988).

Several lime-based materials can be used in neutralising procedures including dolomite ($CaMg(CO_3)_2$), olivine ((Mg,Fe)2SiO4), or limestone ($CaCO_3$). The more soluble soda ash (Na_2CO_3), quick-lime (CaO), or hydrated/slacked lime ($Ca(OH)_2$), or a combination of the above, have also been used (Pawlowski, 1997; Swedish Ministry of Agriculture, 1982). For example, Prepas *et al.* (2001) used a combination of the more expensive slacked lime and the less expensive limestone. Kreutzer (1994) recommended siliceous lime and ore-melting products, in light of their low solubility, and hence long-lasting effect, whilst Davison and House (1988) argued that addition of sodium hydroxide or sodium carbonate would result in higher alkalinity than application of calcium hydroxide or calcium carbonate.

For terrestrial strategies the dissolution rate of lime is further influenced by a number of soil properties including the pH of the soil solution, the removal of dissolution products by drainage, the CO_2 content of the soil air and the soil temperature (Kreutzer, 1994).

2.5 Biological effects of liming

In some cases, the rises in pH following liming have been accompanied by significant increases in the alkalinity and calcium concentration of streams compared to control sites (Hasselrot and Hultberg, 1984; Fjellheim and Raddum, 1992; Rundle *et al.*, 1995; Hindar *et al.*, 1995) and many authors observe reductions in the concentration of toxic metals, such as aluminium, through precipitation (Fjellheim and Raddum, 1992; Hindar *et al.*, 1995; Rundle *et al.*, 1995). Following such alterations, many liming programmes have recorded changes in biological assemblages, including the appearance of acid-sensitive species (Rosseland *et al.*, 1986; Weatherley *et al.*, 1989; Fjellheim and Raddum,, 1992; Howells *et al.*, 1992; Lacroix, 1992; Weatherley and Ormerod, 1992; Rundle *et al.*, 1995; Clayton *et al.*, 1998) (Table 2).

Following streambed application Rosseland *et al.* (1986) recorded reduced mortalities of brown trout and Atlantic salmon (*Salmo salar* Linnaeus) in treated streams. Lacroix (1992) observed an increase in redd size and increased frequency (by three times) of redd digging by Atlantic salmon, accompanied by an increase in alevin and fry survival, and increased trout fry density. In some Pennsylvanian streams, however, streambed limestone sand application resulted in no change in fish populations (LeFevre and Sharpe, 2002). Similarly, Keener and Sharpe (2005) did not find any significant change in macroinvertebrate density or diversity following this treatment, despite the appearance of previously absent acid-sensitive species.

Macroinvertebrate assemblages in acid streams are often species poor and devoid of sensitive taxa, with pH and other acid-base parameters recognised as key determinants of assemblage composition (Townsend *et al.*, 1983; Wright *et al.*, 1984; Sutcliffe and Hildrew, 1989; Rutt *et al.*, 1990; Weatherley and Ormerod, 1991). Thus this group is often used as indicators of the effects of lime treatment. In some cases of direct dosing, acid-sensitive macroinvertebrate species have been observed at limed sites, where they were previously absent or scarce (Fjellheim and Raddum, 1992; Clayton and Menendez, 1996; Raddum and Fjellheim, 2003). However, abundance was often low relative to controls, and colonisation only partial and short-lived (Fjellheim and Raddum 1992; Clayton and Menendez, 1996). As with streambed application, salmonid mortalities can be reduced and density increased following direct stream dosing (Weatherley *et al.*, 1989; Degerman and Appelberg, 1992; Clayton *et al.*, (1998). As part of the U.S Fish and Wildlife Service's Acid Precipitation Mitigation Program, addition of 56 tonnes of powdered limestone to streams in Massachusetts using a stream doser, allowed the target pH of 6.5 to be achieved, despite high flows during the post-treatment study period (Simmons and Cieslewicz, 1996). Brook trout density increased significantly during treatment, and rainbow trout (*Oncorhynchus mykiss* Walbaum) and brown trout mortality were reduced (Simmons *et al.*, 1996). In contrast, as part of the same programme in Appalachian catchments, Eggleton *et al.* (1996) found minimal effects on macroinvertebrates and fish following treatment using a ‘hydropowered doser’.

Following catchment liming, acid-sensitive macroinvertebrate species including mayflies (*Caenis horaria* (Linnaeus), *Baetis rhodani* (Pictet), *Heptagenia* spp.) and stoneflies (e.g. *Brachyptera risi* (Morton)) have been recorded at sites in limed catchments, where they were previously absent or found in only low abundance (Brown *et al.*, 1988; Weatherley and Ormerod, 1992; Rundle *et al.*, 1995; Bradley and Ormerod, 2002) and higher taxon richness and abundance have been observed (Rundle *et al.*, 1995). However, as with many direct dosing experiments, colonisation of these species is often only patchy and short-lived (Fjellheim and Raddum 1992; Rundle *et al.*, 1995; Bradley and Ormerod, 2002), with acid-tolerant species continuing to dominate even after treatment, and assemblage composition usually remaining unaltered (Weatherly and Ormerod, 1992; Bradley and Ormerod, 2002), even in cases where changes in chemistry have been sustained. For example, long-term monitoring over more than 20 years at the Llyn Brianne catchment revealed prolonged changes in chemistry following liming, including increases in mean pH and calcium concentration, and decreases in mean aluminium (Rundle *et al.*, 1995; Bradley and Ormerod, 2002; Kowalik and Ormerod, 2006). Biological responses however were more moderate, with increases in the abundance of acid-sensitive taxa only significant in the first two years after treatment and increases in frequency of occurrence being confined to two species (Bradley and Ormerod, 2002). During a catchment liming study on the river Esk, Diamond *et al.* (1992) found no significant difference in the changes in invertebrate abundance occurring at limed sites. With regard to fish populations, reduced brown trout mortality and increased salmonid density and spawning have been recorded (Weatherley *et al.*, 1989; Degerman and Appelberg, 1992; Dalziel *et al.*, 1994; Miller *et al.*, 1995) following catchment liming.

Effects on epilithic algae are less well documented than for other groups and much of the research into the effects of lime treatment on aquatic macrophytes and phytobenthos has been focused on lake and pond habitats. For example, a study of diatom assemblages conducted at moorland pools in the Netherlands found that the acid-tolerant *Eunotia paludosa* (Grunow) was replaced by eutraphentic and saprophilous taxa, particularly in permanent pools (Bellemakers and Vandam, 1992) whilst in a Scottish lake liming experiment previously acidobiontic assemblages, dominated by *Tabellaria quadriseptata* (Knudson), were dominated by acidophilous species (*Eunotia incisa* (Gregory) and *Peronia fibula* (Ross)) following treatment, although these changes were not consistent across all habitat types (Cameron, 1995). Wilkinson and Ormerod (1994) found that one species of Bryophyte (*Nardia compressa*) declined significantly in response to liming with no other taxa increasing in abundance following the loss of *Nardia*.

Decomposition rates of organic matter, especially plant detritus, in acid streams and lakes are often retarded (Grahn *et al.*, 1974; Traaen, 1980, cited in Weatherly, 1988). Following liming, acid streams, which may otherwise be dominated by fungi, can have increased abundance of sediment bacteria (Traaen, 1980) and thus increased decomposition rates and primary productivity. In a recent study at sites on the river Wye in mid-Wales, liming increased the decomposition rate of Beech leaves (F*agus sylvatica* L.) when submerged for 20 days in treated streams (Merrix *et al.*, submitted). However, there are currently few data on the effects of liming on ecosystem processes and function.

2.6 Limitation to recovery

Although chemical changes following liming have been observed for long periods after treatment, in some cases decades (Driscoll *et al.*, 1996; Bradley and Ormerod, 2002), the biological response is less comprehensive and often patchy and short-lived. Several hypotheses regarding the factors limiting recovery have been proposed including: i) that continued acid episodes prevent acid-sensitive species from persisting in limed streams (Weatherley and Ormerod, 1991; Bradley and Ormerod, 2002; Lepori *et al.*, 2003; Kowalik *et al.*, in press); (ii) limited dispersal abilities of acid-sensitive taxa may prevent the re-colonisation of previously excluded species (Weatherley and Ormerod, 1992; Rundle *et al.*, 1995); (iii) lack of food sources or habitat types for colonising species (Rundle *et al.*, 1995); (iv) acid-sensitive species may be competitively excluded from returning to recovering locations (Ledger and Hildrew, 2005); (v) natural variation may mask changes resulting from liming (Weatherley and Ormerod, 1992; Bradley and Ormerod, 2001). There is increasing evidence for the first of these hypotheses, that acid-sensitive species are prevented from recolonising recovering streams because of the continued effects of acid episodes (Lepori *et al.*, 2003; Lepori *et al.*, 2005; Kowalik and Ormerod, 2006; Kowalik *et al.*, in press). These 'acid episodes' are described as short-term chemical changes resulting from increases in discharge, which include base cation dilution and the addition of strong acid anions (Wigington *et al.*, 1996; Tranter *et al.*, 1994) and thus decreases in ANC to below zero (Davies *et al.*, 1992). The biological effects of such episodes have been demonstrated to some extent, for example through reduced survival and depleted densities of sensitive species, and altered assemblage composition (Ormerod *et al.*, 1987; Hirst *et al.*, 2004; Kowalik and Ormerod, 2006).

Further to the direct link between high discharge and episodes of acidity (Soulsby, 1995; Davies *et al.*, 1992), contact time between the limestone and the stream water during high flow periods may be limited (Gunn and Keller, 1984), further decreasing the buffering capacity of the system during these episodes. Examination of the literature suggests that, with respect to upland areas, palliative liming through application to hydrological source areas of the catchment has potential for altering the acid-base status. However, acid episodes can persist even after this treatment and addressing such episodes may be essential for comprehensive and prolonged biological recovery to be achieved (Jenkins *et al.*, 1991; Bradley and Ormerod 2002; Kowalik and Ormerod, 2006).

2.7 Conclusions

Liming has been widely tested and implemented across areas of Europe and North America to counteract the effects of acidity on water quality and aquatic biota. In many of the examples discussed, liming has increased the acid-base status of the study rivers, and both immediate and longer-lasting changes in water chemistry have resulted. In some cases, such changes may be an indication of long-term alterations in water chemistry, for example through calcium incorporation into the upper parts of the catchment soil (Kvaerner and Kraft, 1995). For upland streams, such as those in mid-Wales, catchment liming may be the most suitable and effective method for achieving long-lasting chemical changes.

Despite the obvious potential for liming to modify the chemistry of acidified waters towards pre-acidification status, possible negative effects on non-target organisms have been highlighted, including effects on terrestrial groups such as Bryophytes

(Wilkinson and Ormerod, 1994) and terrestrial invertebrates (Ormerod and Rundle, 1998). Such changes in non-target groups has possible implications for the whole-ecosystem and conservation issues such as these need to be considered when planning and implementing a liming programme (Farmer, 1992; Wilkinson and Ormerod, 1994), along with consideration of costs and duration. In addition to the discussed conservation concerns over liming, various authors have pointed out additional issues. For example that habitat availability in mixing zones may be limited (Clayton and Menendez, 1996) and that increased turbidity and hence reduced light penetration can result from lime addition (Brocksen *et al.*, 1992). Knowledge of the catchment hydrology flow pathways and other pre-treatment catchment characteristics is thus important (Jenkins *et al.*, 1991). Discrepancies remain over whether liming methods result in pre-acidification chemistry. Furthermore, the biological consequences of liming appear inconsistent and the reasons for limited biological restoration in otherwise recovering systems are still less than explicit. Large scale experiments into catchment-wide responses in water quality and biota are required for the mechanisms and potential extent of recovery to be more comprehensively appraised and understood.

2.8 References

Bellemakers, M.J.S. and Vandam, H. 1992. Improvement of breeding success of the Moor frog (*Rana-arvalis*) by liming of acid moorland pools and the consequences of liming for water chemistry and diatoms. *Environmental Pollution*, **78**, 165-171.

Bradley, D.C. and Ormerod, S.J. 2001. Community persistence among stream invertebrates tracks the North Atlantic Oscillation. *Journal of Animal Ecology*, **70**, 987-996.

Bradley, D.C. and Ormerod, S.J. 2002. Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater biology*, **47**, 161-171.

Brandrud, T.E. 2002. Effects of liming on aquatic macrophytes, with emphasis on Scandinavia. *Aquatic Botany*, **73**, 395-404.

Brown, D.J.A. 1988. The Loch Fleet and other catchment liming programs. *Water, Air, and Soil Pollution*, **41**, 409-415.

Brown, D.J.A., Howells, G.D., Dalziel, T.R.K. and Stewart, B.R. 1988. Loch fleet - a research watershed liming project. *Water, Air, and Soil Pollution*, **41**, 25-41.

Brocksen, R.W., Marcus, M.D. and Olem, H. 1992. *Practical guide to managing acidic surface waters and their fisheries*. Lewis Publishers Inc., Michigan, U.S.A.

Buckton, S. T. and Ormerod, S. J. 1997. Effects of liming on the Coleoptera, Hemiptera, Araneae and Opiliones of catchment wetlands in Wales. *Biological Conservation*, **79**, 43-57.

Cameron, N.G. 1995. The representation of diatom communities by fossil assemblages in a small acid lake. *Journal of Paleolimnology*, **14**, 185-223.

Clayton, J.L. and Menendez, R. 1996. Macroinvertebrate responses to mitigative liming of Dogway Fork, West Virginia. *Restoration Ecology*, **4**, 234-246.

Clayton, J.L., Dannaway, E.S., Menendez, R., Rauch, H.W., Renton, J.J., Sherlock, S.M. and Zurbuch, P.E. 1998. Application of Limestone to restore fish communities in acidified streams. *North American Journal of Fisheries Management*, **18**, 347-360.

Dahl, K. 1927. The effects of acid water on trout fry. *Salmon and Trout Magazine,* **46,** 35-43.

Dalziel, T.R.K., Wilson, E.J. and Proctor, M.V. 1994. The effectiveness of catchment liming in restoring acid waters at Loch-Fleet, Galloway, Scotland. *Forest Ecology and Management,* **68,** 107-117.

Davies, T.D., Tranter, M., Wigington Jr., P.J. and Eshleman, K.N. 1992. 'Acidic episodes' in surface waters in Europe. *Journal of Hydrology*, **132**, 25-69.

Davies, J.J.L., Jenkins, A., Monteith, D.T., Evans, C.D. and Cooper, D.M. 2005. Trends in surface water chemistry of acidified UK Freshwaters, 1988–2002. *Environmental Pollution*, **137**, 27-39.

Davison, W. and House, W.A. 1988. Neutralising strategies for acid waters: sodium and calcium products generate different acid neutralising capacities. *Water Research*, **22**, 577-583.

Degerman, E. and Appelberg, M. 1992. The response of stream-dwelling fish to liming. *Environmental Pollution,* **78,** 149-155.

Diamond, M., Hirst, D., Winder, L., Crawshaw., D.H. and Prigg, R.F. 1992. The effect of liming agricultural land on the chemistry and biology of the River Esk, north-west England. *Environmental Pollution*, **78**, 179-185.

Donald, A. P. and Gee, A. S. 1992. Acid waters in upland Wales: causes, effects and remedies. *Environmental Pollution*, **78**, 141-148.

Donnelly, A., Jennings, E. and Allot, N. 2003. A review of liming options for afforested catchments in Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy*, **103**, 91-105.

Downey, D.M. 1994. Low-cost limestone treatment of acid-sensitive trout streams in the Appalachian mountains of Virginia. *Water, Air, and Soil Pollution,* **77**, 49-77.

Driscoll, C.T., Cirmo, C.P., Fahey, T.J., Blette, V.L., Bukaveckas, P.A., Burns, D.A., Gubala, C.P., Leopold, D.J., Newton, R.M., Raynal, D.J., Schofield, C.L, Yavitt, J.B. and Porcella, D.B. 1996. The experimental watershed liming study: Comparison of lake and watershed neutralization strategies. *Biogeochemistry*, **32**, 143-174.

Duliere, J.F., De Bruyn, R. and Malaisse, F. 2000. Changes in the moss layer after liming in a Norway spruce (*Picea abies* (L.).Karst.) stand of eastern Belgium. *Forest Ecology and Management*, **136**, 97-105.

Dupont, J., Clair, T.A., Gagnon, C., Jeffries, D.S., Kahl, J.S., Nelson, S.J. and Peckenham, J.M. 2005. Estimation of critical loads of acidity for lakes in north-eastern United States and eastern Canada. *Environmental Monitoring and assessment,* **109**, 275-291.

Eggleton, M.A., Morgan, E.L. and Pennington, W.L. 1996. Effects of liming on an acid-sensitive southern Appalachian stream. *Restoration Ecology*, **4**, 247-263.

Evans, C.D., Cullen, J.M., Alewell, C., Kopacek, J., Marchetto, A., Moldan, F., Prechtel, A., Rogora, M., Vesely, J. and Wright, R. 2001. Recovery from acidification in European surface waters. *Hydrology and Earth System Sciences*, **5**, 283-297.

Farmer, A.M. 1992. Catchment liming and nature conservation. *Land Use Policy*, **9**, 8-10.

Fiance, S. B. 1978. Effects of pH on the biology and distribution of *Ephemerella-Funeralis* (Ephemeroptera). *Oikos*, **31**, 332-339.

Fjellheim, A. and Raddum, G.G. 1992. Recovery of acid-sensitive species of Ephemeroptera, Plecoptera and Trichoptera in River Audna after liming. *Environmental Pollution*, **78**, 173-178.

Fölster, J. and Wilander, A. 2002. Recovery from acidification in Swedish forest streams. *Environmental Pollution*, **117**, 379-389.

Fowler, D., Coyle, M., ApSimon, H.M., Ashmore, M.R., Bareham, S.A., Battarbee, R.W., Derwent, R.G., Erisman, J.-W., Goodwin, J., Grennfelt, P., Hornung, M., Irwin, J., Jenkins, A., Metcalfe, S.E., Ormerod, S.J., Reynolds, B., and Woodin, S. 2001. Transboundary Air Pollution: acidification, eutrophication and ground-level ozone in the UK, Rep. No. EPG 1/3/153. DEFRA, Edinburgh.

Fowler, D., Smith, R.I., Muller, J.B.A., Hayman, G. and Vincent, K.J. 2005. Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, **137**, 15-25.

Grahn, O., Hultberg, H. and Landner, L. 1974. Oligotrophication – a self accelerating process in lakes subjected to excessive supply of acid substances. *Ambio*, **3**, 93-94.

Gunn, J. M. and Keller, W. 1984. In situ manipulation of water chemistry using crushed limestone and observed effects on fish. *Fisheries*, **9**, 19-24.

Hall, R.J., Likens, G.E, Fiance, S.B. and Hendrey, G.R. 1980. Experimental acidification of a stream in the Hubbard-Brook-Experimental-Forest, New-Hampshire. *Ecology*, **61**, 976-989.

Hall, L.W., Killen, W.D., Fischer, S.A., Ziegenfuss, M.C., Anderson, R.D. and Klauda, R.J. 1994. The efficacy of a limestone doser to mitigate stream acidification in a Maryland coastal-plain stream - implications for migratory fish species. *Environmental Monitoring and Assessment*, **31**, 233-257.

Hasselrot, B. and Hultberg, H. 1984. Liming of Swedish lakes and streams and its consequences for aquatic ecosystems. *Fisheries*, **9**, 4-9.

Herrmann, J. and Andersson, K.G. 1986. Aluminium impact on respiration of lotic mayflies at low pH. *Water, Air, and Soil Pollution*, **30**, 703-709.

Hildrew, A. G. and Ormerod, S. J. 1995. Acidification: causes, consequences and solutions. In *The Ecological Basis for River Management* (eds Harper, D.M. and Ferguson, A.J.D.). John Wiley and Sons Ltd, Chichester.

Hindar, A., Nilsen, P., Skiple, A. and Hogberget, R. 1995. Counteractions against acidification in forests ecosystems: effects on stream water quality after dolomite application to forest soil in Gjerstad, Norway. *Water, Air, and Soil Pollution,* **85,** 1027-1032.

Hindar, A. 1996. Liming of wetlands in the acidified Lake Roynelandsvatn catchment in southern Norway: Effects on stream water chemistry. *Canadian Journal of Fisheries and Aquatic Sciences*, **53** 985-993.

Hindar, A. 2005. Whole-catchment application of dolomite to mitigate episodic acidification of streams induced by sea-salt deposition. *Science of the Total Environment*, **343**, 35-49.

Hirst, H., Chaud, F., Delabie, C., Jüttner, I., and Ormerod, S.J. 2004. Assessing the short-term response of stream diatoms to acidity using inter-basin transplantations and chemical diffusing substrates. *Freshwater Biology*, **49**, 1072-1088.

Howells, G. and Brown, D.J.A. 1988. The Loch Fleet Project, SW Scotland. *Transactions of the Royal Society of Edinburgh, Earth Sciences*, **78**, 241-248.

Howells, G., Dalziel, T.R.K., and Turnpenny, A.W.H. 1992. Loch Fleet: liming to restore a brown trout fishery. *Environmental Pollution*, **78**, 131-139.

Huettl, R.F. and Zoettl, H.W. 1993. Liming as a mitigation tool in Germany's declining forests - reviewing results from former and recent trials. *Forest Ecology and Management*, **61**, 325-338.

Hultberg, H. and Alenäs, I. 1981. *Swedish Water, Air and Pollution Research Institute, Götenborg*, 65.

Jenkins, A., Waters, D. and Donald, A. 1991. An assessment of terrestrial liming strategies in upland Wales. *Journal of Hydrology*, **124**, 243-261.

Jüttner, I., Lintelmann, J., Michalke, B., Winkler, R., Steinberg, C.E.W. and Kettrup, A. 1997. The acidification of the Herrenwieser See, Black Forest, Germany, before and during industrialisation. *Water Research,* **31,** 1194-1206.

Keener, A.L. and Sharpe, W.E. 2005. The effects of doubling limestone sand applications in two acidic Southwestern Pennsylvania streams. *Restoration Ecology*, **13**, 108-119.

Kowalik, R.A. and Ormerod, S.J. 2006. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology*, **51**, 180-191.

Kowalik, R.A., Cooper, D.M. and Ormerod, S.J. Acid episodes retard the biological recovery of upland British streams from acidification. *Global Change Biology,* In press.

Kreutzer, K. 1994. The influence of catchment management processes in forests on the recovery in fresh waters. In *Acidification of Freshwater Ecosystems* (Eds C. E. W. Steinberg and R. F. Wright). John Wiley and Sons, Chichester.

Kvaerner, J. and Kraft, P. 1995. Liming of wetlands in the Røynelandsvatn catchment - effects on soil chemistry and neutralisation properties in the soil profile. *Water, Air, and Soil Pollution*, **85**, 985-990.

Lacroix, G. L. 1992. Mitigation of low stream pH and its effects on salmonids. *Environmental Pollution*, **78,** 157-164.

Ledger, M.E. and Hildrew, A.G. 2001. Growth of an acid-tolerant stonefly on epilithic biofilms from streams of contrasting pH. *Freshwater Biology*, **46**, 1457-1470.

Ledger, M.E. and Hildrew, A.G. 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution*, **137**, 103-118.

LeFevre, S.R. and Sharpe, W.E. 2002. Acid stream water remediation using limestone sand on Bear Run in southwestern Pennsylvania. *Restoration Ecology*, **10**, 223-236.

Lepori, F., Barbieri, A. and Ormerod, S.J. 2003. Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams. *Freshwater Biology*, **48**, 1873-1885.

Lepori, F. and Ormerod, S.J. 2005. Effects of spring acid episodes on macroinvertebrates revealed by population data and in situ toxicity tests. *Freshwater Biology*, **50,** 1568-1577.

Mackun, I.R., Leopold, D.J. and Jraynal, D.J. 1994. Short-term responses of wetland vegetation after liming of an Adirondack watershed. *Ecological Applications*, **4** 535-543.

Menendez, R., Clayton, J. L. and Zurbuch, P. E. 1996. Chemical and fishery responses to mitigative liming of an acidic stream, Dogway Fork, West Virginia. *Restoration Ecology*, **4,** 220-233.

Merrix, F.L., Lewis B.R. and Ormerod, S.J. submitted. Experimental liming offsets the impact of low pH on Beech litter decomposition in acid-sensitive streams.

Miller, J.D. 1995. The consequences of liming a highly acidified catchment in central Scotland. *Water, Air, and Soil Pollution*, **85,** 1015-1020.

Monteith, D.T. and Evans, C.D. 2005. The United Kingdom Acid Waters Monitoring Network: a review of the first 15 years and introduction to the special issue. *Environmental Pollution,* **137**, 3-13.

Newton, R.M., Burns, D.A., Blette, V.L. and Driscoll, C.T. 1996. Effect of whole catchment liming on the episodic acidification of two Adirondack streams. *Biogeochemistry*, **32**, 299-322

Orendt, C. 1998. Macroinvertebrates and diatoms as indicators of acidification in forest spring brooks in a region of eastern Germany (Leipzig-Halle-Bitterfeld) highly impacted by industrial activities. *Archiv für Hydrobiologie,* **143,** 435-467.

Ormerod, S.J., Boole, P., McCahon, C.P., Weatherley, N.S., Pascoe, D. and Edwards, R.W. 1987. Short-term experimental acidification of Welsh streams: comparing the biological effects of hydrogen ions and aluminium. *Freshwater Biology*, **17**, 341-356.

Ormerod, S. J. and Edwards, R. W. 1985. Stream acidity in some areas of Wales in relation to historical trends in afforestation and the usage of agricultural limestone. *Journal of Environmental Management*, **20,** 189-197.

Ormerod, S.J., Weatherley, N.S., Merrett, W.J., Gee, A.S. and Whitehead, P.G. 1990. Restoring acidified streams in upland Wales - a modelling comparison of the chemical and biological effects of liming and reduced sulphate deposition. *Environmental Pollution*, **64**, 67-85.

Ormerod, S.J. and Rundle, S.D. 1998. Effects of experimental acidification and liming on terrestrial invertebrates: implications for calcium availability to vertebrates. *Environmental Pollution*, **103**, 183-191.

Pawlowski, L. 1997. Acidification: its impact on the environment and mitigation strategies. *Ecological Engineering*, **8**, 271-288.

Prepas, E.E., Pinel-Alloul, B., Chambers, P.A., Murphy, T.P., Reedyk, S., Sandland, G. and Serediak, M. 2001. Lime treatment and its effects on the chemistry and biota of hardwater eutrophic lakes. *Freshwater Biology*, **46,** 1049-1060.

Raddum, G.G. and Fjellheim, A. 2003. Liming of River Audna, southern Norway: A large-scale experiment of benthic invertebrate recovery. *Ambio*, **32**, 230-234.

Raulund-Rasmussen, K. 1989. Effect of artificial acid rain and liming on the base status in an acid forest (*Picea abies* (L.), Karst.) soil (Typic Haplohumod). *Scandinavian Journal of Forest Research*, **4**, 417-425.

Rosseland, B. O. and Skogheim, O. K. 1984. Attempts to reduce effects of acidification on fishes in Norway by different mitigation techniques. *Fisheries*, **9**, 10-16.

Rosseland, B.O., Skogheim, O.K., Abrahamsen, H. and Matzow, D. 1986. Limestone slurry reduces physiological stress and increases survival of Atlantic salmon (*Salmo salar*) in an acidic Norwegian river. *Canadian Journal of Fisheries and Aquatic Sciences*, **43,** 1888-1893.

Rundle, S. D., Weatherley, N. S. and Ormerod, S.J. 1995. The effects of catchment liming on the chemistry and ecology of upland Welsh streams: testing model predictions. *Freshwater Biology,* **34**, 165-175.

Rutt, G.P., Weatherley, N.S. and Ormerod, S.J. 1990. Relationships between the physiochemistry and macroinvertebrates of British upland streams: the development of modelling and indicator systems for predicting fauna and detecting acidity. *Freshwater Biology*, **24**, 463- 480.

Schofield, C.L. and Keleher, C. 1996. Comparison of brook trout reproductive success and recruitment in an acidic Adirondack lake following whole lake liming and watershed liming. *Biogeochemistry*, **32**, 323-337.

Shore, R.F. and Mackenzie, S. 1993. The effects of catchment liming on shrews (*Sorex spp.*) *Biological Conservation*, **64**, 101-111.

Simmons, K.R. and Cieslewicz, P.G. 1996. Limestone treatment of Whetstone Brook, Massachusetts. 1. Treatment methodology and water-chemistry changes during treatment. 1996. *Restoration Ecology*, **4**, 264-272.

Simmons, K.R., Cieslewicz, P.G. and Zajicek, K. 1996. Limestone treatment of Whetstone Brook, Massachusetts .2. Changes in the brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) fishery. *Restoration Ecology*, **4**, 273-283.

Skjelkvåle, B.L., Stoddard, J.L., Jeffries, D.S., Tørseth, K., Høgåsen, T., Bowman, J., Mannop, J., Monteith, D.T., Mosello, R., Rogara, M., Ryzchon, D., Vesely, J., Wieting, J., Wilander, A. and Worsztynowicz, A. 2005. Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, **137**, 165-176.

Soulsby, C. 1995. Contrasts in storm event hydrochemistry in an acidic afforested catchment in upland Wales. *Journal of Hydrology*, **170**, 159-179.

Stoddard, J.L., Jeffries, D. S., Lükewille, A., Clair, T. A., Dillon, P. J., Driscoll, C. T., Forsius, M., Johannessen, M., Kahl, J. S., Kellogg, J. H., Kemp, A., Mannio, J., Monteith, D. T., Murdoch, P. S., Patrick, S., Rebsdorf, A., Skjelkvåle, B. L., Stainton, M. P., Traaen, T., van Dam, H., Webster, K. E., Wieting, J. and Wilander, A. 1999. Regional trends in aquatic recovery from acidification in North America and Europe. *Nature,* **401**, 575-578.

Sutcliffe, D.W. and Carrick, T.R. 1973. Studies on mountain streams in the English Lake District. *Freshwater Biology*, **3**, 437-462.

Sutcliffe, D. W. and Hildrew, A. G. 1989. Invertebrate communities in acid streams. In *Acid Toxicity and Aquatic Animals* (Eds R. Morris, E. W. Taylor, D.J.A. Brown and J.A. Brown). Cambridge University Press, Cambridge.

Swedish Ministry of Agriculture, Environment '82 Committee. 1982. *Acidification Today and Tomorrow*. Riebergs Tryckeri AB, Uddevalla, Sweden.

Townsend, C.R., Hildrew, A.G. and Francis, J. 1983. Community structure in some southern English streams: The influence of physicochemical factors. *Freshwater Biology,* **13**, 521-544.

Traaen, T.S. 1980. Effect of acidity on the decomposition of organic matter in aquatic environments. In *Ecological Impact of Acid Precipitation* (Eds D. Drablos and T. Tollan). SNSF Project, Oslo.

Traaen, T.S. 1997. Whole-catchment liming at Tjonnstrond, Norway: an 11-year record. *Water, Air, and Soil Pollution*, **94**, 163-180.

Tipping, E., Bass, J.A.B., Hardie, D., Haworth, E.Y., Hurley, M.A. and Wills, G. 2002. Biological responses to the reversal of acidification in surface waters of the English Lake District. *Environmental Pollution*, **116**, 137-146.

Tranter, M., Davies, T.D., Wigington, P.J. and Eshleman, K.N. 1994. Episodic acidification of freshwater systems in Canada - physical and geochemical processes. *Water Air, and Soil Pollution*, **72**, 19-39.

Waters, D., Jenkins, A., Staples, T. and Donald, A.P. 1991. The importance of hydrological source areas in terrestrial liming. *Journal of the Institution of Water and Environmental* Management, **5**, 336-341.

Weatherley, N.S. 1988. Liming to mitigate acidification in freshwater ecosystems: a review of the biological consequences. *Water, Air, and Soil Pollution*, **39**, 421-437.

Weatherley N.S., Thomas, S.P. and Ormerod, S.J. 1989. Chemical and biological effects of acid, aluminium and lime additions to a Welsh hill-stream. *Environmental Pollution*, **56**, 283-297.

Weatherley, N.S. and Ormerod, S.J. 1991. The importance of acid episodes in determining faunal distributions in Welsh streams. *Freshwater Biology*, **25**, 71-84.

Weatherley, N.S., Rutt, G.P., Thomas, S.P. and Ormerod, S.J. 1991. Liming acid streams - aluminium toxicity to fish in mixing zones. *Water Air, and Soil Pollution*, **55**, 345-353.

Weatherley, N.S. and Ormerod, S.J. 1992. The biological response of acidic streams to catchment liming compared to the changes predicted from stream chemistry. *Journal of Environmental Management*, **34**, 105-115.

Weatherley, N.S., Jenkins, M.J., Evans, D.M., Rogers, A.P. and Gee, A.S. 1995. Options for liming rivers to ameliorate acidity - a UK perspective. *Water, Air, and Soil Pollution*, **85**, 1009-1014.

Weiher, E. and Boylen, C.W. 1994. Patterns and prediction of alpha-diversity and beta-diversity of aquatic plants in Adirondack (New-York) lakes. *Canadian Journal of Botany*, **72**, 1797-1804.

Wilkinson, S.M. and Ormerod, S.J. 1994. The effect of catchment liming on bryophytes in upland welsh streams, with an assessment of the communities at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **4**, 297-306.

Wright, J.R., Moss, D., Armitage, P.D. and Furse, M.T. 1984. A preliminary classification of running water sites in Great Britain based on macroinvertebrate species and the prediction of community type using environmental data. *Freshwater Biology*, **14**, 221-256.

Zurbuch, P. E. 1984. Neutralization of acidified streams in West Virginia. *Fisheries*, **9**, 42-47.

Zurbuch, P.E., Menendez, R., Clayton, J.L. 1996. Limestone neutralization of Dogway Fork, West Virginia, by means of a rotary-drum system. *Restoration Ecology*, **4**, 206-219.

2.9 List of tables

Table 1. Comparison of advantages and disadvantages of different liming methods (References: Gunn and Keller, 1984; Brown *et al.*, 1988; Weatherley, 1988; Jenkins *et al.*, 1991; Waters *et al.*, 1991; Donald and Gee, 1992; Shore and Mackenzie, 1993; Downey *et al.*, 1994; Weatherley *et al.*, 1995; Buckton and Ormerod, 1997; Ormerod and Rundle 1998; Duliere *et al.*, 2000; Bradley and Ormerod, 2002; LeFevre and Sharpe, 2002; Keener and Sharpe, 2005).

Method	Advantages	Disadvantages
Streambed liming	Inexpensive	Effectiveness may diminish rapidly over time
	Easy to implement	Limited contact time between lime and water
		Lime particles may become coated in precipitate and thus inactive
		Can not control pH depressions during high flow events
		Remobilization and flushing of aluminium and Iron may occur
Direct dosing	Application can be regulated by stream flow and hence allow targeting of high flow events.	Only treats main river sites and installation streams
	Cost effective	Difficult to install in remote areas
	Avoids some logistical difficulties associated with catchment liming	Equipment may require maintenance
Catchment liming	Allows targeting of hydrological source areas	Possible conservation issues for non-target terrestrial species
	Chemical changes may be more prolonged than for other methods	Expensive in some catchments
	Allows treatment of several streams consecutively	

Table 2. Examples of the biological responses of stream taxa to experimental liming.

Authors	Location	Treatment method	Biological response
Lacroix (1992)	Nova Scotia. Canada	Streambed liming	Atlantic salmon: Increase in redd size, frequency of redd digging, and alevin and fry survival. Trout fry: Increased density
Keener and Sharpe (2005)	Pennsylvania, U.S.A.	Streambed liming	Appearance of previously absent acid-sensitive invertebrate species
Fjellheim and Raddum (1992)	Norway	Direct Dosing	Colonisation by acid-sensitive macroinvertebrate species
Clayton and Menendez (1996)	Virginia, USA	Direct dosing	Appearance of previously absent acid-sensitive invertebrate species
Menendez *et al.* (1996)	Virginia, USA	Direct dosing	Appearance of eight fish species in previously fishless streams
Simmons and Cieslewicz (1996)	Massachusetts	Direct dosing	Increased brook trout density and reduced rainbow trout and brown trout mortality
Rosseland *et al.* (1986)	Norway	Direct dosing	Reduced mortality of Atlantic salmon
Rundle *et al.* (1995)	Wales	Catchment liming	Higher taxon richness and abundance of acid-sensitive macroinvertebrate taxa
Bradley and Ormerod (2002)	Wales	Catchment liming	Acid sensitive macroinvertebrate taxa increased in abundance and richness for first two years after treatment. Two mayfly species occurred significantly more often in limed streams after treatment.
Weatherley *et al.* (1989)	Wales	Catchment liming	Reduced brown trout mortality

Chapter 3

Comparative assessment of stream acidity using diatoms and macroinvertebrates: implications for river management and conservation

3.1 Summary

1. Macroinvertebrates and phytobenthic organisms (e.g. diatoms) are frequently used as bioindicators of water quality, yet few studies compare their effectiveness despite both being emphasised in the EU Water Framework Directive.
2. Here, as a case study, the efficacy of each group in assessing acid-base status in the catchment of the Welsh River Wye was evaluated from surveys in two years.
3. Ordination showed that both diatom and macroinvertebrate assemblages varied highly significantly with pH, alkalinity and calcium concentrations. Moreover, ordination scores were highly inter-correlated between these groups in both study years.
4. There were also contrasts, with diatoms and macroinvertebrates changing in differing ways with catchment land-use and channel hydromorphology. These differing responses suggest complementary indicator value, while variation in generation times between diatoms and macroinvertebrates suggests potentially contrasting speeds of response to variations over different time scales.
5. These data reveal that significant water quality problems in the River Wye, a Special Area of Conservation, are generated from the continued acidification of low-order, headwater streams and this has considerable significance for the objectives of the Water Framework Directive, and Habitats and Species Directive.

Key words: *acidification, biological indicators, Water Framework Directive, water quality, upland streams.*

3.2 Introduction

The EU Water Framework Directive (WFD) (Council of the European Union, 1979) aims to fulfil the need for sustainable, catchment-based protection and management of surface waters and ground waters. More significantly, the Directive will set targets for the measurement of inland surface waters against chemical, hydromorphological and biological criteria that should be achieved through programmes of management measures that deliver good ecological status. The Directive focuses on phytoplankton, phytobenthos, macroinvertebrates, aquatic macrophytes and fish as key elements in bio-assessment and resource value (Council of the European Union, 1979). While each of these groups will be of significance because of their intrinsic importance in indicating ecological conditions, there would clearly be added benefit if their bio-indicator values were additive or complementary. Alternatively, similarity in response to variations in water quality could result in some redundancy (Kaesler *et al.*, 1974). Furthermore, any group that could indicate habitat quality for other taxa would be of particular importance in conservation assessment (Swengel and Swengel, 1999, Sauberer *et al.*, 2004).

Surprisingly, correspondence or redundancy among different groups of aquatic biological indicators has been appraised in only a small array of organisms (e.g. Jackson and Harvey, 1993; Heino *et al.*, 2005). For example, Hirst *et al.* (2002) demonstrated that both macroinvertebrate and diatom assemblages responded to increasing metal concentrations, but macroinvertebrates varied more in diversity while diatoms varied in species composition. While responses to water quality are well known for groups of bio-indicator taxa individually (Dahl, 1927; Rosseland *et al.*, 1986; Sutcliffe and Hildrew, 1989; Jüttner *et al.*, 1997; Orendt, 1998; Jüttner *et al.*, 2003), there has been little investigation into the value of including an array of indicator groups as required by the WFD.

Here, the parallel response of diatoms and macroinvertebrates to variations between streams in water quality is assessed, with particular reference to acidification. The aims were to assess any complementary effects of using both groups in assessing river quality, to detect any redundancy, and to establish any associations between the two indicator groups. In addition, since the WFD will emphasize hydromorphology as an element underpinning quality, River Habitat Survey data (Environment Agency, 2003) were used to appraise any modification in bio-indicator response due to river habitat structure. Finally, an assessment was made of the current distribution of any acidification problems in the catchment of the River Wye, a Special Area of Conservation (SAC; http://www.jncc.gov.uk/page-1458).

3.3 Study Area

The study was conducted during 2003 and 2004 in two sub-catchments of the River Wye referred to subsequently as the upper Wye (174 km^2) and the Irfon (244 km^2). From an altitude of 677m O.D on Plynlimon in Mid Wales (52°28'N, 3°45'W), the Wye drains approximately 4180 km^2 and flows for 250 km before joining the Severn estuary at Chepstow (Figure 1). The geology is predominantly Lower Palaeozoic (mudstones and shales) and Upper Palaeozoic (marls and sandstone) rocks, which are poor in calcium carbonate (Edwards and Brooker, 1982). Catchment land-use is dominated by acid grassland, rough pasture and coniferous forest, the latter of which covers 14% and 25% of the upper Wye and Irfon catchments, respectively. The Wye has been considered, historically, one of the most productive salmonid fishing rivers in Europe. Fish numbers, however, have declined over the last 20 years. A range of factors is involved, but in the higher reaches, continued acid deposition (i.e. 'acid rain') has significant effects on salmonid carrying capacity (Fowler *et al.,* 2001; Monteith and Evans, 2001). This is of potential importance with respect to good ecological status and also to the quality of the Wye as a candidate Special Area of Conservation under the EU Habitats Directive (http://europa.eu.int/comm/environment/nature/).

In total, 42 sites were investigated, 19 of which were located in the Irfon catchment, and 23 in the upper Wye catchment (Figure 1). Sites were selected to incorporate a range of catchment sizes, pH values, and land-use, and ranged from small low-order tributaries to large main river sites (Table 1). At the end of July 2003, six of the study streams in the upper Wye catchment were treated with limestone as part of a large-scale mitigation programme (WY31, WY33, WY38, WY47, WY48 and WY52) but for these streams and main river sites downstream from these confluences (WY30, W34 and WY53), only data collected prior to liming have been used in this study.

3.4 Methods

3.4.1 Physical and chemical data

Habitat Survey

Habitat features in the stream channel and bank, as well as land-use within 50 m of the bank top, were assessed in April 2003 using a modified version of the UK Environment Agency's River Habitat Survey (RHS; see Environment Agency, 2003 for full methods). At each of the 42 study sites, data were collected on 35 attributes describing the character of the banks, riparian zone, channel and flow, over a 150m reach (i.e. 'sweep-up'; Environment Agency, 2003) and at four individual locations (i.e. spot checks). The normal RHS method based on ten spot checks was reduced to four for logistical reasons and because many streams were low-order.

Stream order (after Strahler, 1952) and stream link magnitude (Shreve, 1967) were calculated based on the 1:50,000 stream network and an O.S. topographical map was used to calculate catchment areas (in km^2).

Water chemistry

At each site pH, temperature and conductivity were measured using a pH/Cond 340i meter (WTW Weilheim) during February, April, July and October in each year. Two samples of stream water were collected at all sites on each visit, one filtered (0.45 μm pore size, Whatman sterile membrane filters) and one unfiltered. Unfiltered samples were analysed in the laboratory for alkalinity using the Gran titration. Filtered samples were analysed for calcium (Ca^{2+}), sodium (Na^+), potassium (K^+) and magnesium (Mg^{2+}) by atomic absorption spectrophotometry (AAS, Perkin Elmer). Silicon (Si) was assessed by the molybdenum blue method. Chloride (Cl^-), nitrate-nitrogen (NO_3^--N) and sulphate (SO_4^{2-}) were determined by ion chromatography (Dionex), and DOC was measured by continuous flow colorimetry with UV digestion (Skalar autoanalyser system). Aluminium was determined colorimetrically using the pyrocatechol violet method (Dougan and Wilson, 1974).

3.4.2 Biological sampling

Biological samples were collected in April 2003 and 2004. For macroinvertebrates, a 2-min kick sample was taken from mid-channel riffles and a 1-min kick sample taken from margins, over a 5 m reach, using a standard Freshwater Biological Association (FBA) pond net (0.9 mm mesh). Samples were preserved immediately in ethanol. In the laboratory, macroinvertebrates were sorted, identified where possible to species (Hynes, 1977; Elliot *et al.*, 1988; Friday, 1988; Edington and Hildrew, 1995; Wallace *et al.*, 2003) and counted. Prior to analyses, riffle and margin data were pooled. Macroinvertebrate abundance values were transformed logarithmically prior to statistical analysis to homogenise variances.

Diatoms were collected from stone surfaces in riffle areas using toothbrushes and preserved in ethanol. Samples were processed using hot peroxide oxidation to remove organic material and permanent slides were prepared using Naphrax as mountant. From each sample, at least 500 valves were identified to species (Nikon Eclipse E600, DIC, x1000) and relative abundances calculated (Krammer and Lange-Bertalot, 1986; Round and Bukhtiyarova, 1996; Krammer, 1997; Lecointe *et al.*, 1999; Reichardt, 1999).

3.4.3 Data analysis

Multivariate analysis was used to identify patterns among biological assemblages (CANOCO for Windows 4.0; ter Braak and Šmilauer, 1998). Diatoms and macroinvertebrates were ordinated separately using Detrended Correspondence Analysis (DCA; Hill and Gauch, 1980), an indirect gradient analysis that relates patterns in species assemblages to theoretical axes that are unconstrained to fit any measured chemical variable. This approach was used so that trends for diatoms and macroinvertebrates could subsequently be examined for inter-correlation. Ordination scores for each site were then related to water quality using Spearman's rank correlation analysis (MINITAB, version 14). Principal Components Analysis (PCA) was used to derive three sets of habitat principal components respectively to describe land-use within 50 m of the bank top (Land-use PCA), the character of the bank (Bank PCA) and the character of the stream channel (Channel PCA). Water width and depth were log-transformed ($Log_{10}x$) prior to ordination. To investigate changes in assemblage composition with habitat character for both taxonomic groups, DCA site scores for 2003 were related to habitat PCA scores using correlation analysis. Paired t-tests were used to assess differences in chemical descriptors between years.

3.4.4 Comparing chemical and biological monitoring using diatoms and macroinvertebrates

Maps were produced to illustrate the distribution in acid-base conditions and biological assemblages across the two sub-catchments. Sites were categorised according to the chemical conditions suggested by each indicator group. For diatoms, three groups reflected the relative abundance of acidobiontic, acidophilic and typically circumneutral species. Acidobiontic taxa are defined as those abundant at pH ≤ 5.5, acidophilic taxa occur dominantly between 5.5 and 7, whilst circumneutral taxa are most abundant at pH ≥ 7 (Hustedt, 1937). The most acidic sites had relative abundances of acid-tolerant species of > 40%; intermediate sites had *Achnanthidium minutissimum* (Kützing) Czarnecki at > 25% relative abundance, while acid-tolerant species were still present at > 20%; non acidified sites were dominated by circumneutral species (> 60% relative abundance), while acid-tolerant species were scarce or absent. For macroinvertebrates, ordination scores were regressed against pH and then threshold ordination sample scores expected to indicate three pH categories (pH < 5.5, pH 5.5-6.5, pH > 6.5) were identified. Although somewhat circular, the final aim of this analysis was to appraise the apparent classification of streams on biological indicators alone.

3.5 Results

3.5.1 Water chemistry

As intended from stream selection, pH and alkalinity varied strongly among sites, with corresponding changes in monomeric aluminium and calcium (Table 2). At all sites, pH varied significantly over the study period, often ranging over 1.5-2 pH units (Figure 2).

Several Irfon sites (IF06, IF07, IF21, IF24, IF25) had alkalinity values < 10 μeq L^{-1} in April 2003, when flow was relatively low, and negative alkalinities (-0.7 – -33.2 μeq L^{-1}) during higher flow in April 2004. Four upper Wye sites (WY36, WY39, WY47 and WY56) had negative alkalinities during April of both years. In both catchments, pH, alkalinity, conductivity, Na, Ca and Mg were significantly higher in 2003 than 2004, whereas aluminium (Al) and DOC were lower ($p < 0.05$), reflecting differences in flow. In combination, this range of chemical conditions indicated marked acidification and would be expected to cause marked biological variability across sites.

3.5.2 Biological assemblages

Diatoms (n = 71) were dominated by species of the Eunotiaceae, *Achnanthidium* or *Fragilaria* spp. DCA axes 1 and 2 explained 22.7% and 8.6%, respectively, of the variation among diatoms for 2003 (Figure 3 a), and 24.7% and 8.9%, respectively for 2004 (Figure 3 b). The most common and abundant diatom species at sites with high axis 1 scores were *Eunotia exigua* (Brébisson) Rabenhorst and *Eunotia subarcuatoides* Alles, Nörpel and Lange-Bertalot, while *Tabellaria flocculosa* (Roth) Kützing and *Brachysira vitrea* (Grunow) Ross characterised intermediate sites. At sites with lower axis 1 scores, *A. minutissimum*, *Fragilaria* cf. *capucina* v. *gracilis* (Oestrup) Hustedt and *Gomphonema parvulum* Kützing were most abundant.

Macroinvertebrate species (n = 77) predominantly represented Trichoptera, Plecoptera and Ephemeroptera. DCA axes 1 and 2 explained 10.1% and 7.5 %, respectively, of the variation for 2003 data (Figure 3 c), and 11.6% and 7.8%, respectively, for 2004 (Figure 3 d). Sites with high scores had abundant *Nemurella pictetii* (Klapálek), *Amphinemura sulcicollis*

(Stephens) and *Nemoura cinerea* (Retzius) whilst low-scoring sites, had abundant *Heptagenia lateralis* (Curtis), *Baetis rhodani* (Pictet), and *Rhithrogena semicolorata* (Curtis).

Both macroinvertebrate and diatom assemblages varied significantly with pH and alkalinity along DCA axis 1 in both years, reflecting the changing acid sensitivities of the species along the ordination axes (Table 3; Figure 4). Axis 1 scores for diatoms and macroinvertebrates were significantly inter-correlated in both study years (2003 $r = 0.604$, $p < 0.05$; 2004 $r = 0.610$, $p < 0.05$; Figure 5). Axis 2 scores for both groups varied positively with DOC.

At a large proportion of sites (>30%) severe or moderate acidification were indicated by both groups (Figure 6, black symbols; IF6, IF7, IF10, IF21, IF22, IF23, IF24, IF25, WY36, WY39, WY47, WY48, WY56), usually where *E. exigua* or *E. subarcuatoides* were the abundant diatom species. All acid-sensitive macroinvertebrate species were absent from these sites, and abundances of the acid-tolerant *A. sulcicollis* were greatest (Figure 7). With exceptions (WY48, IF10), these streams were mostly first-order tributaries draining small catchments (<6 km^2) with conifer plantation as the dominant land-use (Table 1). Interestingly, however, diatoms indicated severe impacts by acidification at six sites (IF02, IF08, WY33, WY35, WY50, WY52) whilst macroinvertebrates suggested little effect.

3.5.3 Effects of catchment character and hydromorphology

There were major variations among the sites in habitat structure with respect to land-use, bank and channel character (Table 4). Trends in land-use reflected changes from rough grassland or wetland to improved grassland or broadleaf/mixed woodland (Land-use PC1), and from conifer forest to grassland or broadleaf/mixed woodland (Land-use PC2, Figure 8). Changes in bank character included a gradient from unshaded banks with simple vegetation structure to shaded banks with tree-related features (overhanging boughs; bank-side roots, fallen trees and woody debris; Bank PC1). Bank PC2 reflected a change from gentle or composite banks with vegetated point bars and earth as the dominating bank material, to steep banks with exposed bedrock, stable cliffs and cobbles as the main bank material. Trends in channel structure reflected changes from bedrock substrate with chute flow to cobble substrate and unbroken waves (Channel PC 1), and from rippled or smooth flow with gravel and pebble substrate to coarser substrates with broken waves or chute flow (Channel PC 2).

Among these habitat trends, macroinvertebrate DCA axis 1 correlated with Land-use PC 2 (Table 4). This represented a change from abundant *A. sulcicollis* and *Plectrocnemia conspersa* (Curtis) under conifer, to abundant *Isoperla grammatica* (Poda) and *B. rhodani* under broadleaf/mixed woodland. Macroinvertebrate DCA axis 2 correlated with Land-use PC 1, for example representing a change from abundant *N. pictetii* in rough grassland to a more diverse assemblage in streams draining broadleaf/mixed woodland. Macroinvertebrate DCA axis 2 was also correlated with Bank PC1 with *A. sulcicollis*, Simuliidae and *N. pictetii* associated with shading, and *I. grammatica* associated with sites without shade. Diatom axis 2 scores, representing a change from acid-tolerant species (*E. exigua, E. subarcuatoides*) to species typical at moderately acid conditions (*B. vitrea*, *T. flocculosa*), were associated with Channel PC 1 representing a change from rapid to moderate flow.

3.6 Discussion

A crucial element in planning any river assessment, management or conservation programme is the selection of the biological indicators used to appraise biodiversity and environmental conditions. The efficacy of diatoms and macroinvertebrates for these purposes has been widely demonstrated separately (Rutt *et al.,* 1990; Soininen, 2002; Potapova and Charles, 2003), but the two have been compared less frequently. The generally congruent changes in their assemblage composition in the Wye shows clearly that they respond in consistent ways to the effects of acid-base status.

The effects of acidification on the water quality and biological character of fresh waters are now among the better known of all pollution problems. Among diatoms, taxa such as *Eunotia* spp. typically characterise acidified hill-streams in Western Europe while *Achnanthidium minutissimum* and *Fragilaria* spp. are more common at higher pH (Hirst *et al.*, 2002, 2004). Similarly, the shift with increasing pH from acid-tolerant plecopterans (*Amphinemura* spp., *Leuctra* spp., *Nemoura* spp.) or trichopterans (*Plectrocnemia* spp.) to a range of more acid-sensitive ephemeropterans (e.g. *Baetis* spp., *R. semicolorata, H. lateralis*), trichopterans (e.g. Hydropsychidae) or coleopterans (e.g. Elminthidae, *Hydraena gracilis*) is highly predictable (Rutt *et al.*, 1990). Perhaps more interesting, given increasingly strong evidence across Europe of chemical recovery from acidification in the form of increased mean pH, alkalinity and acid neutralising capacity, is that the biological effects of low pH are still apparent across such large areas as those in this study (Davies *et al.*, 2005). These data therefore support indications that biological recovery from acidification has been only gradual, partial or patchy (Monteith *et al.*, 2005). Apparently a large proportion of the upper-catchment tributaries of the Wye and Irfon still have assemblages of organisms typical of acid conditions, and one possible explanation may be the effects of flow-related variability in pH – in other words acid episodes – of the type indicated by Figure 2 in Kowalik *et al.* (in press): nearly all sites approached or exceeded pH 6 at low flows, but almost two-thirds fell below pH 5.7 at high flow. Further evidence of episodic effects also came from slight contrasts in the maps of acid conditions produced from invertebrates and diatoms. While trends in invertebrates were generally well predicted by diatoms (Figures 5, 6), the latter sometimes indicated severe impacts by acidification where macroinvertebrates implied more moderate effects. Diatoms can respond extremely rapidly to varying pH, and variation in life cycles between diatoms and invertebrates suggest potentially contrasting speed of response (Hirst *et al.,* 2004).

The Wye was one of the first whole-river Sites of Special Scientific Interest (SSSI) in the UK, and is now an SAC (i.e. a potential member of the Natura 2000 network). SSSIs are habitats, geological features or landforms notified under the Wildlife and Countryside Act 1981 for their importance to conservation, while Special Areas of Conservation are designated under the EU Habitats Directive based on important habitats and species present. Therefore evidence of continued acidification effects in the Wye catchment has wider conservation significance. Although acidification was previously known in the Wye system (Ormerod and Edwards, 1987), two results from this survey have particular importance. First, the proportion of the upper-catchment still affected by acidification is large. Effects scale-up to directly influence the main river at least as far as WY53, hence affecting the SAC. Second, the effects are apparently generated entirely by headwater streams of relatively small catchment area, often < 10km^2. Not only do such catchment headwaters have particular biological significance (e.g. inter-basin dispersal; particular species assemblages; important fish spawning habitat), but this study illustrates the well-known principle that their quality can affect conditions downstream. Such effects have relevance beyond the Habitats Directive – with respect to the management of river SACs – in illustrating how the pursuit of good ecological status under the EU Water Framework Directive should involve the management of headwaters as much as larger, main-river reaches.

While their general responses to water quality were similar, there were some apparent contrasts between diatoms and macroinvertebrate assemblages to variations in land-use and stream hydromorphology. An encouraging part of the study was that these effects could be captured by RHS methods. At the same time, however, further work is needed to assess how these effects detected in the Wye were independent of variations in chemistry. For example, changing macroinvertebrate assemblages reflected aspects of land-use, and in particular trends from conifer forest to grassland or mixed woodland. While forestry can influence macroinvertebrate assemblages independently of chemistry (Clenaghan *et al.,* 1998; Stewart *et al.,* 2000; Sponseller *et al.,* 2001) in the Wye over 50% of the acid–affected streams drained conifer plantations at various stages in the forest rotation. In turn, large-scale conifer afforestation in acid sensitive areas has well-known effects on both pH and invertebrate abundance, at least in areas receiving acid deposition. The main mechanism is through increased scavenging and deposition of acidic anions, so there is some potential for effects that confound chemical and habitat effects (Mayer and Ulrich, 1977; Neal *et al.,* 1986; Ormerod *et al.,* 1989; Clenaghan *et al.*, 1998; Goulding and Blake, 1998; Ormerod *et al.,* 2004). The diatom taxa that varied with channel and flow character were also those linked to acid-base conditions and might have proliferated in the chute- and bedrock-dominated habitats likely in smaller, acidified headwaters. While some studies have shown diatom species composition to be related to current velocities (e.g. Peterson and Stevenson, 1990) or substrate composition (Jüttner *et al.,* 2003), others found no response to hydraulic gradients (Biggs and Hickey, 1994). Further investigations of all of these effects would be valuable given the potentially important link between organisms and hydromorphology implied in the WFD. Models that inter-related chemical and habitat-effects on organisms would be particularly powerful.

A final important conservation dimension to these data is the extent to which both diatoms and invertebrates faithfully acted as bio-indicators for each other. While biodiversity surveys often include only a single taxonomic group (e.g. birds, angiosperms, insects, molluscs), an important question is whether such individual taxa can be 'surrogates' that give some indication of the diversity or ecological conditions for other groups (Lawton *et al.,* 1998; Reid, 1998). Taxa that indicated each other's status could clearly lead to cost savings, or could allow some indirect assessment for scarce or difficult taxa in which surveys were not straightforward. In rivers, data that examine this issue have been surprisingly few given the long history of organisms as biological indicators of water quality. For example, Heino *et al.* (2003) showed some lack of concordance in distribution patterns across riverine taxa, thus highlighting uncertainties in this approach. In this case, at least with respect to the dominant acid-base gradient, diatoms and macroinvertebrates were generally good (though not perfect) mutual indicators. Further data are required to appraise similar patterns in relation to other environmental stresses.

3.7 Acknowledgements

This study was partly funded by the Wye and Usk Foundation in collaboration with the School of Biosciences, Cardiff University, and the Centre for Ecology and Hydrology CEH, Bangor, and by a Grant from the Welsh Assembly Government to the Department of Biodiversity and Systematic Biology, National Museum Wales, Cardiff. The author would like to thank Annie Britton, CEH Bangor, for the chemical analysis of the water samples.

3.8 References

Biggs, B.J.F. and Hickey, C.W. 1994. Periphyton response to a hydraulic gradient in a regulated river in New Zealand. *Freshwater Biology,* **32**, 49-59.

Clenaghan, C., Giller, P.S., O'Halloran, J. and Hernan, R. 1998. Stream macroinvertebrate communities in a conifer-afforested catchment in Ireland: relationships to physico-chemical and biotic factors. *Freshwater Biology,* **40**, 175-193.

Council of the European Union. 1979. Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Europa, Brussels.

Council of the European Communities. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities*, **L327**, 1-72.

Dahl K. 1927. The effects of acid water on trout fry. *Salmon and Trout Magazine,* **46**, 35-43.

Davies, J.J.L., Jenkins, A., Monteith, D.T., Evans, C.D. and Cooper, D.M. 2005.
Trends in surface water chemistry of acidified UK Freshwaters, 1988-2002
Environmental Pollution, **137**, 27-39.

Dougan, W.K. and Wilson, A.L. 1974. The absorptiometric determination of aluminium in water: A comparison of some chromogenic reagents and the development of an improved method. *Analyst,* **99**, 413-430.

Edington, J.M. and Hildrew, A.G. 1995. *Caseless Caddis Larvae of the British Isles*. Scientific Publication **53**. Freshwater Biological Association, Ambleside.

Edwards, R.W. and Brooker, M.P. 1982. *The Ecology of the Wye*, Monographiae Biologicae, Volume 50. Junk Publishers, The Hague.

Elliot, J.M., Humpesh, U.H. and Macan, T.T. 1988. *Larvae of the British Ephemeroptera: A key with Ecological notes, Scientific Publication* 49. Freshwater Biological Association, Ambleside.

Environment Agency. 2003. *River Habitat Survey in Britain and Ireland, Field Survey Guidance Manual*. Environment Agency, Bristol.

Fowler, D., Coyle, M., ApSimon, H.M., Ashmore, M.R., Bareham, S.A., Battarbee, R.W., Derwent, R.G., Erisman, J-W., Goodwin, J., Grennfelt, P., *et al.* 2001. Transboundary Air Pollution: acidification, eutrophication and ground-level ozone in the UK, Rep. No. EPG 1/3/153. Centre for Ecology and Hydrology, Edinburgh.

Friday, L.E. 1988. *A Key to the Adults of British Water Beetles*, Publication 189. Field Studies Council, Preston Montford.

Goulding, K.W.T. and Blake, L. 1998. Land use, liming and the mobilization of potentially toxic metals. *Agriculture, Ecosystems and Environment*, **67**, 135-144.

Heino, J., Muotka, T., Paavola, R. and Paasivirta, L. 2003. Among-taxon congruence in biodiversity patterns: can stream insect diversity be predicted using single taxonomic groups? *Canadian Journal of Fisheries and Aquatic Sciences,* **60**, 1039-1049.

Heino, J., Paavola, R., Virtanen, R. and Muotka, T. 2005. Searching for biodiversity indicators in running waters: do bryophytes, macroinvertebrates, and fish show congruent diversity patterns? *Biodiversity and Conservation,* **14**, 415-428.

Hill. M.O. and Gauch, H.G. 1980. Detrended correspondence-analysis - an improved ordination technique. *Vegetatio,* **42**, 47-58.

Hirst, H., Jüttner, I. and Ormerod, S.J. 2002. Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology,* **47**, 1752-1765.

Hirst, H., Chaud, F., Delabie, C., Jüttner, I. and Ormerod, S.J. 2004. Assessing the short-term response of stream diatoms to acidity using inter-basin transplantations and chemical diffusing substrates. *Freshwater Biology,* **49**,1072-1088.

Hustedt, F. 1937. Ökologische und systematische Untersuchungen über die Diatomeenflora von Java, Bali und Sumatra. *Archiv für Hydrobiologie Supplementbände.* 15/16.

Hynes, H.B.N. 1977. *A key to the Adults and Nymphs of the British Stoneflies (Plecoptera), Scientific Publication* 17. Freshwater Biological Association, Ambleside.

Jackson, D.A. and Harvey, H.H. 1993. Fish and benthic invertebrates: community concordance and community-environment relationships. *Canadian Journal of Fisheries and Aquatic sciences,* **50,** 2641-2651.

Jüttner, I., Lintelmann, J., Michalke, B., Winkler, R., Steinberg, C.E.W. and Kettrup, A. 1997. The acidification of the Herrenwieser See, Black Forest, Germany, before and during industrialisation. *Water Research,* **31**, 1194-1206.

Jüttner, I., Sharma, S., Dahal, B.M., Ormerod, S.J., Chimonides, P.J. and Cox, E.J. 2003. Diatoms as indicators of stream quality in the Kathmandu Valley and Middle Hills of Nepal and India. *Freshwater Biology,* **48**, 2065-2084.

Kaesler, R.L., Cairns, J. and Crossman, S. 1974. Redundancy in data from stream surveys. *Water Research,* **8**, 637-642.

Kowalik, R.A., Cooper, D.M., Evans, C. D. and Ormerod, S.J (in press). Acid episodes retard the biological recovery of upland British streams from acidification. Global Change Biology.

Krammer, K. and Lange-Bertalot, H. 1986. *Bacillariophyceae, Süßwasserflora von Mitteleuropa*, Vol. 2, Parts 1-4, Ettl H, Gerloff J, Heynig H and Mollenhauer D (eds). Gustav Fischer Verlag, Stuttgart.

Krammer, K. 1997. *Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Arten.*, Vol. 1,2. J. Cramer, Berlin.

Lawton, J.H., Bignell, D.E., Bolton, B., Bloemers, G.F., Eggleton, P., Hammond, P.M., Hodda, M., Holt, R.D., Larsen, T.B., Mawdsley, N.A., *et al.* 1998. Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. *Nature,* **391**, 72-75.

Lecointe, C., Coste, M. and Prygiel, J. 1999. *OMNIDA version 3: notice d'utilisation - CLC1* Monbazillac.

Mayer. R. and Ulrich, B. 1977. Acidity of precipitation as influenced by the filtering of atmospheric S and N compounds – its role in the element balance and effect on soil. *Water, Air and Soil Pollution,* **9**, 409-416.

Monteith, D.T. and Evans, C.D. 2001. *United Kingdom Acid Waters Monitoring Network (UKAWMN): Summary Report.* Department of the Environment, Transport, and the Regions, United Kingdom

Monteith, D.T., Hildrew, A.G., Flower, R.J., Raven, P.J., Beaumont, W.R.B., Collen, P., Kreiser, A.M., Shilland, E.M. and Winterbottom, J.H. 2005. Biological responses to the chemical recovery of acidified fresh waters in the UK. *Environmental Pollution,* **137**, 83-101.

Neal, C., Whitehead, P.G., Neal, R. and Crosby, B.J. 1986. Modelling the effects of acid deposition and conifer afforestation on stream acidity in the British Uplands. *Journal of Hydrology,* **86**, 15-26.

Orendt, C. 1998. Macroinvertebrates and diatoms as indicators of acidification in forest spring brooks in a region of eastern Germany (Leipzig-Halle-Bitterfeld) highly impacted by industrial activities. *Archiv für Hydrobiologie,* **143**, 435-467.

Ormerod, S.J. and Edwards, R.W. 1987. The ordination and classification of macroinvertebrate assemblages in the catchment of the River Wye in relation to environmental factors. *Freshwater Biology,* **17**, 533-546.

Ormerod, S.J., Donald, A.P. and Brown, S.J. 1989. The influence of plantation forestry on the pH and Aluminium concentration of upland welsh streams - a re-examination. *Environmental Pollution,* **62**, 47-62.

Ormerod, S.J., Jones, M.E., Jones, M.C. and Phillips, D.R. 2004. The effects of riparian forestry on invertebrate drift and brown trout in upland streams of contrasting acidity. *Hydrology and Earth System Science,* **8**, 578-588.

Peterson, C.G. and Stevenson, R.J. 1990. Post-spate development of epilithic algal communities in different current environments. *Canadian Journal of Botany,* **68**, 2092-2102.

Potapova, M. and Charles, D.F. 2003. Distribution of benthic diatoms in U.S. Rivers in relation to conductivity and ionic composition. *Freshwater Biology,* **48**, 1311-1328.

Reichardt, E. 1999. Zur Revision der Gattung Gomphonema. Die Arten um G.affine/insigne, G. angustum/micropus, G. accuminatum sowie gomphonemoide Diatomeen aus dem

Oberoligozän in Böhmen. In *Iconographia Diatomologica 8, A.R.G*, Lange-Bertalot H. (ed). Gantner, Rugell

Reid, W.V. 1998. Biodiversity hotspots. *Trends in Ecology and Evolution,* **13**, 275-280.

Rosseland, B.O., Skogheim, O.K. and Sevaldrud, I.H. 1986. Acid deposition and effects in Nordic Europe – damage to fish populations in Scandinavia continue to apace. *Water, Air and Soil Pollution,* **30**, 65-74.

Round, F.E. and Bukhtiyarova, L. 1996. *Diatom Research,* **11**, 345-361.

Rutt, G.P., Weatherley, N.S. and Ormerod, S.J. 1990. Relationships between the physiochemistry and macroinvertebrates of British upland streams: the development of modelling and indicator systems for predicting fauna and detecting acidity. *Freshwater Biology,* **24**, 463-480.

Sauberer, N., Zulka, K.P., Abensperg-Traun, M., Berg, H.M., Bieringer, G., Milasowszky, N., Moser, D., Plutzar, C., Pollheimer, M., Storch, C., *et al.* 2004. Surrogate taxa for biodiversity in agricultural landscapes of eastern Austria. *Biological Conservation,* **117**,181-190.

Skjelkvåle, B.L., Stoddard, J.L., Jeffries, D.S., Tørseth, K., Høgåsen, T., Bowman, J., Mannop, J., Monteith, D.T., Mosello, R., Rogara, *et al.* 2005. Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, **137**, 165-176.

Soininen, J. 2002. Response of epilithic diatom communities to environmental gradients in some Finish rivers. *International Review of Hydrobiology,* **87**, 11-24.

Sponseller, R.A., Benfield, E.F. and Valett, H.M. 2001. Relationships between land use, spatial scale and stream macroinvertebrate communities. *Freshwater Biology,* **46**, 1409-1424.

Stewart, P.M., Butcher, J.T. and Swinford, T.O. 2000. Land use, habitat, and water quality effects on macroinvertebrate communities in three watersheds of a Lake Michigan associated marsh system. *Aquatic Ecosystem Health and Management,* **3**, 179-189.

Sutcliffe, D.W. and Hildrew, A.G. 1989. Invertebrate communities in acid streams. In *Acid Toxicity and Aquatic Animals,* Morris R, Taylor EW, Brown DJA, Brown JA. (eds). Cambridge University Press, Cambridge.

Swengel, S.R. and Swengel, A.B. 1999. Correlations in abundance of grassland songbirds and prairie butterflies. *Biological Conservation,* **90**, 1-11.

ter Braak, C.J.F. and Šmilauer, P. 1998. *CANOCO Reference Manual and Users Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4).* Microcomputer Power, Ithaca, New York.

Wallace, I.D., Wallace, B. and Philipson, G.N. 2003. *Keys to the Case-bearing Caddis larvae of Britain and Ireland, Scientific Publication 61*. Freshwater Biological Association, Ambleside.

3.9 List of tables

Table 1. Stream order, stream-link magnitude (S.L.M), drainage area and land use (within 50 m of bank top) of 42 sites on the River Wye. (BL = broadleaf/mixed woodland, CP = coniferous plantation, MH = moorland/heath, IG = improved/semi-improved grassland, RP = rough/unimproved grassland/pasture, WL = wetland).

Site Code	Stream order	S.L.M.	Drainage area (km^2)	Land use
WY 30	5	102	45	IG
WY 31	3	17	12	IG
WY 33	2	4	4	RP
WY 34	5	74	22	IG
WY 35	2	4	3	IG
WY 36	1	1	2	RP
WY 37	1	1	1	WL
WY 38	3	12	6	CP
WY 39	2	3	2	CP
WY 43	4	32	12	RP
WY 44	1	1	1	CP
WY 45	2	3	1	RP
WY 46	3	11	4	RP
WY 47	3	12	3	CP
WY 48	4	32	11	CP
WY 49	2	2	1	RP
WY 50	3	10	3	RP
WY 51	2	3	2	RP
WY 52	2	8	5	RP
WY 53	5	69	24	IG
WY 54	3	12	8	IG
WY 55	1	1	5	IG
WY 56	1	1	2	CP
IF 01	4	72	59	IG
IF 02	3	5	4	CP
IF 03	2	3	4	CP
IF 05	2	2	1	BL
IF 06	2	3	1	IG
IF 07	1	1	1	CP
IF 08	4	66	47	IG
IF 09	2	3	2	RP
IF 10	4	30	34	BL
IF 11	1	1	1	RP
AC 15	2	4	2	CP
AC 16	1	1	1	BL
AC 17	2	2	1	BL
AC 18	3	10	8	RP
IF 21	1	1	1	RP
IF 22	3	15	6	RP
IF 23	3	7	2	BL
IF 24	1	1	1	CP
IF 25	1	1	1	CP

Table 2. Mean, standard deviation and range of chemical variables at sites in the upper Wye and River Irfon catchments, measured in 2003 and 2004.

Variable	2003		2004	
pH	6.3 ± 0.5;	4.8 -7.1	5.8 ± 0.5;	4.7-6.5
Conductivity (µS cm^{-1})	50.6 ± 7.6;	32.5-66.5	43.7 ± 5.6;	30.0-53.5
Alkalinity (ueq L^{-1})	53.3 ± 44.7;	-7.5-149.1	28.8 ± 33.4;	-25.0-117.6
Na (mg L^{-1})	4.8 ± 0.8;	3.6-7.1	4.1 ± 0.6;	3.0-5.4
K (mg L^{-1})	0.2 ± 0.1;	0.1-0.6	0.2 ± 0.1;	0.1-0.4
Ca (mg L^{-1})	2.1 ± 0.8;	0.9-5.1	1.6 ± 0.5;	0.6-3.0
Mg (mg L^{-1})	1.1 ± 0.2;	0.7-1.7	0.9 ± 0.2;	0.6-1.3
Al (µg L^{-1})	55 ± 45.6;	20.0-225.0	56.6 ± 72.2;	5.0-302.5
Si (mg L^{-1})	1.4 ± 0.3;	0.8-2.2	1.3 ± 0.3;	0.6-2.3
Cl (mg L^{-1})	8.3 ± 1.5;	6.1-12.6	6.9 ± 1.0;	5.2-9.1
NO_3^--N (mg L^{-1})	0.3 ± 0.2;	0.0-0.9	0.3 ± 0.2;	0.1-0.8
SO_4^{2-} (mg L^{-1})	5.1 ± 0.7;	3.1-6.7	4.3 ± 0.8;	2.5-7.0
DOC (mg L^{-1})	2.2 ± 0.8;	1.1-4.1	2.9 ± 0.8;	1.1-5.3

Table 3. Significant correlation coefficients between macroinvertebrate and diatom assemblages (as DCA axis scores) and chemical variables in the Wye catchment in 2003 and 2004 (* p < 0.05, ** p < 0.01, *** p< 0.001, NS = not significant).

		Diatoms		Macroinvertebrates	
Variable	Year	Axis 1	Axis 2	Axis 1	Axis 2
Alkalinity	2003	-0.84 ***	0.38 *	-0.64 ***	NS
pH	2003	-0.82 ***	0.38 *	-0.65 ***	0.38 *
Ca	2003	-0.54 ***	NS	-0.36 *	NS
Mg	2003	-0.49***	0.38 *	NS	NS
Al	2003	0.49 **	-0.47 **	0.54 ***	NS
DOC	2003	NS	0.42 **	NS	0.42 ***
K	2003	-0.41 **	NS	-0.43 **	NS
Alkalinity	2004	-0.71 ***	NS	-0.77 ***	NS
pH	2004	-0.70 ***	0.34 **	-0.78 ***	NS
Ca	2004	-0.69 ***	0.35 *	-0.60 ***	NS
Al	2004	0.61 ***	-0.43 *	0.67 ***	NS
Mg	2004	-0.55***	0.40 *	-0.47 **	NS
K	2004	-0.49 **	NS	-0.44 *	NS
DOC	2004	NS	-0.52 **	NS	NS
Si	2004	NS	NS	NS	0.50 **

Table 4. Significant correlations (Spearman's rank correlation values) between DCA ordination axes and PCA gradients of habitat character (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS = not significant).

Principal Component	Gradient	Macroinvertebrate Axis 1	Macroinvertebrate Axis 2	Diatom Axis 2
Land use PC1 38.0% variance	Rough grassland/wetland to improved grassland or broadleaf/mixed woodland		-0.41 **	
Land use PC2 21.6% variance	Conifer forest to grassland or broadleaf/mixed woodland	0.40 **		
Bank PC1 32.1% variance	Unshaded banks to shaded banks		-0.53 ***	
Channel PC1 55.2% variance	Bedrock substrate and chute flow to cobble substrate and unbroken waves			0.43 **

3.10 List of Figures

Figure 7. Variations in the abundances (mean ± SD) of the acid-tolerant *Amphinemura sulcicollis* (Plecoptera; solid bars) and acid-sensitive *Baetis rhodani* (Ephemeroptera; open bars) at sites in the upper Wye and Irfon catchments as indicated by diatoms to be severely, moderately or un-impacted by acidity in (a) 2003 and (b) 2004.

Figure 8. Principal Components Analysis (PCA) plot of loading values for land-use variables at sites on the river Wye according to River Habitat Survey data. (AW = Artificial open water; BL = broadleaf/mixed woodland; CP = coniferous plantation; IG = improved/semi-improved; MH = moorland/heath, grassland; OW = Natural open water; RD = Rock, scree or sand dunes; RP = rough/unimproved grassland/pasture; SH = Scrub and shrubs; TH = Tall herb/rank vegetation; WL = wetland).

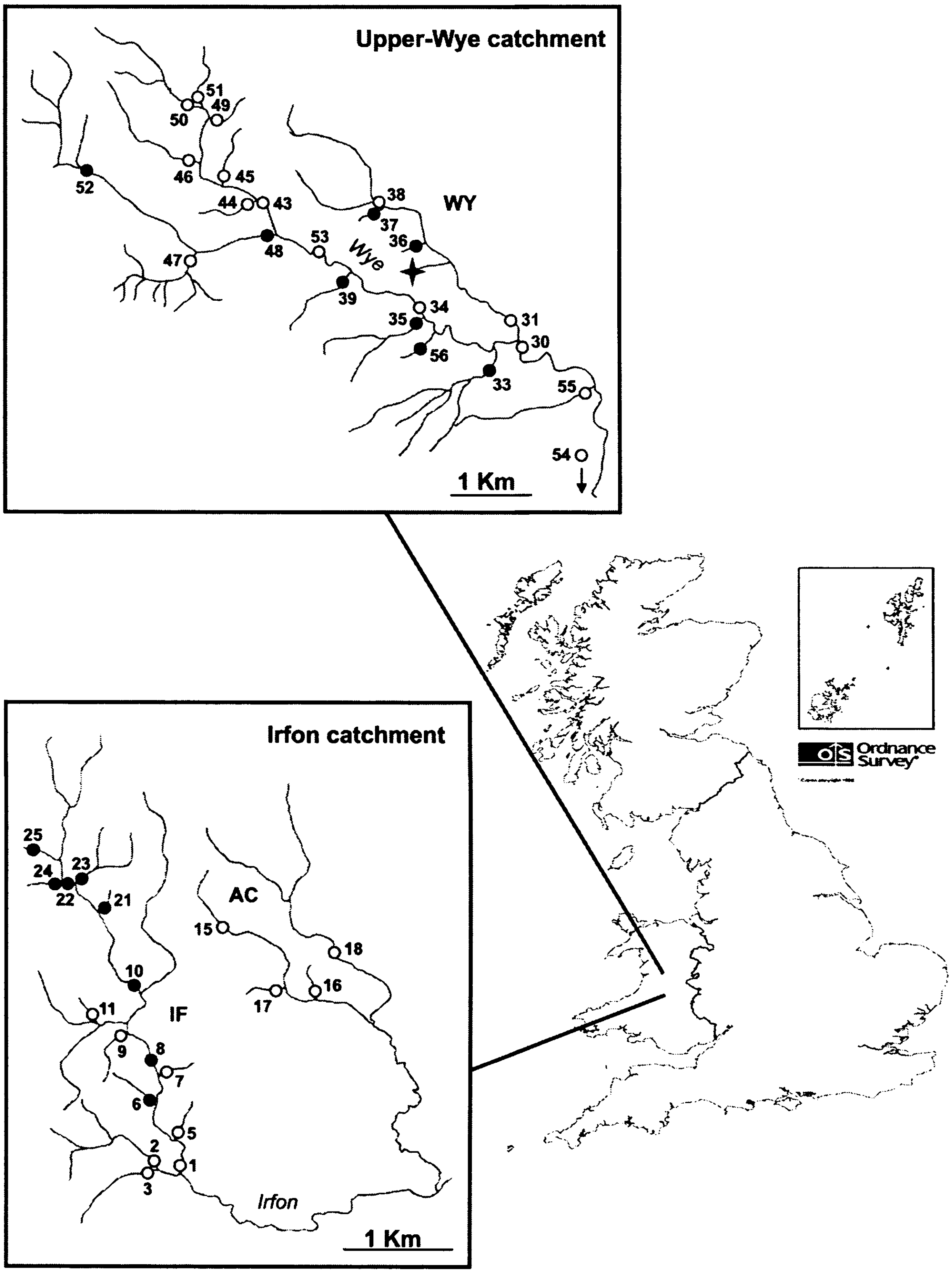

Upper-Wye catchment
51
50
49
46
45
52
44
43
38
WY
37
36
53
47
48
Wye
39
34
35
31
30
56
33
55
54
1 Km
Irfon catchment
25
24
23
22
21
AC
15
18
10
16
17
11
IF
9
8
7
6
5
2
1
3
Irfon
1 Km
Ordnance Survey

Figure 1.

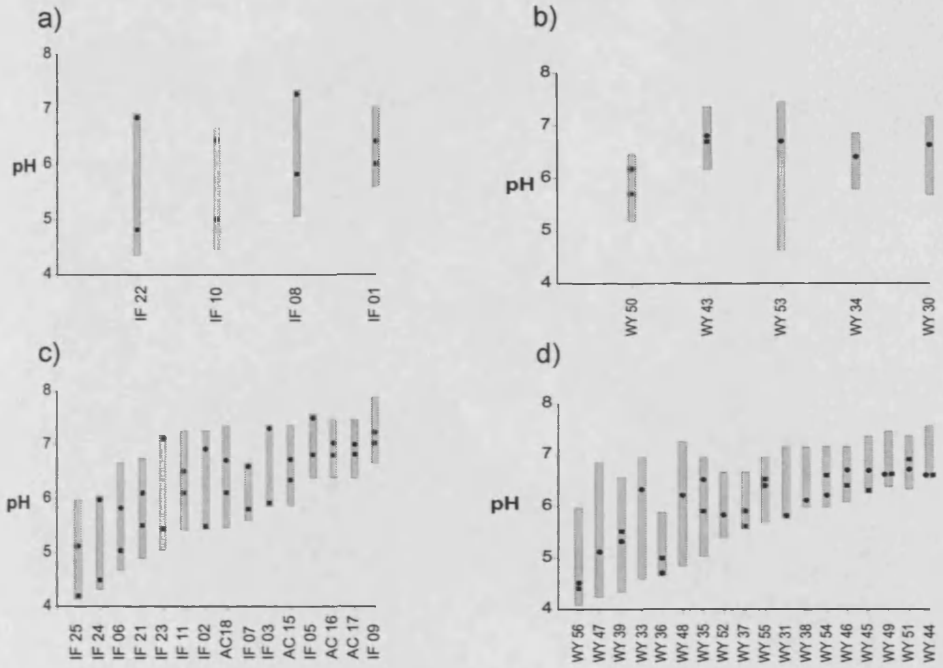
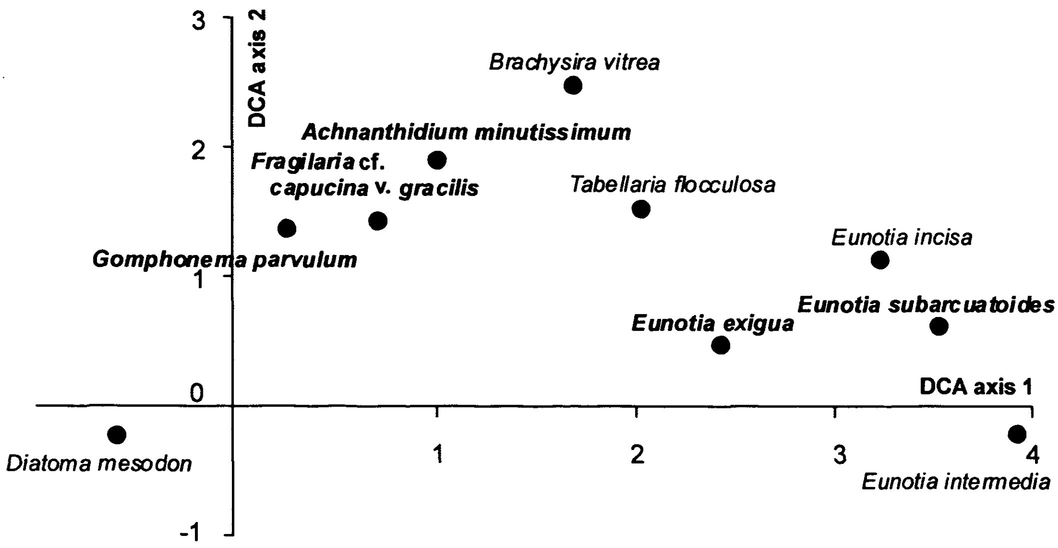
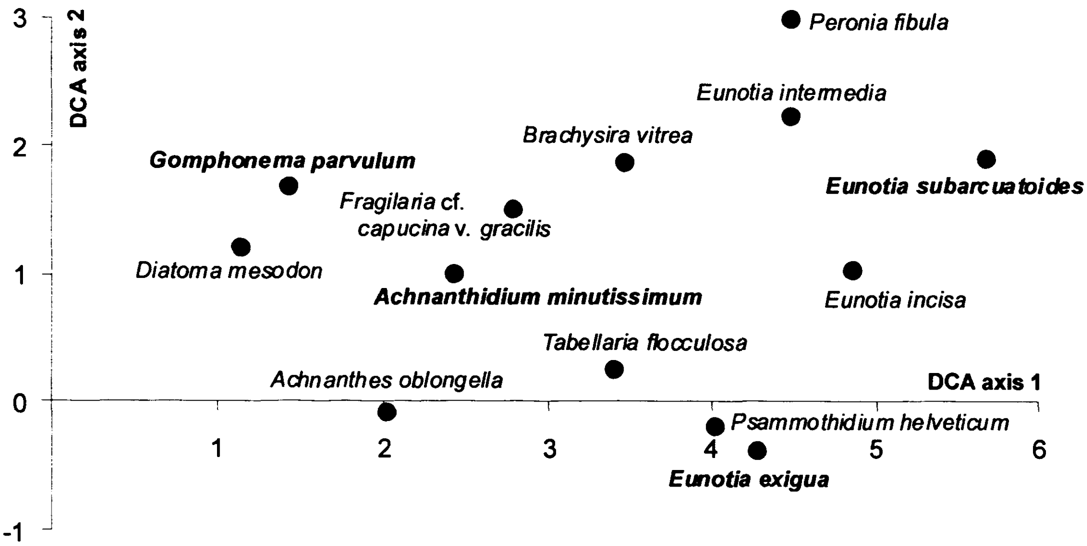

a)
b)
c)
d)
pH
IF 22
IF 10
IF 08
IF 01
WY 50
WY 43
WY 53
WY 34
WY 30
IF 25
IF 24
IF 06
IF 21
IF 23
IF 11
IF 02
AC18
IF 07
IF 03
AC 15
IF 05
AC 16
AC 17
IF 09
WY 56
WY 47
WY 39
WY 33
WY 36
WY 48
WY 35
WY 52
WY 37
WY 55
WY 31
WY 38
WY 54
WY 46
WY 45
WY 49
WY 51
WY 44

Figure 2


DCA axis 2
DCA axis 1
Brachysira vitrea
Achnanthidium minutissimum
Fragilaria cf.
capucina v. gracilis
Tabellaria flocculosa
Eunotia incisa
Gomphonema parvulum
Eunotia subarcuatoides
Eunotia exigua
Diatoma mesodon
Eunotia intermedia
3
2
1
0
-1
1
2
3
4

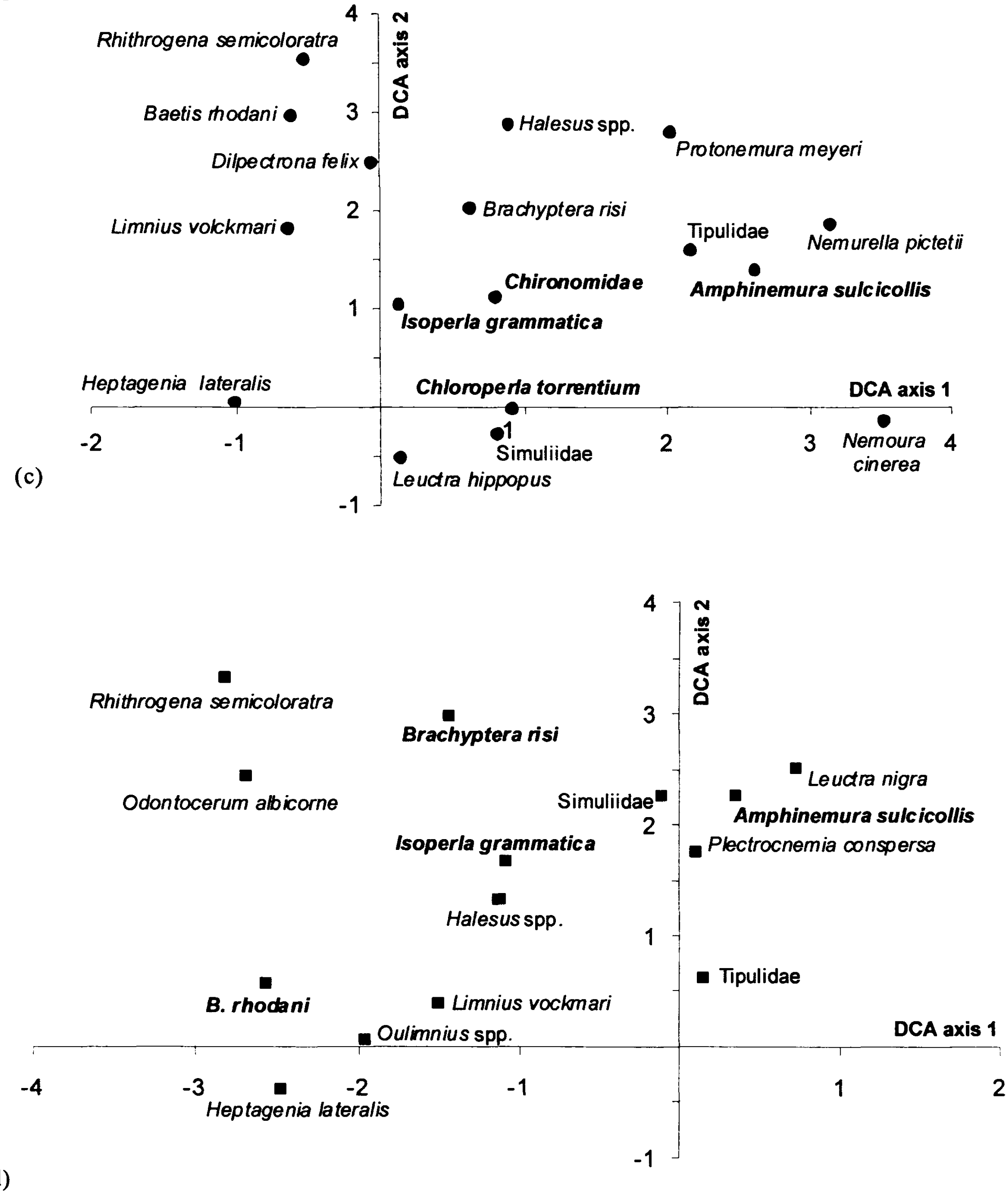
(a)

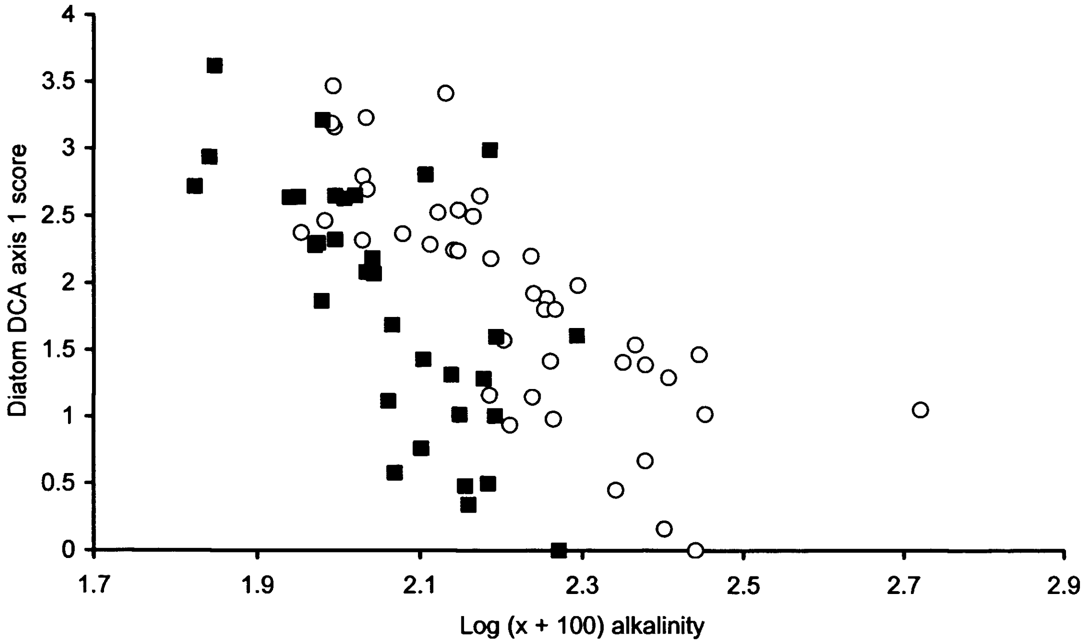

DCA axis 2
DCA axis 1
Peronia fibula
Eunotia intermedia
Brachysira vitrea
Gomphonema parvulum
Eunotia subarcuatoides
Fragilaria cf.
capucina v. gracilis
Diatoma mesodon
Achnanthidium minutissimum
Eunotia incisa
Tabellaria flocculosa
Achnanthes oblongella
Psammothidium helveticum
Eunotia exigua
3
2
1
0
-1
1
2
3
4
5
6

(b)

Figure 3.

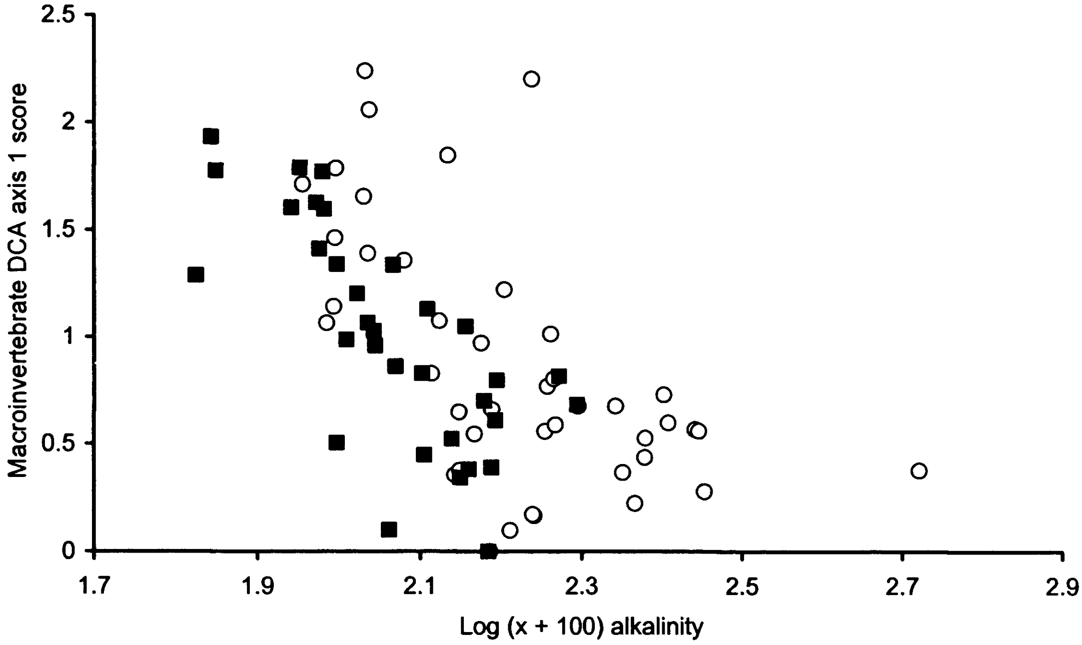
Figure 3. cont.





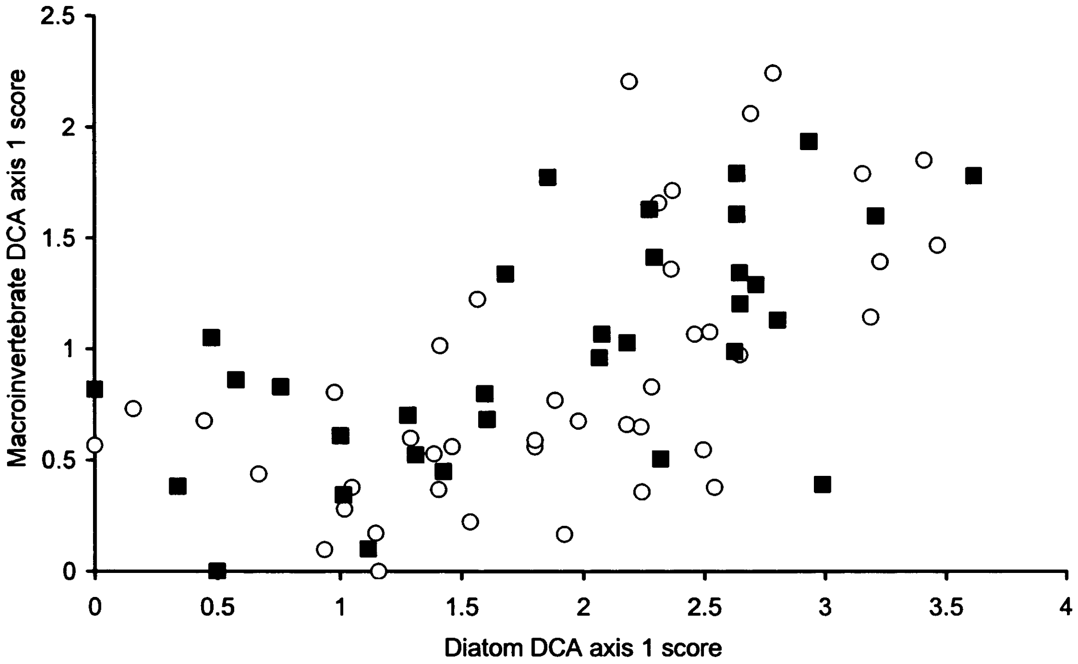
4
3.5
3
2.5
2
1.5
1
0.5
0
Diatom DCA axis 1 score
1.7
1.9
2.1
2.3
2.5
2.7
2.9
Log (x + 100) alkalinity

(a)



2.5
2
1.5
1
0.5
0
Macroinvertebrate DCA axis 1 score
1.7
1.9
2.1
2.3
2.5
2.7
2.9
Log (x + 100) alkalinity

(b)
Figure 4.



Macroinvertebrate DCA axis 1 score
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
Diatom DCA axis 1 score

Figure 5.

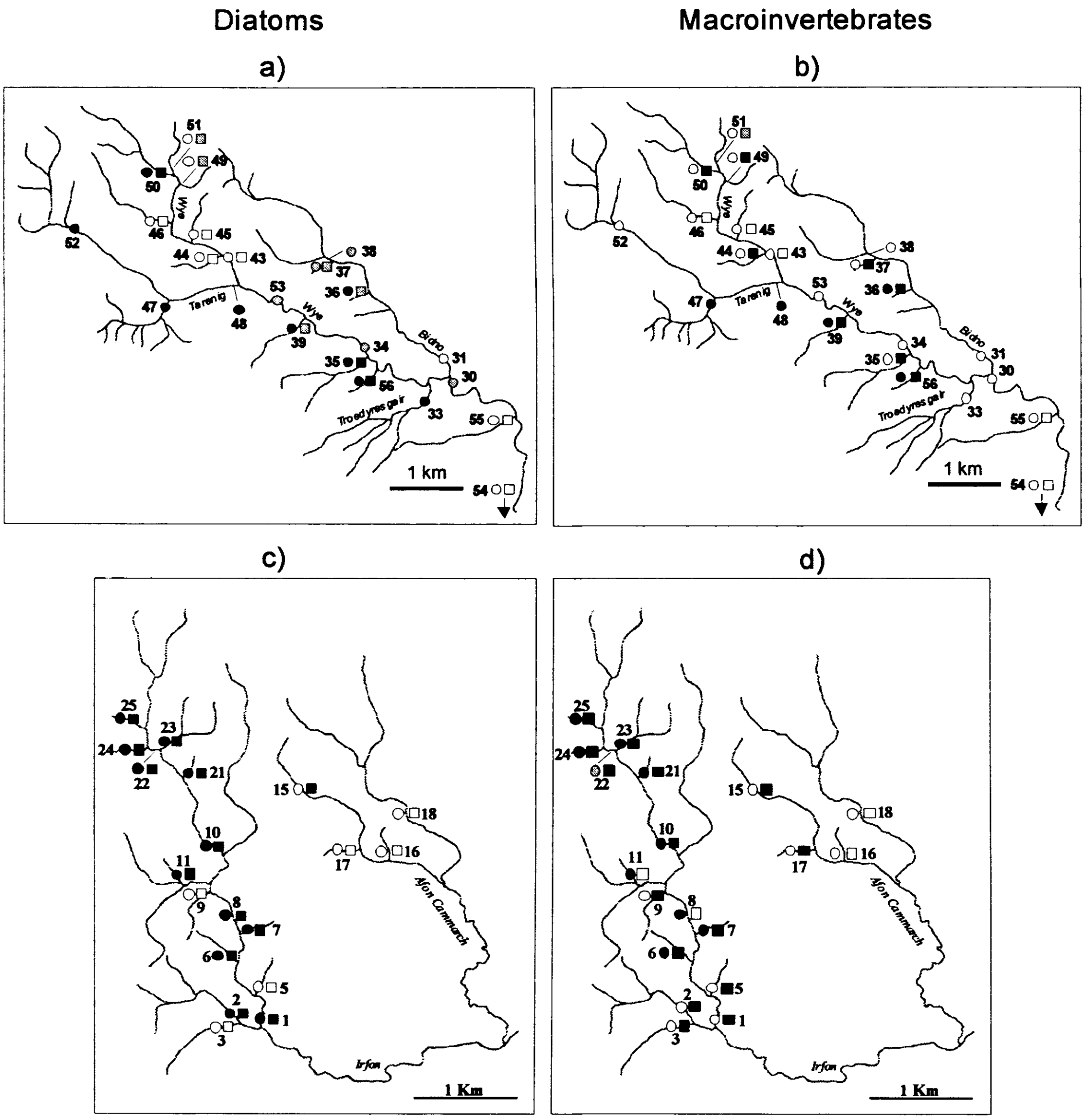
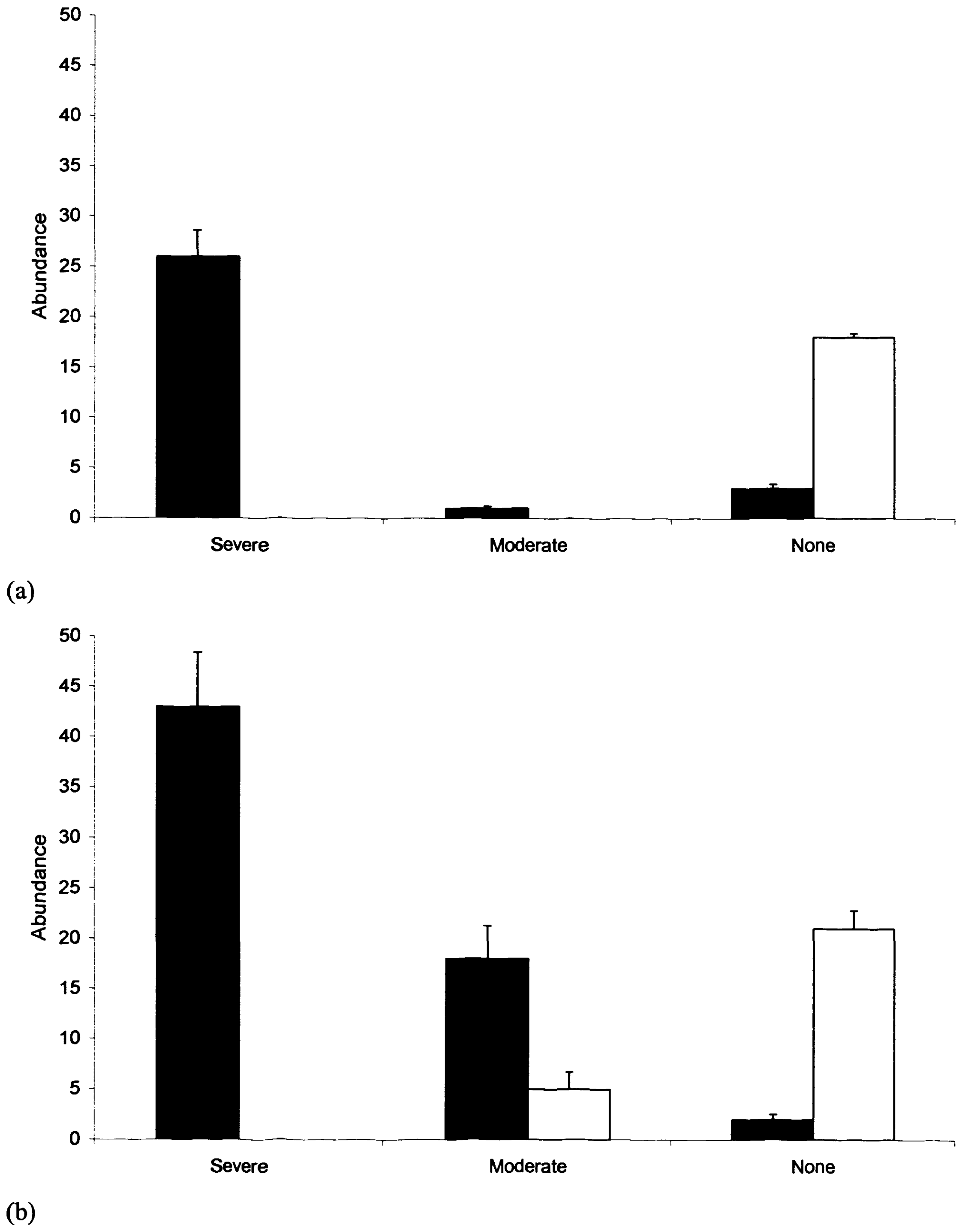
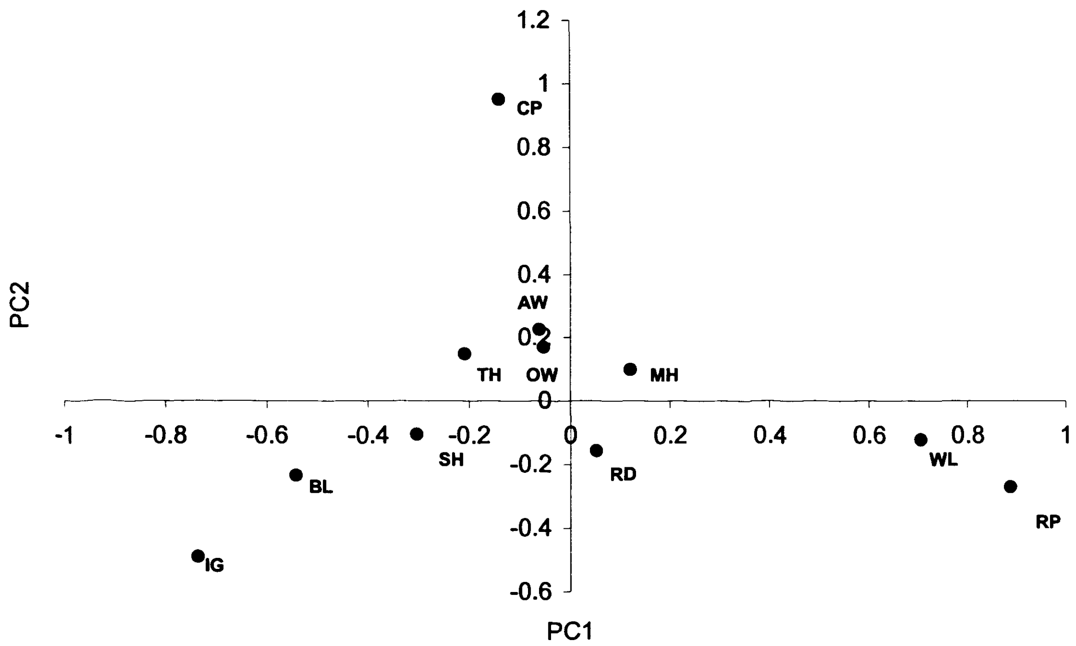

Diatoms
Macroinvertebrates
a)
b)
c)
d)
Wye
Tarenig
Bidno
Troedyresgair
Afon Cammarch
Irfon
1 km
1 Km

Figure 6.



50
45
40
35
30
25
20
15
10
5
0
Abundance
Severe
Moderate
None
(a)
50
45
40
35
30
25
20
15
10
5
0
Abundance
Severe
Moderate
None
(b)

Figure 7.



PC2
PC1
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
CP
AW
TH
OW
MH
SH
RD
BL
WL
RP
IG

Figure 8.

Chapter 4

The chemical response of tributaries of the Welsh river Wye to catchment liming.

4.1 Summary

1. Limestone powder ($CaCO_3$) is used widely to accelerate the recovery of acidified rivers where responses to decreasing acid deposition is slow. However, in few instances have the chemical effects of wide-scale catchment lime additions been assessed in whole river basins.
2. This chapter assesses the chemical response of upland tributaries of the Wye catchment in mid-Wales to experimental liming. Data from quarterly streamwater sampling over three years at 42 sites, were used to compare changes at limed sites with acid controls and circumneutral reference streams in a Before-After-Control-Impact (BACI) experiment with replicate treatment, control and reference sites.
3. In the year (2003) prior to treatment, mean pH in tributaries across the study area ranged from 4.6-7.3. pH, alkalinity, acid neutralising capacity and base cations (calcium and magnesium) were significantly higher at circumneutral sites (mean pH range 6.3-7.3) than at acid controls (pH range 5.0-6.7) and streams targeted for liming (pH range 5.8-6.7). Conifer afforested sites had significantly elevated NO_3^- by comparison with other sites while conductivity was significantly higher at sites draining improved grassland and broadleaf/mixed woodland.
4. During sampling in the first year after treatment (2004), discharge was higher (mean average daily flow 1.42 $cm^3\ sec^{-1}$) than in the pre-treatment period (mean ADF 0.22 $cm^3\ sec^{-1}$) reflecting drought conditions in the latter. There were no significant changes in overall mean chemistry attributable to liming probably because effect-sizes were small and because of large inter-annual variations in discharge during the experiment.

5. Many low-order tributaries in the Wye catchment are still impacted by acidification, with liming so far insufficient to substantially increase pH, particularly at high flow. More intensive liming, targeted at further acidic headwaters, would be required to increase effects.

Key words: *acidification, discharge, neutralisation, pH, recovery, water quality.*

4.2 Introduction

There is now unequivocal evidence that the emissions and deposition of acidifying compounds are declining across much of Europe from their peak in the late 1970s. Emissions of sulphur dioxide fell by 82% between 1970 and 1999, and oxides of nitrogen by 30% (Fowler *et al.*, 2001) with some areas now below critical loads of acid deposition (http://critloads.ceh.ac.uk). The reversal of acidification has been observed in some UK freshwaters, including increases in acid neutralising capacity (ANC), pH and alkalinity (Evans *et al.*, 2001; Fowler *et al.*, 2001; Davies *et al.*, 2005, Monteith and Evans, 2005; Skjelkvåle *et al.*, 2005). Despite this, it is estimated that over 12,000 km of Welsh rivers, including many upland sites, are still acidified (Rimes *et al.*, 1994; Stevens *et al.*, 1997; Kowalik *et al.*, in press; Lewis *et al.*, in press). Moreover, rates of pH and ANC increase are generally slow and incremental (typically < 0.01 to 0.03 pH units per year, 1.3 μeq L^{-1} $year^{-1}$; Evans *et al.* 2001; Harrman *et al.*, 2000; Reynolds *et al.*, 2004). In northern Welsh streams, for example, mean pH has risen from pH 4.6 to 5.1 over an 18 year study (Reynolds *et al.*, 2004).

The chemical characteristics of acidified streams have been well described. The pattern and extent of acidification is strongly linked to physio-chemical attributes of the catchment, including geology, soil characteristics and land-use. For example streams underlain by reactive rock types such as carbonates have higher base-cation concentrations than those underlain by igneous and matamorphosed rocks, while areas underlain by slow-weathering granite are vulnerable to acid inputs (Hornung *et al.*, 1990; Kram *et al.*, 1997; Thornton and Dise, 1998; Williard *et al.*, 2005). Soil type and land-use also affect runoff chemistry, with thin siliceous clay or sandy soils often highly susceptible to acidification. Low pH with elevated metals is linked to conifer afforestation in sensitive areas (Ormerod *et al.,* 1989; Donald and Gee, 1992; Weatherley *et al.*, 1993; Clenaghan *et al.*, 1998; Goulding and Blake, 1998; Thornton and Dise, 1998).

Water chemistry in base-poor locations also tracks discharge, with increased runoff often accompanied by base-cation dilution, increased H^+ concentration and decreased pH (Davies *et al.*, 1992; Anderson *et al.*, 1993; Giller and Malmqvist, 2002). Base-flows are often relatively alkaline, positive in ANC and enriched in Ca, whilst high flow chemistry tends to be acidic and enriched in DOC (Dissolved Organic Carbon) and aluminium. In temperate streams or those affected by snowmelt, periods of high discharge are associated with 'acid episodes'. Davies *et al.* (1992) describe such events as short-term chemical changes resulting from increased discharge which result in decreased acid neutralising capacity (ANC) through base-cation dilution and the addition of strong acid anions (Wigington *et al.*, 1996; Tranter *et al.*, 1994). Such effects still occur widely in Europe and have biological effects (Lepori *et al.*, 2003; Kowalik and Ormerod, submitted) that are sufficient to retard the recovery of upland Welsh streams (Kowalik *et al.*, in press).

The slow response of streams to reduced emissions and deposition have led to the continued examination of intervention methods, such as liming, that might accelerate the recovery process. Catchment liming has so far yielded mostly positive results with regards to chemical change. In some Norwegian catchments, experimental liming of watersheds has increased pH, calcium and ANC while decreasing reactive Al (Hindar, 1996; 2005; Traaen, 1997). In Welsh and Scottish catchments, similar changes have been observed (Brown 1988; Ormerod *et al.*, 1990; Jenkins *et al.,* 1991; Donald and

Gee, 1992; Howells *et al.*, 1992) with effects often sustained for years to decades after treatment (Rundle *et al.*, 1995; Bradley and Ormerod, 2002). Effects have been particularly marked when treatment is targeted at hydrological source areas (Donald and Gee, 1992). However, the extent to which these effects scale-up to be applicable to extensive upland watersheds still requires elucidation.

In July 2003, limestone powder was applied extensively to wetland areas on the upper reaches of the river Wye in mid-Wales. In this large-scale study, the response of headwaters, low order tributaries and main river sites within the catchment, to lime treatment, was assessed. Forty-two sites were monitored over three years to assess water quality changes following treatment in comparison to untreated control streams. The central hypotheses of this study were that (i) the chemistry of upland streams will be influenced by physical attributes of the catchment; (ii) application of limestone, to the catchment of acidified, streams will lead to increases in mean stream pH, alkalinity and calcium concentration, and reduced aluminium concentration relative to control sites; (iii) stream pH will be strongly influenced by stream discharge. The experiment is based on a Before-After-Control-Impact (BACI) design which allows comparison of temporal trends in treated streams with unmanipulated control sites while avoiding pseudo-replication (Stewart-Oaten *et al.*, 1986). The inclusion of multiple treatment, control and reference sites, and multiple sampling times, allows multiple BACI (MBACI) to be performed using repeated measures ANOVA (Underwood, 1993; Paine, 1996; Niemi *et al.*, 1999).

4.3 Methods

4.3.1 Study area

The River Wye rises at 677m O.D on Plynlimon in mid-Wales (52°28'N, 3°45'W) and drains approximately 4180 km^2 before joining the Severn estuary at Chepstow (Figure 1). The source area receives an annual rainfall of 2500 mm (Reynolds *et al.*, 1997). In the upper reaches, soils consist of peats, stagnopodzols, acid brown earths and stagnogleys (Reynolds *et al.*, 1997) and are underlain predominantly by Lower Palaeozoic (mudstones and shales) and Upper Palaeozoic rocks (marls and sandstone) (Edwards and Brooker, 1982). Acid grassland, rough pasture and coniferous forest, dominate the land-use. The latter makes up approximately 14% and 25% of the upper Wye and Irfon catchments respectively and is dominated by Sitka spruce (*Picea sitchensis*) and Norway spruce (*Picea abies*).

A total of 42 sites were selected for this investigation, mostly comprising individual streams, but 10 main river locations downstream of other sites were also included. The sites thus ranged from first to fourth order streams and main river sites with 23 on the upper Wye (catchment area 174 km^2) and 19 on the River Irfon, a major tributary of the Wye (244 km^2) (Figure 1). Sites were chosen to represent a range of physical characteristics and land-uses, and ranged from first order headwaters draining small catchments (1-5 km^2) at high altitudes, to higher order sites draining large areas (up to 59 km^2) at lower altitudes (Table 1).

In July 2003, wetland areas on the upper Wye were treated with limestone as part of the pHISH (Powys Habitat Improvement Scheme), which aims to improve water quality and restore salmonid populations in Welsh rivers. A total of 750 tonnes of limestone was applied by tractor and by hand at 17 locations (Figure 1). Application sites were selected based on previous assessment of the catchment chemistry and accessibility. The treatment affected nine of the study sites (Figure 1), with 13 remaining as acid control sites and 20 as circumneutral reference streams. The later two groups were categorised on the basis of minimum pH below or above 5.7, respectively.

4.3.2 Chemical sampling

Chemical data were collected quarterly between February 2003 and April 2005 to represent all seasons and a range of discharges. At each site, stream pH, temperature and conductivity were measured directly using a pH/Cond 340i meter (WTW Weilheim). Two streamwater samples were collected using 180 ml polyethylene bottles at all sites on each visit, one of which was filtered (0.45 μm pore size; Whatman sterile membrane filters) and one unfiltered. Unfiltered samples were analysed in the laboratory for alkalinity using Gran titration. Filtered samples were analysed for cations (calcium, sodium, potassium and magnesium) by atomic absorption spectrophotometry (AAS, Perkin Elmer) whilst silicon was assessed by the molybdenum blue method and anions (chloride, nitrate (NO_3^-N) and sulphate) determined by ion chromatography (Dionex). DOC was measured by continuous flow colorimetry with UV digestion (Skalar autoanalyser system) and aluminium was determined colorimetrically using the pyrocatechol violet method (Dougan and Wilson, 1974). Acid neutralising capacity (ANC) was calculated as the sum of base cations ($Ca^{2+} + Mg^{2+} + Na^+ + K^+$) minus the sum of acid anions ($Cl^- + SO_4^{2-} + NO_3^-$). All sample bottles were rinsed with deionsied water and then sample site water prior to sampling.

4.3.3 Stream and catchment character

Stream order and stream link magnitude were calculated after Strahler (1952) and Shreve (1967), respectively. An O.S. topographical map was used to calculate catchment areas for each site (to the nearest km^2) and to verify the altitude readings provided by a Garmin GPS (GPS 12 XL) recorder used on-site. A modified version of the UK Environment Agency's River Habitat Survey (see Environment Agency, 2003 for full methods) was used to assess land-use features in April 2003. At each of the study sites, data were collected over a 150m reach (i.e. 'sweep-up'; Environment Agency, 2003) and at four individual locations (i.e. spot checks), describing land-use (e.g. conifer plantation, broadleaf/mixed woodland) within 50 m of the bank top described.

Discharge was measured in the upper Wye over all sampling periods, using a Campbell Scientific CR10 data logger situated at a monitoring station at a site on Plynlimon (Figure 1). Values here were assumed to represent all sites, an assumption validated by strong inter-correlation with an adjacent gauged site on the Cothi (upper Afon Tywi). The time (within one hour) at which each site was sampled was recorded and the corresponding flow was used as an estimate to compare discharge across the sampling periods (average distance from gauging station to survey sites = 16km).

4.3.4 Statistical analysis

One-way ANVOA was used compare the concentration of chemical determinands between experimental groups (i.e. acid, limed, circumneutral) in the pre-treatment period. For land-use data, Principal Components Analysis (PCA; CANOCO for

Windows 4.0; ter Braak and Šmilauer, 1998) was used to describe variations in land-use between sites, with the resulting axes taken to represent gradients in land-use between sites. Relationships between pre-treatment chemistry and stream or catchment attributes were then assessed using Spearman's rank correlation (SPSS, version 12).

Variation in chemistry before and after liming, at treatment, control and reference sites were assessed using repeated measures ANOVA (SPSS, version 12) to include the effects of treatment (limed vs acid; limed vs circumneutral), time (Before/After), and time x treatment interactions, with discharge included as a covariate. This model tested the effect of all measured sources of variation and the effects of interactions (Underwood, 1993; Paine, 1996; Niemi *et al.*, 1999).

Chemical data were collected over multiple seasons and were thus expected to vary with flow. As a consequence, variations in discharge between sampling times might confound or obscure any treatment effects. Moreover, changes in the relationship between pH and discharge as a consequence of liming could be indicative of treatment effects. pH at each site was therefore plotted against discharge at the time of sampling (i.e. on Plynlimon), and Pearson correlation analysis used to obtain an r-value for this relationship at each site. These calculations were performed for the pre- and post-treatment periods separately, and the r value for each site, along with the slope and intercept of the trend lines describing the pH/discharge relationship, were compared using ANOVA (MINITBAB, version 13) across treatments (acid/lime/circumneutral) and times (before/after liming).

4.4 Results

4.4.1 Pre-treatment chemistry

Pre-treatment mean pH ranged across sites from 4.6 to 7.3 and alkalinity from -12.6 to 203.5 µeq L^{-1} (Table 2). As expected from the study design, pH ($F_{1,40} = 36.53, p <$ 0.05), alkalinity ($F_{1,40} = 31.39, p < 0.05$), ANC ($F_{1,40} = 34.40, p < 0.05$), Ca ($F_{1,40} =$ 13.34, $p < 0.05$) and Mg ($F_{1,40} = 25.95, p < 0.05$) were all higher at circumneutral streams that at other sites, whilst DOC did not differ significantly between groups (Table 2). A total of 17 sites had minimum pH below 5.7 of which 13 sites had mean pH below 5.7.

In the PCA of land-use data, PC1 (38.0% of variance) reflected a change from improved/semi-improved grassland or broadleaf/mixed woodland to rough grassland or wetland (Land-use PC1), whilst PC2 represented a gradient from grassland or broadleaf/mixed woodland to conifer plantations (Land-use PC2; 21.6% variance) (Table 3). Correlation analysis revealed significant relationships between silicon and stream order ($r = -0.33, p < 0.05$) and altitude ($r = -0.38, p < 0.05$). NO_3^- was weakly but positively correlated with land-use PC2, with sites of high NO_3^- concentration associated with conifer afforestation ($r = 0.36, p < 0.05$). Conductivity was negatively correlated with land-use PC1 and thus conductivity was greater in improved/semi-improved grassland and broadleaf/mixed woodland ($r = -0.31, p < 0.05$).

4.4.2 Changes following treatment

In the first year after treatment, mean pH and alkalinity were lower at all sites than in the previous year (Figure 2) probably as a consequence of variations in discharge. In repeated measures ANOVA, using flow as a significant covariate ($p < 0.05$), limed streams had significantly greater alkalinity, ANC, K, Mg, Ca, conductivity, and pH than acid streams (at $p < 0.05$) (Table 4). Additionally, Mg, Ca, K, SO_4^{2-}, conductivity and pH decreased between the years of the experiment, whilst NO_3^- increased. However, there were no significant time x treatment interactions, and hence no strong indications of any effects on mean chemistry due to liming. Al (lower at circumneutral sites) and Ca, pH, alkalinity, K, NO_3^-, SO_4^{2-}, conductivity and Mg also differed in repeated measures ANOVA between limed sites and circumneutral sites. However, again, there were no significant time x treatment interactions for any determinand.

Reflecting drought conditions in 2003, discharge was generally higher during sampling events in 2004 than in the pre-treatment year (Figure 3); mean average daily flow over the 2003 study periods was 0.22 $cm^3\ sec^{-1}$ whilst mean average daily flow during the 2004 study periods was 1.42 $cm^3\ sec^{-1}$. Correlations, slopes and intercepts for the relationship between pH and discharge varied between groups of sites in the post-treatment period. In particular, intercepts were higher at circumneutral sites than acid sites in the pre- ($F = 6.62$; $p < 0.05$) and post-treatment ($F = 7.45$; $p < 0.05$) periods (Table 5). The mean r-value for circumneutral sites were higher in the post-treatment period then before treatment ($F_{1,32} = 1.9$, $p < 0.05$), whilst the mean intercept was lower ($F_{1,32} = 15.2$, $p < 0.05$). The intercepts for lines describing the relationship between discharge and pH at limed sites were statistically higher than at acid sites ($F = 12.6$; $p < 0.05$) after treatment, whereas before treatment they were not significantly different ($F = 5.33$; $p < 0.05$) (Table 5). There was not a significant change in the slope or the strength of the correlation describing the relationship between pH and discharge at limed sites.

4.5 Discussion

Despite significant declines in the deposition of acidifying compounds across much of Europe and North America (Pawlowski, 1997; Fowler *et al.*, 2001), upland Welsh streams are still affected by acidification (Monteith and Evans, 2005; Kowalik *et al.*, in press). Recent data from the Llyn Brianne catchment in mid-Wales and the adjacent river Wye show that both catchments are impacted (Kowalik *et al.*, in press; Lewis *et al.*, in press).

In this study, initial analysis of the water chemistry at stream sites on the Wye was focused on the relationship between physical attributes and water chemistry as, for example, biological, chemical and physical processes within soils (and thus run-off chemistry) are known to be strongly influenced by different land-uses (Goulding and Blake, 1998). In this study, the strongest gradients in land-use appeared to be from sites in improved/semi-improved grassland or broadleaf/mixed woodland to those in wetlands or rough grassland, and from those in improved/semi-improved grassland to those draining conifer plantations. These land-use gradients were related to gradients in water chemistry, with high nitrate concentrations associated with sites draining conifer plantations. In neighbouring catchments, conifer forestry has been linked, locally, to increased nitrates, through felling (Neal *et al.*, 2004a,b), with the authors suggesting that current UK forestry guidelines which incorporate 'phased harvesting of catchments' over several years, may diminish the extent to which felling promotes stream acidification (Neal *et al.*, 1998). With regard to acidity, depleted acid-base status has been linked to conifer afforestation (Ormerod *et al.,* 1989; Weatherley *et al.*, 1993; Clenaghan *et al.*, 1998) through elevated loading of acidic anions (Donald and Gee, 1992), changes in the drainage efficiency of soil, and increased surface area available for scavenging of atmospheric pollutants (Goulding and Blake, 1998). It thus appears that liming should be undertaken with consideration of the effects of other land-use manipulations, as these may have a large and even over-riding effects on chemistry.

This study incorporated stream sites within the upper reaches of the Wye and ranged from low order streams at high altitudes, to high order main river sites draining large areas. Many of the low order sites had low mean pH (see chapter 3) and respectively over 40% and 30% of streams had minimum and mean pH below 5.7, and thus below a critical level for sensitive species (Feldman and Connor, 1992; Masters, 2002; Kowalik and Ormerod, 2006). Although causative treatment is the favourable method for restoring acidified catchments, the time scales involved often lead to large lags between treatment and results, and short falls in chemical recovery have thus led to development, testing and implementation of intervention methods, such as liming. An earlier literature review (Chapter 2) highlighted the advantages and disadvantages of different liming methods, and it is apparent that catchment liming can achieve sustained changes in water quality. In the Wye catchment, application of lime to the watershed was aimed at aiding and accelerating chemical recovery, to allow re-colonisation by acid-sensitive taxa. Hydrological source areas were targeted, as there is evidence that this can increase the efficacy of treatment (Jenkins *et al.*, 1991; Waters *et al.*, 1991; Donald and Gee, 1992).

During the first post-treatment study year (2004), mean pH (5.5-6.3) across the catchment was lower than in the previous year (6.1-6.3). Between limed sites and acid controls or circumneutral reference sites there were obvious time and treatment effects for acid-base variables including pH and Ca. However, the lack of any time x treatment interactions indicated that differences between sites over time were independent of treatment, and the changes at limed sites could therefore not be attributed to intervention. It was apparent that the differences over time were strongly associated with flow. Water is increasingly derived from acidic and organic rich, upper soil horizons as discharge increases (Reid *et al.*, 1981) and base flow chemistry therefore tends to be alkaline, with positive ANC, and enriched in Ca, whilst high flow chemistry will be acidic, and enriched in DOC and Al. Periods of pronounced high flow have distinct chemical features, with such episodes characterised by ANC below 0 μeqL^{-1-} and lower concentrations of weathering products compared to cation and bicarbonate rich base-flow water (Reid *et al.*, 1981; Davies *et al.*, 1992). Current data suggest that the effects of high flow episodes still occur widely (Lepori *et al.*, 2003; Kowalik and Ormerod, submitted) and recent evidence is emerging that such episodes retard the recovery of upland Welsh streams (Kowalik *et al.*, in press). In this study mean average daily flow during sampling was more than 600% higher in 2004 than 2003, with the lowest daily flows recorded in April 2003 and the highest in October 2004. At acid sites and limed sites, there was a strong negative correlation between pH and discharge, with pH particularly low during the high flow periods of February and October 2004.

Despite sustained changes to mean chemistry recorded in other catchment liming experiments (Rundle *et al.*, 1995; Bradley and Ormerod, 2002; Hindar *et al.*, 2003), this method does not allow targeting of high flow periods, when acidity may be most pronounced (Soulsby, 1995; Davies *et al.*, 1992), and addressing the issue of high-flow-chemistry may now be essential for substantial and comprehensive changes in chemistry to occur (Jenkins *et al.*, 1991; Bradley and Ormerod, 2002; Kowalik *et al.*, in press).

Although the focus of many liming studies is to detect changes in pH, the importance of other variables has been highlighted. DOC has ecological implications for aquatic biota, for example through effects on the supply, bioavailability and toxicity of metals (e.g. copper, Sciera *et al.*, 2004; methylmercury, Waters *et al.*, 1998; Kullberg *et al.*, 1993) and interest in DOC trends during recovery from acidification is growing. Studies in Welsh catchments and other areas of Europe and North America have shown similar means to those recorded here (mean DOC of Wye 2.2-2.9 mg L^{-1}). Long-term data sets have revealed increasing stream DOC (e.g. 0.055 mg C l^{-1} $year^{-1}$) (McCartney *et al.*, 2003; Neal *et al.*, 2005; Skjelkvåle *et. al*, 2005). However, within the relatively small time-scales involved in this study, no significant changes in DOC were detected at any of the stream sites and more prolonged investigation may elucidate patterns in DOC at acidified sites in this area .

Additional influences on stream chemistry which were not addressed in this study include geology and soil characteristics. For example minimum, maximum and mean concentrations for some determinands can be strongly linked to soil characteristics (Billett and Cresser, 1992) and soils with low buffering capacities such as thin siliceous clay, sandy soils, or those overlaying granite bedrock, can be highly susceptible to acidification. Geology has been identified as a strong predictor of stream nitrate, calcium and magnesium concentrations in acidified waters (Stutter *et al.*, 2003; Williard *et al.*, 2005) and can be used in predicting sensitivity and mapping critical loads (Langan and Wilson, 1992; Hall *et al.*, 1995).

The study included sampling over a large range in flows, and the pre-treatment study year (2003) was the driest in the UK for over 40 years (http://www.metoffice.com/). The changes in chemistry at treated sites in subsequent years were thus difficult to detect against such large background variations if flow. Further, short-term trends may be difficult to separate from the effects of climatic cycles (Bradley and Ormerod 2001). It appears that more intensive and widespread application of limestone to the upper reaches of the Wye as part of a long-term investigation will be necessary to produce and detect significant alterations in mean chemistry. In particular, targeting of treatment during high flow periods could prove important.

4.6 Acknowledgments

Funding for this work was provided by The Wye and Usk Foundation. Water samples were analysed by Annie Britton and discharge data were provided by Simon Grant, both of CEH Bangor.

4.7 References

Anderson, A.R., Adamson, J.K., Pyatt, D.G. and Hornung, M. 1993. pH, aluminium and calcium concentrations of streams draining acidic soils on calcareous till at Kershope, Cumbria. *Journal of Hydrology*, **149**, 27-37.

Billett, M.F. and Cresser, M.S. 1992. Predicting stream-water quality using catchment and soil chemical characteristics. *Environmental Pollution*, **77**, 263-268.

Bradley, D.C. and Ormerod, S.J. 2001. Community persistence among stream invertebrates tracks the North Atlantic Oscillation. *Journal of Animal Ecology*, **70**, 987-996.

Bradley, D.C. and Ormerod, S.J. 2002. Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater Biology*, **47**, 1961-171.

Brown, D.J.A. 1988. The Loch Fleet and other catchment liming programs. *Water, Air, and Soil Pollution*, **41**, 409-415.

Clayton, J.L., Dannaway, E.S., Menendez, R., Rauch, H.W., Renton, J.J., Sherlock, S.M. and Zurbuch, P.E. 1998. Application of Limestone to Restore Fish Communities in acidified Streams. *North American Journal of Fisheries Management*, **18**, 347-360.

Clenaghan, C., Giller, P.S., O'halloran, J. and Hernan, R. 1998. Stream macroinvertebrate communities in a conifer-afforested catchment in Ireland:

relationships to physico-chemical and biotic factors. *Freshwater Biology*, **40**, 175-193.

Davies, T.D., Tranter, M., Wigington Jr., P.J. and Eshleman, K.N. 1992. 'Acidic episodes' in surface waters in Europe. *Journal of Hydrology*, **132**, 25-69.

Davies, J.J.L., Jenkins, A., Monteith, D.T., Evans, C.D. and Cooper, D.M. 2005. Trends in surface water chemistry of acidified UK Freshwaters, 1988–2002. *Environmental Pollution*, **137**, 27-39.

Donald, A.P. and Gee, A.S. 1992. Acid waters in upland Wales - causes, effects and remedies. *Environmental Pollution*, **78**, 141-148.

Dougan, W.K. and Wilson, A.L. 1974. The absorptiometric determination of aluminium in water: a comparison of some chromogenic reagents and the development of an improved method. *Analyst,* **99**, 413-430.

Edwards, R.W. and Brooker, M.P. 1982. *The Ecology of the Wye*, Monographiae Biologicae, Volume 50. Junk Publishers, The Hague.

Evans, C.D., Cullen, J.M., Alewell, C., Kopacek, J., Marchetto, A., Moldan, F., Prechtel, A., Rogora, M., Vesely, J. and Wright, R. 2001. Recovery from acidification in European surface waters. *Hydrology and Earth System Sciences*, **5**, 283-297.

Environment Agency. 2003. *River Habitat Survey in Britain and Ireland, Field Survey Guidance Manual*. Environment Agency, Bristol.

Feldman, R.S. and Connor, E.F. 1992. The relationship between pH and community structure of invertebrates in streams of the Shenandoah-national-park, Virginia, USA. Freshwater Biology, **27,** 261-276.

Fowler, D., Coyle, M., ApSimon, H.M., Ashmore, M.R., Bareham, S.A., Battarbee, R.W., Derwent, R.G., Erisman, J.-W., Goodwin, J., Grennfelt, P., Hornung, M., Irwin, J., Jenkins, A., Metcalfe, S.E., Ormerod, S.J., Reynolds, B. and Woodin, S. 2001. Transboundary Air Pollution: acidification, eutrophication and ground-level ozone in the UK, Rep. No. EPG 1/3/153. DEFRA, Edinburgh.

Giller, P.S. and Malmqvist, B. 2002. *The Biology of Streams and Rivers*. Oxford University Press, Oxford.

Goulding, K.W.T. and Blake, L. 1998. Land use, liming and the mobilization of potentially toxic metals. *Agriculture, Ecosystems and Environment,* **67,** 135-144.

Hall, J.R., Wright, S.M., Sparks, T.H., Ullyett, J., Allott, T.E.H. and Hornung, M. 1995. Predicting freshwater critical loads from national data on geology, soils and land use. *Water, Air, and Soil Pollution*, **85,** 2443-2448.

Harriman, R., Watt, A.W., Christie, A.E.G., Collen, P., Moore, D.W., McCartney, A.G., Taylor, E.M. and Watson, J. 2000. Interpretation of trends in acidic deposition and surface water chemistry in Scotland during the past three decades. *Hydrology and Earth System Sciences*, **5**, 407-420.

Hindar, A. 1996. Liming of wetlands in the acidified Lake Roynelandsvatn catchment in southern Norway: Effects on stream water chemistry. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 985-993.

Hindar, A., Wright, R.F., Nilsen, P., Laessen, T. and Hogberget, R. 2003. Effects on stream water chemistry and forest vitality after whole-catchment application of dolomite to a forest ecosystem in southern Norway. *Forest Ecology and Management*, **80**, 509-525.

Hindar, A. 2005. Whole-catchment application of dolomite to mitigate episodic acidification of streams induced by sea-salt deposition. *Science of the Total Environment*, **343**, 35-49.

Hornung, M., Le-Grice, S., Brown, N. and Norris, D. 1990. *The role of geology and soils in controlling surface water acidity in Wales*. In Acid Waters in Wales (Eds R. W. Edwards, J. H. Stoner and A. S. Gee). Kluwer Academic Publishers, Dordrecht, pp 55-66.

Howells, G., Dalziel, T.R.K. and Turnpenny, A.W.H. 1992. Loch Fleet: liming to restore a brown trout fishery. *Environmental Pollution*, **78**, 131-139.

Huettl, R.F. and Zoettl, H.W. 1993. Liming as a mitigation tool in Germany's declining forests - reviewing results from former and recent trials. *Forest Ecology and Management,* **61**, 325-338.

Jenkins, A., Waters, D. and Donald, A. 1991. An assessment of terrestrial liming strategies in upland Wales. *Journal of Hydrology*, **124**, 243-261.

Kowalik, R.A. and Ormerod, S.J. 2006. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology*, **51**, 180-191.

Kowalik, R.A., Cooper, D. M. and Ormerod, S. J. In press. Acid episodes retard the biological recovery of upland British streams from acidification. *Global Change Biology*.

Kowalik, R.A. and Ormerod, S.J. Submitted. Stream invertebrate communities and continued episodic acidification in regions of contrasting acid deposition. Manuscript submitted.

Kram, P., Hruska, J., Wenner, B.S., Driscoll, C.T. and Johnson, C.E. 1997. The biogeochemistry of basic cations in two forest catchments with contrasting lithology in the Czech Republic. *Biogeochemistry*, **37**, 173-202.

Kullberg, A., Bishop, K.H., Hargeby, A., Jansson, M. and Petersen, R.C. 1993. The ecological significance of dissolved organic-carbon in acidified waters. *Ambio*, **22**, 331-337.

Langan, S.J. and Wilson, M.J. 1992. Predicting the regional occurrence of acid surface waters in Scotland using an approach based on geology, soils and land-use. *Journal of Hydrology*, **138**, 515-528.

Lepori, F., Barbieri, A. and Ormerod, S.J. 2003. Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams. *Freshwater Biology*, **48**, 1873-1885.

Lewis, B.R., Jüttner, I., Reynolds, B. and Ormerod, S.J. In press. Comparative assessment of stream acidity using diatoms and macroinvertebrates: implications for river management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems.*

Masters, Z. 2002. *Factors affecting Stream Macroinvertebrate Recovery from Acidification.* Unpublished. PhD Thesis, Cardiff University, Cardiff.

McCartney, A.G., Harriman, R., Watt, A.W., Moore, D.W., Taylor, E.M., Collen, P. and Keay, E.J. 2003. Long-term trends in pH, aluminium and dissolved organic carbon in Scottish fresh waters: implications for brown trout (*Salmo trutta*) survival. *Science of the Total Environment*, **310,** 133-141.

Miller, J.D., Anderson, H.A., Harriman, R. and Collen, P. 1995. The consequences of liming a highly acidified catchment in central Scotland. *Water, Air, and Soil Pollution*, **85**, 1015-1020.

Monteith, D.T. and Evans, C.D. 2005. The United Kingdom Acid Waters Monitoring Network: a review of the first 15 years and introduction to the special issue. *Environmental Pollution,* **137**, 3-13.

Neal, C., Reynolds, B., Adamson, J.K., Stevens, P.A., Neal, M., Harrow, M. and Hill, S. 1998. Analysis of the impacts of major anion variations on surface water acidity particularly with regard to conifer harvesting: case studies from Wales and Northern England. *Hydrology and Earth System Sciences*, **2**, 303-322.

Neal, C., Reynolds, B., Neal, M., Wickham, H., Hill, L. and Williams, B. 2004a. The water quality of streams draining a plantation forest on gley soils: the Nant Tanllwyth, Plynlimon mid-Wales. *Hydrology and Earth System Sciences*, **8**, 485-502.

Neal, C., Reynolds, B., Neal, M., Wickham, H., Hill, L. and Williams B. 2004b. The impact of conifer harvesting on stream water quality: the Afon Hafren, mid-Wales. *Hydrology and Earth System Sciences*, **8,** 503-520.

Neal, C., Robson, A.J., Neal, M. and Reynolds, B. 2005. Dissolved organic carbon for upland acidic and acid sensitive catchments in mid-Wales. *Journal of Hydrology*, **304**, 203-220.

Niemi, G.J., Hershey, A.R., Shannon, L., Hanowski, J.M., Lima, A., Axler, R.P. and Regal, R.R. 1999. Ecological effects of mosquito control on zooplankton, insects and birds. *Environmental Toxicology and Chemistry*, **18**, 549-559.

Ormerod, S.J., Donald, A.P. and Brown, S.J. 1989. The influence of plantation forestry on the pH and Aluminium concentration of upland welsh streams - a re-examination. *Environmental Pollution,* **62**, 47-62.

Ormerod, S.J., Weatherley, N.S., Merrett, W.J., Gee, A.S. and Whitehead, P.G. 1990. Restoring acidified streams in upland Wales - a modelling comparison of the chemical and biological effects of liming and reduced sulphate deposition. *Environmental Pollution*, **64**, 67-85.

Paine, M.D. 1996. Repeated measures designs. *Environmental Toxicology and Chemistry*, **15**, 1439-1441.

Pawlowski, L. 1997. Acidification: its impact on the environment and mitigation strategies. *Ecological Engineering,* **8**, 271-288.

Reid, J.M., Macleod, D.A. and Cresser, M.S. 1981. Factors affecting the chemistry of precipitation and river water in an upland catchment. *Journal of Hydrology*, **50**, 129-145.

Reynolds, B., Fowler, D., Smith, R.I. and Hall, J.R. 1997. Atmospheric inputs and catchment solute fluxes for major ions in five Welsh upland catchments. *Journal of Hydrology*, **194**, 305-329.

Reynolds, B., Stevens, P.A., Brittain, S.A., Norris, D.A., Hughes, S. and Woods, C. 2004. Long-term changes in precipitation and stream water chemistry in small forest and moorland catchments at Beddgelert Forest, north Wales. *Hydrology and Earth System Sciences*, **8**, 436-448

Rimes, C.A., Farmer, A.M. and Howell, D. 1994. A survey of the threat of surface-water acidification to the nature conservation interest of fresh-waters on sites of special scientific interest in Britain. *Aquatic Conservation: Marine and Freshwater ecosystems*, **4**, 31-44.

Rundle, S. D., Weatherley, N.S. and Ormerod, S.J. 1995. The effects of catchment liming on the chemistry and ecology of upland Welsh streams: testing model predictions. *Freshwater Biology*, **34**, 165-175.

Sciera, K.L., Isely, J.J., Tomasso, J.R., and Klaine, S.J. 2004. Influence of multiple water-quality characteristics on copper toxicity to fathead minnows (*Pimephales promelas*). *Environmental Toxicology and Chemistry*, **23**, 2900-2905.

Shreve, R.L. 1967. Infinite topographically random channel networks. *Journal of Geology* , **75**, 178-186.

Skjelkvåle, B.L., Stoddard, J.L., Jeffries, D.S., Tørseth, K., Høgåsen, T., Bowman, J., Mannop, J., Monteith, D.T., Mosello, R., Rogara, M., Ryzchon, D., Vesely, J., Wieting, J., Wilander, A. and Worsztynowicz, A. 2005. Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, **137**, 165-176.

Soulsby, C. 1995. Contrasts in storm event hydrochemistry in an acidic afforested catchment in upland Wales. *Journal of Hydrology*, **170**, 159-179.

Stevens, P.A., Ormerod, S.J. and Reynolds, B. 1997. Final Report on the Acid Waters Survey for Wales. CEH Bangor, Bangor.

Stewart-Oaten, A., Murdoch, W.W. and Parker, K.R. 1986. Environmental impact assessment: 'pseudoreplication in time'? *Ecology*, **67**, 929-940.

Strahler, A.N. 1952. Hypsometric (area-altitude) analysis of erosional topography. *Bulletin of the Geographical Society of America*, **63**, 1117-1142.

Stutter, M., Langan, S., Cresser, M. 2003. Weathering and atmospheric deposition signatures of base cations in upland soils of NE Scotland: their application to critical load assessment. *Geoderma*, **116**, 301-324.

ter Braak, C.J.F. and Šmilauer P. 1998. *CANOCO Reference Manual and Users Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4).* Microcomputer Power, Ithaca: New York.

Thornton, G.J.P. and Dise, N.B. 1998. The influence of catchment characteristics, agricultural activities and atmospheric deposition on the chemistry of small streams in the English Lake District. *Science of the Total Environment*, **216**, 63-75.

Traaen, T.S. 1997. Whole-catchment liming at Tjonnstrond, Norway: an 11-year record. *Water, Air, and Soil Pollution*, **94**, 163-180.

Tranter, M., Davies, T.D., Wigington, P.J. and Eshleman, K.N. 1994. Episodic acidification of fresh-water systems in Canada - physical and geochemical processes. *Water, Air, and Soil Pollution*, **72**, 19-39.

Waters, D., Jenkins, A., Staples, T. and Donald, A.P. 1991. The importance of hydrological source areas in terrestrial liming. *Journal of the Institution of Water and Environmental Management,* **5**, 336-341.

Waters, C.J., Back, R.C., Halvorsen, S., Hudson, R.J.M., Morrison, K.A. and Wente, S.P. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Science of the Total Environment*, **219**, 183-208.

Weatherley, N.S., Lloyd, E.C., Rundle, S.D. and Ormerod, S.J. 1993. Management of conifer plantations for the conservation of stream macroinvertebrates. *Biological Conservation*, **63**, 171-176.

Wigington, P.J., DeWalle, D.R., Murdoch, P.S., Kretser, W.A., Simonin, H.A., VanSickle, J. and Baker, J.P. 1996. Episodic acidification of small streams in the north-eastern United States: Ionic controls of episodes. *Ecological Applications*, **6**, 389-407.

Williard, K.W.J., Dewalle, D.R. and Edwards, P.J. 2005. Influence of bedrock geology and tree species composition on stream nitrate concentrations in mid-Appalachian forested watersheds. *Water, Air, and Soil Pollution*, **160**, 55-76.

Underwood, A.J. 1993. The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. *Australian Journal of Ecology*, **18**, 99-116.

4.8 List of tables

Table 1. Physical attributes of 42 stream sites in the river Wye catchment.
S.L.M. = stream link magnitude; land use = within 50 m of bank top; area = catchment area.
BL = broadleaf/mixed woodland; CP = coniferous plantation; MH = moorland/heath; IG = improved/semi-improved grassland; RP = rough/unimproved grassland/pasture; WL = wetland.

Site	Stream Order	S.L.M.	Altitude (m)	Area (km^2)	Land use
WY36	1	1	332	2	RP
WY37	1	1	343	1	WL
WY44	1	1	357	1	CP
WY55	1	1	290	5	IG
WY56	1	1	321	2	CP
IF07	1	1	231	1	CP
IF11	1	1	286	1	RP
AC16	1	1	252	1	BL
IF21	1	1	327	1	RP
IF24	1	1	330	1	CP
IF25	1	1	456	1	CP
WY33	2	4	307	4	RP
WY35	2	4	307	3	IG
WY39	2	3	325	2	CP
WY45	2	3	357	1	RP
WY49	2	2	393	1	RP
WY51	2	3	392	2	RP
WY52	2	8	410	5	RP
IF03	2	3	225	4	CP
IF05	2	2	329	1	BL
IF06	2	3	244	1	IG
IF09	2	3	261	2	RP
AC15	2	4	328	2	CP
AC17	2	2	269	1	BL
WY31	3	17	298	12	IG
WY38	3	12	343	6	CP
WY46	3	11	367	4	RP
WY47	3	12	355	3	CP
WY50	3	10	393	3	RP
WY54	3	12	256	8	IG
IF02	3	5	250	4	CP
AC18	3	10	241	8	RP
IF22	3	15	328	6	RP
IF23	3	7	260	2	BL
WY43	4	32	346	12	RP
WY48	4	32	314	10	CP
IF01	4	72	249	59	IG
IF08	4	66	261	47	IG
IF10	4	30	267	34	BL
WY30	5	102	288	45	IG
WY34	5	74	299	22	IG
WY53	5	69	314	24	IG

Table 2. Mean (bold) and range (parentheses) of chemical variables before treatment (2003) (n = 3) and in the first two years after (2004/2005) (n = 7) at limed streams (L), acid control sites (A) and circumneutral reference streams (C) in the catchment of the river Wye.

Year	pH	Alkalinity (μeq L^{-1})	ANC (μeq L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	DOC (mg L^{-1})
2003	**5.6** (4.6-6.1)	**5.6** (-12.6 – 36.6)	**11.75** (-36.32 – 50.8)	**1.39** (0.89 - 1.96)	**0.90** (0.71-1.19)	**1.88** (1.20 – 2.91)
2004	**5.2** (4.7-5.7)	**-3.6** (-25.0 – 19.7)	**-7.0** (-42.8 – 22.6)	**1.03** (0.65 - 1.42)	**0.73** (0.58 – 0.90)	**2.89** (1.93 – 4.24)
2005	**5.2** (4.5-5.8)	**5.1** (-13.2 - 24.9)	**-15.3** (-45.5 – 25.2)	**1.04** (0.59 -1.51)	**0.74** (0.60 - 0.86)	**2.04** (1.17 - 2.98)
2003	**6.2** (5.4-6.7)	**29.4** (-2.4 – 45.2)	**30.5** (-5.2 – 65.9)	**2.11** (1.52 – 2.50)	**1.07** (0.79 – 1.25)	**2.15** (1.18 – 3.56)
2004	**5.6** (5.2-6.1)	**15.2** (-3.2 - 36.1)	**26.4** (-8.9 – 55.0)	**1.63** (1.20 – 2.13)	**0.83** (0.62 – 0.95)	**3.06** (2.33 – 4.95)
2005	**5.7** (5.4-6.2)	**23.4** (7.3 - 46.9)	**7.1** (-20.3 – 29.3)	**1.55** (1.05 – 1.92)	**0.83** (0.64 – 0.96)	**1.93** (1.58 – 2.78)
2003	**6.7** (6.2-7.3)	**72.0** (8.3 – 203.5)	**93.1** (32.4 – 219.3)	**2.55** (1.51 – 5.05)	**1.30** (0.99 – 1.70)	**2.37** (1.12 – 4.06)
2004	**6.1** (5.5-6.5)	**53.2** (3.0 - 118.6)	**55.8** (-6.1 – 123.0)	**1.92** (1.21 – 2.99)	**1.02** (0.78 – 1.30)	**2.77** (1.14 – 5.29)
2005	**6.3** (5.7-6.8)	**59.0** (11.4 – 156.0)	**49.5** (-8.1 – 141.5)	**1.96** (1.07 – 3.37)	**1.04** (0.75 – 1.37)	**1.98** (0.91 – 3.40)

Table 3. Loading values for land-use variables along the first two PCA components describing land use in the upper catchment of the river Wye. PC1 and PC2 represent 38.0%and 21.6% of variation in data respectively.

Variable	PC 1 loading value	PC 2 loading value
Improved/semi-improved Grassland	-0.74	-0.4902
Broadleaf/mixed woodland	-0.54	-0.23
Scrub and shrubs	-0.30	-0.10
Tall herb/ rank vegetation	-0.21	0.15
Conifer Plantation	-0.14	0.95
Artificial open water	-0.06	0.23
Natural open water	-0.05	0.17
Rock, scree or sand dunes	0.05	-0.16
Moorland/heath	0.12	0.10
Wetland	0.71	-0.12
Rough/unimproved grassland/pasture	0.89	-0.27

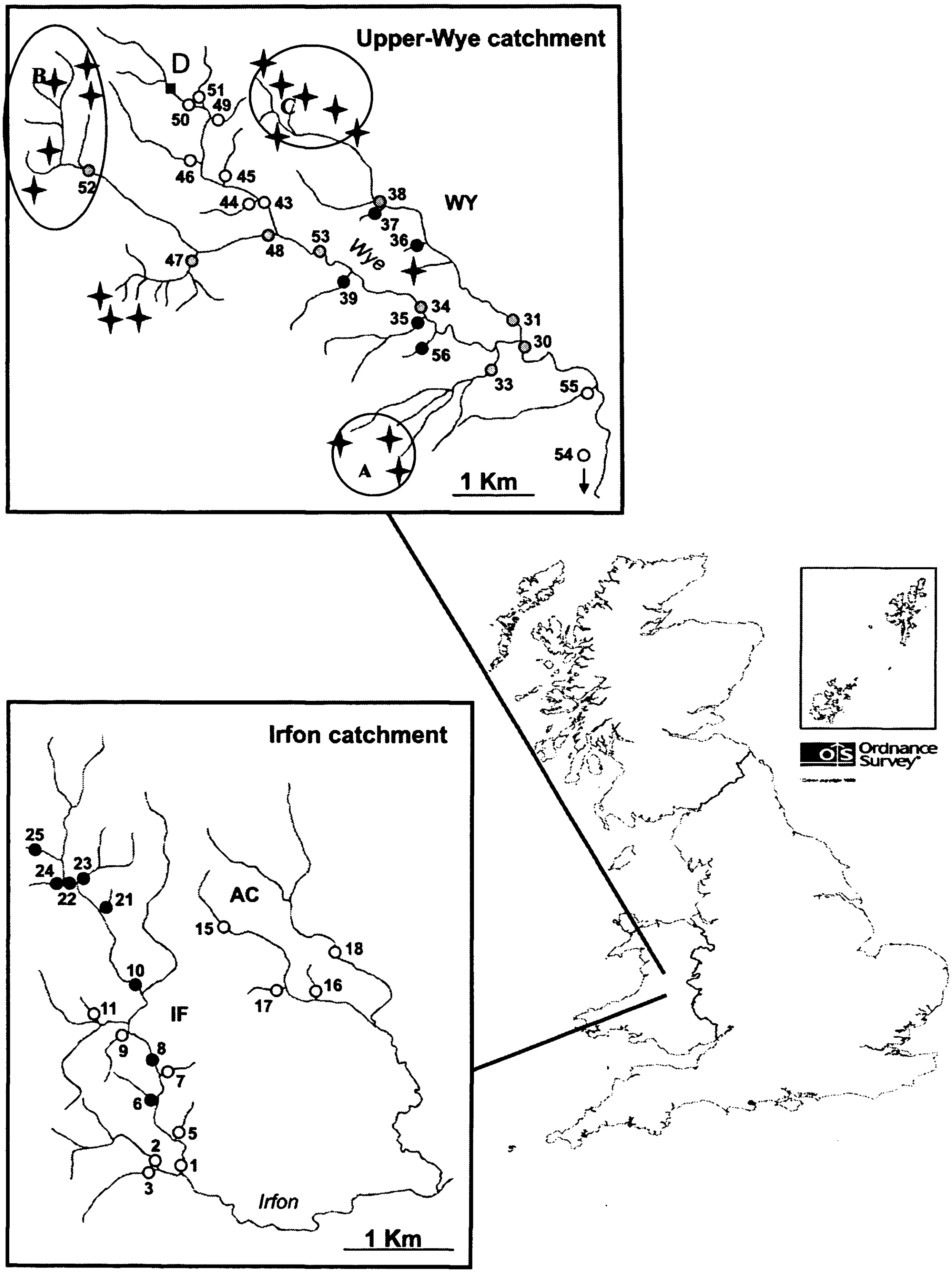
Table 4. Significant sources of variation on means of chemical variables assessed using repeated measures ANOVA. Model included effects of treatment (L = limed streams; A = acid control sites; C = circumneutral streams) and time (before/after treatment) (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; degrees of freedom between groups = 2, within groups = 10). For all chemical variables, flow was a significant covariate (at $p < 0.05$).

Variable	Source of variation	F
a) Limed versus acid streams		
pH	Time	54.7 ***
Ca	Time	350.9 ***
Mg	Time	21.2 ***
K	Time	106.6 ***
NO_3-	Time	24.6 ***
SO_4^{2-}	Time	113.5 ***
Conductivity	Time	282.7 ***
pH	treatment	8.8 **
Alkalinity	treatment	14.0 ***
ANC	treatment	10.6 **
Ca	treatment	21.0 ***
Mg	treatment	4.5 *
K	treatment	28.6 ***
Conductivity	treatment	4.7 *
b) Limed versus circumneutral streams		
pH	Time	12.7 ***
Alkalinity	Time	9.9 **
Ca	Time	6.1 *
Mg	Time	12.0 **
ANC	Time	10.5 **
Al	Time	9.6 *
Cl	Time	5.5 *
pH	treatment	103.2 ***
Alkalinity	treatment	11.4 **
Ca	treatment	67.2 ***
Mg	treatment	37.45 ***
K	treatment	57.0 ***
Al	treatment	33.7 ***
NO_3^-	treatment	18.7 ***
SO_4	treatment	33.7 ***
Conductivity	treatment	7.51 *

Table 5. Mean r (Pearson correlation) value, slope and intercept for relationship between pH and discharge in three groups of streams in the upper Wye and Irfon before (2003) and after (> 2004) liming at limed sites. * $p < 0.05$.

	r value		Slope		Intercept	
Site group	Before	After	Before	After	Before	After
Acid	-0.79* (n= 10)	-0.81* (n= 13)	-3.82	-3.42	6.14	6.10
Limed	-0.72* (n= 7)	-0.78* (n= 9)	-0.31	-1.53	6.21	6.23
Circumneutral	-0.34 (n= 17)	-0.58 (n= 20)	-0.47	-1.38	6.89	6.52

4.9 List of figures



Upper-Wye catchment
B
D
C
A
WY
Wye
51
49
50
46
45
44
43
52
38
37
36
53
48
47
39
34
35
31
30
56
33
55
54
1 Km
Irfon catchment
AC
IF
25
24
23
22
21
15
18
10
17
16
11
9
8
7
6
5
2
1
3
Irfon
1 Km
Ordnance Survey

Figure 1.

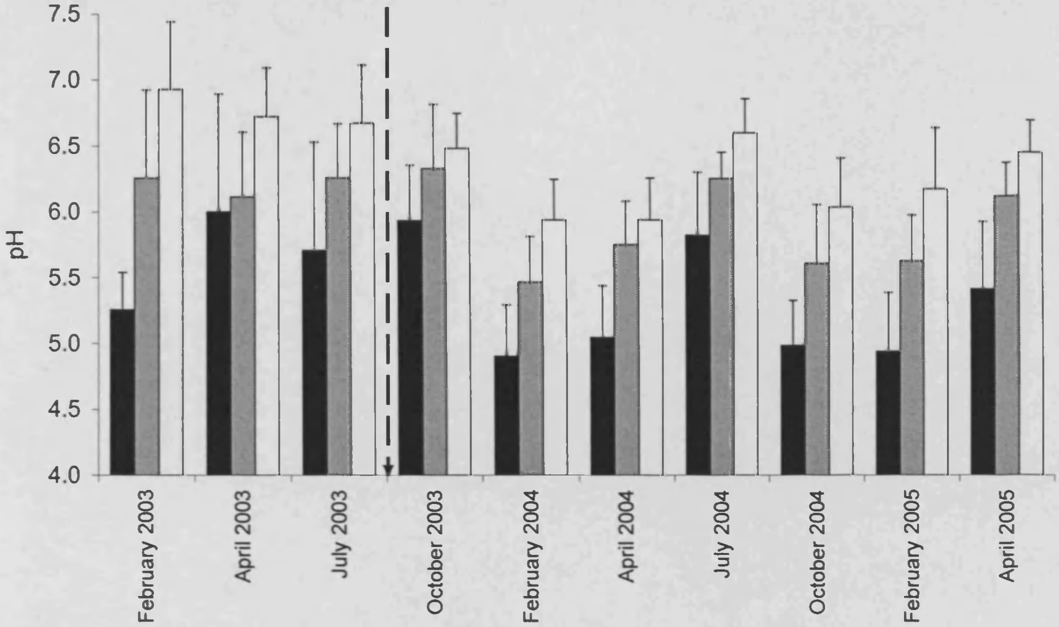

pH
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
February 2003
April 2003
July 2003
October 2003
February 2004
April 2004
July 2004
October 2004
February 2005
April 2005

Figure 2.

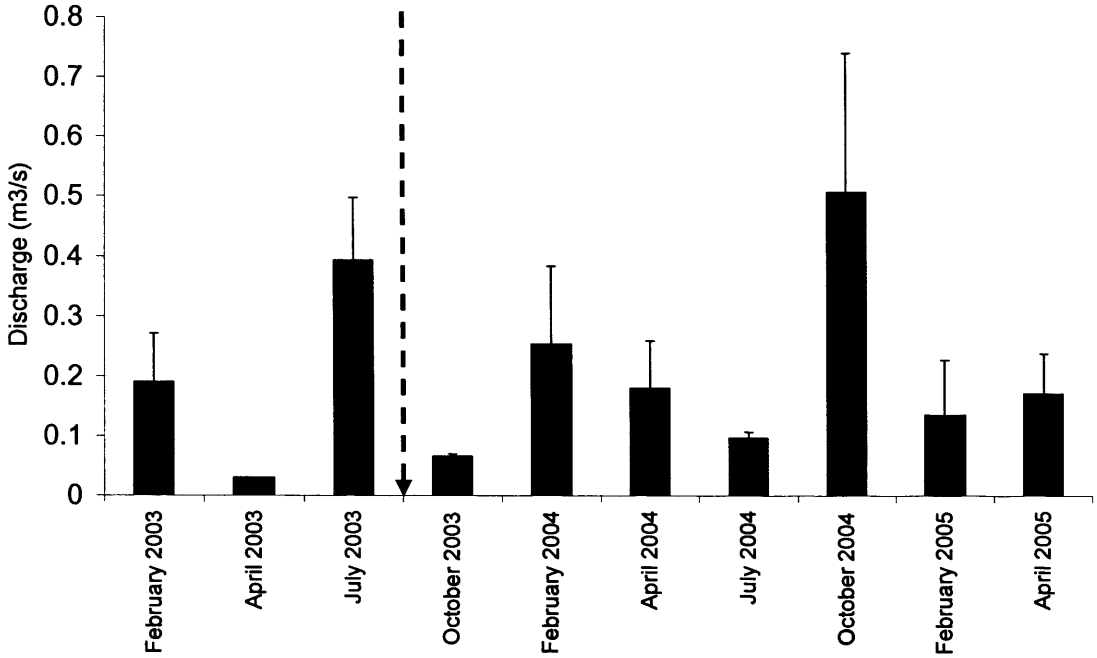

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Discharge (m3/s)
February 2003
April 2003
July 2003
October 2003
February 2004
April 2004
July 2004
October 2004
February 2005
April 2005

Figure 3.

Chapter 5

The response of macroinvertebrates to palliative liming in the upper catchment of the Welsh River Wye

5.1 Summary

1. Catchment liming is used widely to promote the chemical and biological recovery of acidified rivers. Although there is evidence that liming can alter chemical conditions, the biological response is less well understood.
2. In 2003, streams in the upper catchment of the Welsh River Wye were limed. In a replicated BACI experiment, changes in macroinvertebrate assemblages before and over two years after liming were compared with adjacent reference streams. From spring and summer samples, abundance, richness and species composition were assessed in limed streams, acid control streams and circumneutral reference sites. Particular attention was paid to acid-sensitive species, identified though Detrended Correspondence Analysis.
3. There was no significant change in the abundance or richness of macroinvertebrate assemblages at treated sites by comparison with acid control streams in the first two post-liming years. Although some acid-sensitive species, including *Baetis rhodani* and *Rhabdiopterx acuminata* appeared at some limed sites where they were previously absent, they did not increase significantly in frequency of occurrence or abundance.
4. These data suggests that liming has not so far led to the sustained and widespread recovery of macroinvertebrate assemblages in the Wye catchment. As a result, further liming has been advocated and is ongoing.

Keywords: *acidification, acid-sensitive species, BACI, pH, recovery, upland streams.*

5.2 Introduction

The chemical and biological impacts of acidification are some of the most assiduously described issues in freshwater ecosystems. The effects of low pH, associated changes in base cations and increases in toxic anions are known for several aquatic groups including diatoms, macrophytes, macroinvertebrates and fish (Dahl, 1927; Ormerod *et al.*, 1987; Sutcliffe and Hildrew, 1989; Jüttner *et al.,* 1997; Orendt, 1998). Whilst European agreements have led to reductions in UK emissions of acidifying compounds over recent decades (Stoddard *et al.*, 1999; Fowler *et al.*, 2005; Monteith and Evans, 2005; Skjelkvåle, *et al.*, 2005), the magnitude and rate of chemical recovery of upland Welsh streams have been less than expected (Fowler, *et al.*, 2001). Currently, 12,000 km of Welsh rivers are impacted by acidification (Rimes *et al.*, 1994; Stevens *et al.*, 1997) including areas important to fisheries and conservation, such as the upper reaches of the River Wye (Lewis *et al.*, in press). As a result, there are continuing calls for symptomatic treatment to accelerate the restoration process.

Intervention techniques, such as liming, are potentially valuable if acidified systems are to be restored, although examination of the literature reveals inconsistent results (Chapter 2). Some liming programmes have led to extensive chemical recovery (Fjellheim and Raddum, 1992; Hindar, *et al.*, 1995; Lingdell and Engblom, 1995) including increases in pH, calcium and magnesium, and decreases in aluminium. At the Llyn Brianne experimental catchment in mid-Wales, increases in mean pH and calcium, and decreased aluminium concentration were recorded following a single lime application to headwater catchments, with these changes persisting for over 17 years following treatment (Bradley and Ormerod, 2002a; and S. J. Ormerod unpublished data).

In addition to effects on chemistry, an important aspect of liming is the effect on freshwater organisms. Liming can reduce the physiological stresses caused by acidification through raising pH and calcium concentrations, and reducing the mobilisation of toxic metals (Fjellheim and Raddum, 1992; Howells *et al.*, 1992). However, even where chemical conditions suitable for acid-sensitive species have been produced, biological recovery is sometimes less than expected (Fjellheim and Raddum 1992; Weatherley and Ormerod, 1992; Appelberg and Svenson, 2001). Despite the sustained chemical recovery observed at the Llyn Brianne catchment for example, macroinvertebrate data revealed only modest and inconsistent biological changes following liming (Rundle *et al.*, 1995; Bradley and Ormerod, 2002a).

Macroinvertebrates have proven valuable indicators of environmental conditions (Wright *et al.*, 1984; Ormerod and Edwards, 1987; Rosenberg and Resh, 1993) and are effective in assessment of the impact of acidity on freshwaters (Townsend *et al.*, 1983; Sutcliffe and Hildrew, 1989; Ormerod and Wade, 1990; Rutt *et al.*, 1990; Merrett *et al.*, 1991; Lewis *et al.*, in press). They might, therefore, be expected to be good indicators of liming effects. In particular, the range in sensitivities to pH (Wade *et al.*, 1989; Rutt *et al.*, 1990; Davy-Bowker *et al.*, in press) and life cycles which expose much of this group to episodic events (Kowalik and Ormerod, 2006) strengthen their indicator value. Further, most taxa in this group are relatively easy to identify, ubiquitous, and can reflect integrated conditions over relatively long periods of time (Wade *et al.*, 1989).

As part of a catchment restoration programme, hydrological source areas on the upper Wye in mid-Wales were limed in July 2003. This chapter investigates whether biological assemblages have responded to catchment liming through appraisal of changes in macroinvertebrate assemblage composition, and the occurrence and abundance of acid-sensitive species in particular. These data were used to address three hypotheses: (i) acid streams will differ from circumneutral sites with regards to macroinvertebrate assemblage composition; (ii) limestone treatment of the watershed of some sites will be followed by changes in macroinvertebrate assemblage composition relative to acid control sites; (iii) the abundance and richness of acid-sensitive species will increase after liming. Assemblage responses are assessed in multiple limed sites, acid control streams and circumneutral reference sites in the year before liming and the first two years following treatment. The BACI (Before-After-Control-Impact; Stewart-Oaten *et al.*, 1986) design implemented here allowed variations at the impact sites to be distinguished from changes at unmanipulated controls (Downes *et al.*, 2002). This study thus included 42 sites ranging from small, low order streams, which are often ignored by management and conservation programmes despite their indicator and conservation value, and vulnerability to changes resulting from acid inputs (Lewis *et al.*, in press), to larger main river sites.

5.3 Methods

5.3.1 Study area

The study area consists of 23 sites on the upper reaches of the Wye and 19 sites within the catchment of the River Irfon, a major tributary of the Wye (Figure 1). Rising on Plynlimon in mid-Wales, the Wye drains an area of approximately 4180 km^2 before joining the Severn Estuary, 250 km from the source. The sites range from small first order headwaters (catchment < 5 km^2), to large main rivers sites (up to 102 km^2) and represent a range in mean pH, habitat types and land-use. A full description of the study area can be found elsewhere (Lewis *et al.*, in press; Chapter 3). In July 2003, 750 tonnes of limestone were applied to 17 wetland areas on the upper reaches of the Wye, affecting nine of the study sites (chapter 4; Figure 1). The experiment was based on a BACI (Stewart-Oaten *et al.*, 1986; Downes *et al.*, 2002) design with samples taken at treated sites, acid control sites (minimum pH < 5.7) and naturally circumneutral reference streams (minimum pH > 5.7). The collection of data over a three year period and at multiple treatment, control and reference sites allowed use of repeated measures analysis of variance (ANOVA; Paine, 1996) for this multiple-BACI design. The experimental design thus included 9 limed sites, 13 acid sites and 20 circumneutral sites.

5.3.2 Data collection

Physiochemical data

Water samples were collected in February, April, July and October 2003 and 2004, and in February and April 2005. The suite of chemical determinands measured is akin to those recorded by the UK Acid Waters Monitoring Network. For each site, one unfiltered sample was collected on each occasion and measured for alkalinity by Gran titration. A filtered sample (0.45 μm pore size, Watman sterile membrane filters) was also collected and measured for calcium (Ca), sodium (Na), potassium (K) and magnesium (Mg) by atomic absorption spectrophotometry (AAS, Perkin Elmer), silicon (Si) by the molybdenum blue method and chlorine (Cl), nitrate (NO_3^--N) and sulphate (SO_4) by ion chromatography (Dionex). Dissolved Organic Carbon (DOC) was measured by continuous flow colorimetry with UV digestion (Skalar autoanalyser system) whilst aluminium (Al) was determined colorimetrically using the pyrocatechol violet method (Dougan and Wilson, 1974). At each site pH, temperature and conductivity were also measured directly using a pH/Cond 340i meter (WTW Weilheim).

Stream link magnitude is based on Shreve's proposed system (1967) whereby the number of upstream tributaries is taken as a measure of stream order, and was calculated using a topographical map (O.S. 1:50000), whilst slope was measured using a Silva compass. Altitude readings provided by a Garmin GPS (GPS 12 XL) were checked against measurements from an O.S. map. Discharge was measured at a main river site on the upper Wye, near to the source using a Campbell Scientific CR10 data logger (Figure 1). For each site, the relative discharge was then calculated (see Chapter 4).

Biological data

Macroinvertebrate samples were collected in April 2003, 2004 and 2005 using a standard kick sampling procedure (2 minute mid-channel riffles, 1 minute marginal habitats) over a 5 m reach and a standard Freshwater Biological Association (FBA) pond net (230 mm x 250 mm frame, 900 μm mesh), and immediately preserved in ethanol. Prior to analyses, riffle and margin data were pooled. Collection of samples in this way has been demonstrated to be effective for detecting differences between sites (Bradley and Ormerod, 2002b). In the laboratory, macroinvertebrates were sorted and identified, where possible to species (Hynes, 1977; Elliot *et al.*, 1988; Friday, 1988; Edington and Hildrew, 1995; Wallace *et al.*, 2003). For these analyses, data from riffles and margins were combined and then transformed logarithmically prior to statistical analysis to homogenise variances. A sub-set of nine sites representing three of each treatment type (limed, acid, circumneutral) were also sampled in July 2003 and 2004. April and July macroinvertebrate data are hereafter referred to as spring and summer samples, respectively.

5.3.3 Statistical analyses

Mean pH, minimum pH and other chemical variables were compared using a repeated measures ANOVA model (SPSS, version 12), to detect effects of treatment (limed/acid/circumneutral), time (before-after liming) and time-treatment interactions (Underwood 1993; SPSS, version 12). For the ANOVA model, discharge was also included as a covariate.

Patterns in assemblage composition were identified using multivariate techniques (CANOCO for Windows 4.0; ter Braak and Šmilauer, 1998). Detrended correspondence analysis was performed (DCA; Hill and Gauch, 1980), with Eigen values used as measures of the strength of axes. DCA sites scores were then assessed for correlations with measured physio-chemical variables in order to detect the variables that were most strongly associated with variations in species composition. DCA species scores for pre-liming data were used to identify acid-sensitive species and χ^2 tests were performed on the frequency of occurrence of each of these acid-sensitive species at limed sites before and after treatment.

Biological changes between treatments and over time were analysed in terms of total abundance and species richness using repeated measures ANOVA. As with stream chemistry, variations as a result of treatment and time were assessed along with time-treatment interactions. Repeated measures ANOVA was also used to detect changes in the abundance, relative abundance and richness of acid-sensitive species.

Variables which were identified through correlation analysis as biologically important were further used to produce environmental profiles of the sites. The presence or absence of *Baetis rhodani*, an acid-sensitive species mayfly species (Fjellheim and Raddum, 1990; Rundle *et al.*, 1995), at these sites was then plotted allowing production of a polygon outlining the extent of occurrence of this species (Masters, 2002). The location of limed sites within the plot, relative to this polygon could then be assessed.

5.4 Results

5.4.1 Water chemistry

Across all sites, mean pH and alkalinity were lower in the first year after treatment (2004) than before (2003) (Figure 2). Although both treatment and time differences were observed between limed sites and control/reference sites, repeated measures ANOVA did not reveal any significant time x treatment interactions, and hence no strong indications of any effects due to lime treatment. Discharge was generally higher during sampling events in 2004 than in the pre-treatment year (Figure 3), and flow was thus found to be a significant covariate in the repeated measures ANOVA.

5.4.2 Biological change

In 2003, 2004 and 2005, respectively, 77, 71 and 70 macroinvertebrate species were identified within the catchment, with assemblages dominated by Trichoptera (caddisfly), Plectoptera (stonefly) and Ephemeroptera (mayfly) larvae. At acid and limed sites, abundance ranged from 17 to 460 individuals per sample, and richness from 8 to 22 taxa. At circumneutral sites, abundance varied between 34 and 640 individuals, and richness between 9 and 30 species. Effects of treatment and time were apparent for abundance and richness, although there were no significant time x treatment interactions (Table 1) and thus no effect of liming (Figure 4). For July data, there were no significant time, treatment or time- treatment effects on abundance or species richness at the subset of sites tested. *Dixa* spp., *Ephemerella ignita* (Poda), *Siphlonurus armatus* (Eaton), *Tinodes waeneri* (Linnaeus) and *Goeridae* appeared in summer samples only, whilst several other species were present in spring samples but absent in summer (*Apatania* spp., *Amphinemura standfussi* (Ris), *Baetis muticus* (Linnaeus), *Diura bicaudata* (Linnaeus), *Diplectrona felix* (McLachlan), *Esolus parallelepipedus* (Müller), *Isoperla grammatica* (Poda), *Rhithrogena semicolorata* (Curtis), *Rhabdiopteryx acuminata* (Klapálek)).

DCA was performed to assess variation in species composition between sites. DCA axes 1 and 2 respectively explained 10.1% and 7.5 % of the variation for 2003 data (Figure 5). DCA axis 1 was negatively correlated with pH ($r = -0.65, p < 0.001$), alkalinity ($r = -0.64, p < 0.001$), K ($-0.43, p < 0.01$) and Ca ($r = -0.36, p < 0.05$) and positively correlated with Al ($r = 0.54, p < 0.001$), indicating a strong influence of acid-base status on assemblages composition. Axis 1 correlated less strongly with slope ($r = 0.38, p < 0.05$) and altitude ($r = -0.33, p < 0.01$), whilst variation along axis 2 reflected DOC ($r = 0.42, p < 0.001$). At sites with high pH, and thus low DCA axis 1 scores, mayflies such as *Heptagenia lateralis* (Curtis), *B. rhodani* (Pictet) and *R. semicolorata,* and the caddis-larvae *D. felix* and *Odontocerum albicorne* (Scopoli), were common and abundant (Figure 5). Many sites with lower pH, and thus higher axis 1 scores, had *Amphinemura sulcicollis* (Stephens), *Nemurella pictetii* (Klapálek) and *Plectrocnemia conspersa* (Curtis) in abundance, whilst the stoneflies *Leuctra inermis* (Kempny) and *Chloroperla torrentium* (Pictet) were very common across all sites and were collected at 98% and 95% of sites respectively in 2003.

Species scores along the first DCA axis were used to identify which of the species collected were acid-sensitive. As DCA axis 1 was strongly negatively correlated with pH, species with a negative axis 1 score were likely to be associated with sites with higher than average pH, and were unlikely to be present at sites with lower pH values. These species were thus classified as acid-sensitive. This highlighted 23 acid-sensitive species (Table 2) including six mayfly, four stonefly, and eight caddisfly species. Abundance and richness of these species were high in circumneutral streams in all years, remained low in acid and limed stream in each year and were absent from all sites with pH < 5.8 (Figure 6a). There was no significant change in relative abundance or total abundance of these species at limed sites following treatment. For richness of acid-sensitive species, time differences were apparent for all stream types, with an overall decrease across the three study years (Figure 6b), whilst there were no treatment or time x treatment effects. At two limed sites (WY47 and WY48) all acid sensitive species were absent prior to treatment and in the first year following liming, four and three acid-sensitive species respectively appeared. Although nine acid-sensitive species appeared to increase in frequency of occurrence (Table 2), χ^2 tests on spring data revealed that there was no significant increase in the frequency of occurrence of any acid-sensitive species following treatment.

In the year prior to liming, *B. rhodani* was absent from all sites targeted for lime treatment and although there was no significant increase in the frequency of occurrence of acid-sensitive species, this species was recorded at 3 limed sites (WY30, WY31 and WY52 in 2004; WY30, WY31 WY34 in 2005) following treatment, although abundance was low (2-17 individuals). Polygon profiles based on pH and calcium indicated that prior to liming, limed sites did not have pH and calcium concentrations suitable for *B. rhodani* to be present (i.e. limed sites were not within the polygon representing the extent of occurrence of *B. rhodani*; Figure 7a). In 2004, five of the limes sites had pH and calcium values within the range at which *B. rhodani* was found at reference sites and *B. rhodani* did appear at three of these sites (WY30, WY31 and WY52) (Figure 7 b).

5.5 Discussion

Following declines in emissions and deposition of acidifying compounds across much of Europe and North America in recent decades, evidence of chemical recovery of river catchments has been documented (Fowler *et al.*, 2001, Skjelkvåle *et al.*, 2005). Recent surveys of Scottish and Welsh upland catchments indicate, however, that acid chemistry, particularly at high flow, continues to dominate many areas (Kowalik and Ormerod, 2006). Recent assessment of the upper reaches of the Welsh River Wye, through water quality surveys and use of bio-indicator taxa, has revealed continuing and often severe biological impacts of acidification (Lewis *et al.*, in press; Chapter 3).

Experimental liming of the upper reaches of the Wye was thus accompanied by water quality monitoring across the catchment over a range of chemical variables (Chapter 4) and biological parameters. Although water quality attributes are important in assessing the effects of treatments such as liming, changes in biological assemblages also need to be assessed to determine the ecological stream-scale response. Previous studies have shown depauperate invertebrate assemblages at low pH, probably because of a combination of direct physiological effects, altered food resources, predator-prey interactions and changes in growth, respiration, and drift and avoidance behaviour (Sutcliffe and Carrick 1973; Fiance, 1978; Hall *et al.*, 1980; Herrmann and Andersson 1986; Ormerod *et al.*, 1987; Sutcliffe and Hildrew, 1989). Macroinvertebrates are thus considered valuable in assessment of acidification status in lakes and rivers (Sutcliffe and Hildrew, 1989; Rutt *et al.*, 1990; Merrett *et al.*, 1991; Orendt, 1998; Lewis *et al.*, in press). Analysis of pre-treatment assemblages in this study showed a strong association between acid-base status and assemblage composition, with marked differences between acid and circumneutral streams, the latter having higher abundance of acid-sensitive species and the former being abundant in more acid-tolerant macroinvertebrates. Data collected before treatment in limed streams and acid controls during this study showed characteristics similar to those in other studies of acidified upland streams (Stoner *et al.*, 1984; Wade *et al.*, 1989; Rundle *et al.*, 1995; Bradley and Ormerod, 2002a).

In other liming experiments, authors have recorded the recolonisation of limed sites by acid-sensitive species (Fjellheim and Raddum, 1992; Howells *et al.*, 1992; Lacroix, 1992; Lingdell and Engblom, 1995). In many cases however, recovery has only been fragmented and modest (Rundle *et al.*, 1995; Soulsby, *et al.*, 1997; Bradley and Ormerod, 2000a). Following liming of the upper Wye sites during this study, neither abundance nor species richness of macroinvertebrate assemblages changed significantly in the first two years after treatment, with assemblages in limed and acid control sites remaining similarly species-poor in all three study years.

Many of the species identified in this catchment as acid-sensitive have been classified as such previously in an adjacent catchment (Rundle *et al.*, 1995). In 2003, some of these sensitive species, such as the mayfly *B. rhodani*, were absent from acid streams targeted for treatment, whilst highly tolerant species (*A. sulcicollis*, *N. pictetii*) were abundant. At some limed sites, acid-sensitive species did appear where they had previously been absent, and profiling of the chemical character of the 42 study sites in terms of pH and calcium, indicated that these sites had suitable conditions for *B. rhodani* to be present. The appearance of *B. rhodani* at these sites following treatment indicates that this species is able to reach these streams, implying that neither geographical isolation or dispersal are problematic in the colonisation of those particular locations. However, abundance was low, occurrence did not change significantly against background variations between study years, and colonisation was not consistent across treated sites. Moreover, significant increases in relative abundance of acid-sensitive species and changes in their total abundance or species richness were lacking. In a liming study in Norway (Fjellheim and Raddum, 1992), an increase in moderately sensitive Ephemeroptera, Plectoptera and Trichoptera species was recorded in the first year after treatment. However, of the more sensitive *B. rhodani* only occurred two years after treatment. Stable chemical and biological conditions suitable for such species may thus take a long time to establish, and significant changes may not be easily detectable within the short time-scales involved in this study.

Examination of the water chemistry data collected concurrently with biological samples revealed few indications of recovery at treated sites (Chapter 4), with periods of high discharge and associated episodes of acid chemistry persisting (Davies *et al.*, 1992; Lepori, *et al.*, 2003a; Kowalik *et al.*, in press). Periods of high flow are common in upland streams, and in the Wye catchment correlation between discharge and pH are strong at acid sites. If such periods lead to acid episodes, then invertebrate communities will be impacted (Hall *et al.*, 1988; Hirst *et al.*, 2004, Lepori *et al.*, 2003b). In a study of the adjacent Llyn Brianne catchment, an index of 'risk of exposure' to acid episodes was calculated using over 40 years of hydrological data from a monitoring station near the source of the Wye (Kowalik and Ormerod, 2006). During this study of the Wye, discharge was highly variable between seasons, but the afore-mentioned flow index suggests that the sampling period during which biological samples were collected (April) follows antecedent conditions when the risks of episodes are greatest.

Despite the appearance of sensitive species in treated areas, it is clear that liming has not yet achieved consistent, sustained or stable conditions necessary for biological recovery. Thus, while possible reasons for limited recovery in deacidified systems have been evaluated, in the Wye, limited liming effects on acid-base chemistry are at present a sufficient explanation for limited biological change (Rundle *et al.*, 1995; Raddum *et al.*, 2001; Bradley and Ormerod, 200a; Lepori *et al.*, 2003b; Ledger and Hildrew 2005; Kowalik *et al.*, in press). Further liming is advocated to address the overriding effects of acid episodes and to increase the potential for chemical change. Further, elucidation of the response of sensitive species during differing flow patterns could allow prediction of the macroinvertebrate assemblage changes that may follow more comprehensive treatment.

5.6 References

Appelberg, M and Svenson, T. 2001. Long-term ecological effects of liming — The Iselaw Programme. *Water, Air, and Soil Pollution,* **130,** 1745 – 1750.

Bradley, D.C. and Ormerod, S.J. 2002a. Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater biology*, **47**, 161-171.

Bradley, D.C. and Ormerod, S.J. 2002b. Evaluating the precision of kick-sampling in upland streams for assessments of long-term change: the effects of sampling effort, habitat and rarity. *Archives für Hydrobiologie,* **155,** 199-221

Dahl, K. 1927. The effects of acid water on trout fry. *Salmon and Trout Magazine,* **46,** 35-43.

Davies, T.D., Tranter, M., Wigington, P.J. and Eshkeman, K.N. 1992. 'Acidic episodes' in surface waters in Europe. *Journal of Hydrology*, **132**, 25-69.

Davy-Bowker, J., Murphy, J.F., Rutt, G.P., Steel, J.E.C. and Furse, M. T. (in press). The development and testing of a macroinvertebrate biotic index for detecting the impact of acidity on streams. *Archives für Hydrobiologie.*

Dougan, W. K. and Wilson, A. L. 1974. The absorptiometric determination of aluminium in water: a comparison of some chromogenic reagents and the development of an improved method. *Analyst*, **99**, 413-430.

Downes, B. J., Barmuta, L. A., Fairweather, P.G., Faith, D.P., Keough, M.J., Lake, P.S., Mapstone, B.D. and Quinn, G.P. 2002. *Monitoring Ecological Impacts. Cambridge* University Press, Cambridge.

Edington, J.M. and Hildrew, A.G. 1995. *Caseless caddis larvae of the British Isles*, Scientific Publication **53**, Freshwater Biological Association, Ambleside.

Elliot, J.M., Humpesh, U.H. and Macan, T.T. 1988. *Larvae of the British Ephemeroptera: A key with ecological notes*, Scientific Publication **49,** Freshwater Biological Association, Ambleside.

Fiance, S. B. 1978. Effects of pH on the biology and distribution of *Ephemerella-funeralis* (Ephemeroptera). *Oikos*, **31**, 332-339.

Fjellheim, A. and Raddum, G.G. 1990. Acid Precipitation - biological monitoring of streams and lakes. *Science of the Total Environment*, **96**, 57-66.

Fjellheim, A. and G. G. Raddum. 1992. Recovery of acid-sensitive species of Ephemeroptera, Plecoptera and Trichoptera in River Audna after liming. *Environmental Pollution*, **78**, 173-178.

Fowler, D., Coyle, M., ApSimon, H.M., Ashmore, M.R., Bareham, S.A., Battarbee, R.W., Derwent, R.G., Erisman, J.-W., Goodwin, J., Grennfelt, P., Hornung, M., Irwin, J., Jenkins, A., Metcalfe, S.E., Ormerod, S.J., Reynolds, B., and Woodin, S. 2001.

Transboundary Air Pollution: acidification, eutrophication and ground-level ozone in the UK, Rep. No. EPG 1/3/153. DEFRA, Edinburgh.

Fowler, D., Smith, R.I., Muller, J.B.A., Hayman, G. and Vincent, K.J. 2005. Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, **137**, 15-25.

Friday, L.E. 1988. *A Key to the Adults of British Water Beetles,* Field Studies Council, Publications, **189**.

Hall, R. J., Likens, G.E., Fiance, S.B. and Hendrey, G.R. 1980. Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. *Ecology*, **61**, 976-989.

Hall, R.J., Bailey, R.C. and Findeis, J. 1988. Factors affecting survival and cation concentration in the blackflies *Prosimulium fuscus/mixtum* and the mayfly *Leptophlebia cupida* during spring snowmelt. *Canadian Journal of Fisheries and Aquatic Sciences*, **45,** 2123-2132.

Herrmann, J. and Andersson, K.G. 1986. Aluminium impact on respiration of lotic mayflies at low pH. *Water, Air, and Soil Pollution*, **30**, 703-709.

Hill, M.O. and Gauch, H.G. 1980. Detrended correspondence analysis - an improved ordination technique. *Vegetatio*, **42**, 47-58.

Hindar, A., Nilsen, P., Skiple, A. and Hogberget, R. 1995. Counteractions against acidification in forests ecosystems: effects on stream water quality after dolomite application to forest soil in Gjerstad, Norway. *Water, Air and Soil Pollution,* **85,** 1027-1032.

Hirst, H., Chaud, F., Delabie, C., Jüttner, I. and Ormerod, S.J. 2004. Assessing the short-term response of stream diatoms to acidity using inter-basin transplantations and chemical diffusing substrates. *Freshwater Biology*, **49**, 1072-1088.

Howells, G., Dalziel, T.R.K., and Turnpenny, A.W.H. 1992. Loch Fleet: liming to restore a brown trout fishery. *Environmental Pollution*, **78**, 131-139.

Hynes, H.B.N. 1977. *A Key to the Adults and Nymphs of the British Stoneflies (Plecoptera) with Notes on their Ecology and Distribution*. Freshwater Biological Association, Scientific Publication **17**.

Jüttner, I., Lintelmann, J., Michalke, B., Winkler, R., Steinberg, C.E.W. and Kettrup, A. 1997. The acidification of the Herrenwieser See, Black Forest, Germany, before and during industrialisation. *Water Research,* **31**, 1194-1206.

Kowalik, R.A. and Ormerod, S.J. 2006. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology*, **51**, 180-191.

Kowalik, R.A., Cooper, D.M., Evans, C. D. and Ormerod, S.J. (in press). Acid episodes retard the biological recovery of upland British streams from acidification. *Global Change Biology.*

Lacroix, G. L. 1992. Mitigation of low stream pH and its effects on salmonids. *Environmental Pollution,* **78**, 157-164.

Ledger, M.E. and Hildrew, A.G. 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution*, **137**, 103-118.

Lepori, F., Barbieri, A. and S. J. Ormerod, 2003a. Causes of episodic acidification in Alpine streams. *Freshwater Biology*, **48**, 175-189.

Lepori, F., Barbieri, A. and Ormerod, S.J. 2003b. Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams. *Freshwater Biology*, **48**, 1873-1885.

Lewis, B.R., Jüttner, I., Reynolds, B. and Ormerod, S.J. In press. Comparative assessment of stream acidity using diatoms and macroinvertebrates: implications for river management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Lingdell, P.E. and Engblom, E. 1995. Liming restores the benthic invertebrate community to 'pristine' state. *Water, Air, and Soil Pollution*, **85**, 955-960.

Masters, Z. 2002. *Factors affecting Stream Macroinvertebrate Recovery from Acidification.* Unpublished. PhD Thesis, Cardiff University, Cardiff.

Merrett, W.J., Rutt, G.P., Weatherley, N.S., Thomas, S.P. and Ormerod, S.J. 1991. The response of macroinvertebrates to low pH and increased aluminium concentration in Welsh streams: multiple episodes and chronic exposure. *Archives für Hydrobiologie,* **121**, 115-125.

Monteith, D.T. and Evans, C.D. 2005. The United Kingdom Acid Waters Monitoring Network: a review of the first 15 years and introduction to the special issue. *Environmental Pollution,* **137**, 3-13.

Orendt, C. 1998. Macroinvertebrates and diatoms as indicators of acidification in forest spring brooks in a region of eastern Germany (Leipzig-Halle-Bitterfeld) highly impacted by industrial activities. *Archives für Hydrobiologie,* **143**, 435-467.

Ormerod, S.J. and Edwards, R.W. 1987. The ordination and classification of macroinvertebrate assemblages in the catchment of the River Wye in relation to environmental factors. *Freshwater Biology*, **17**, 533-546.

Ormerod, S.J., Boole, P., McCahon, C.P., Weatherley, N.S., Pascoe, D. and Edwards, R.W. 1987. Short-term experimental acidification of Welsh streams: comparing the biological effects of hydrogen ions and aluminium. *Freshwater Biology*, **17**, 341-356.

Ormerod, S. J. and Wade, K. R. 1990. *The role of acidity in the ecology of Welsh lakes and streams*. In Acid Waters in Wales (Eds R. W. Edwards, J. H. Stoner and A. S. Gee). Kluwer Academic Publishers, Dordrecht, pp 93-120.

Paine, M.D. 1996. Repeated measures designs. *Environmental Toxicology and Chemistry*, **15**, 1439-1441.

Raddum, G.G., Fjellheim, A. and Skjelkvåle, B.L. 2001. Improvements in water quality and aquatic ecosystems due to reduction in sulphur deposition in Norway. *Water, Air and Soil Pollution*, **130**, 87-98.

Rimes, C.A., Farmer, A.M. and Howell, D. 1994. A survey of the threat of surface-water acidification to the nature conservation interest of fresh-waters on sites of special scientific interest in Britain. *Aquatic Conservation: marine and freshwater ecosystems*, **4**, 31-44.

Rosenberg, D.M. and Resh, V.H. 1993. *Freshwater Biomonitoring and Benthic Macroinvertebrates*, Chapman and Hall, New York.

Rundle, S. D., Weatherley, N. S. and Ormerod, S.J. 1995. The effects of catchment liming on the chemistry and ecology of upland Welsh streams: testing model predictions. *Freshwater Biology,* **34**, 165-175.

Rutt, G.P., Weatherley, N.S. and Ormerod, S.J. 1990. Relationships between the physiochemistry and macroinvertebrates of British upland streams: the development of modelling and indicator systems for predicting fauna and detecting acidity. *Freshwater Biology*, **24**, 463- 480.

Skjelkvåle, B.L., Stoddard, J.L., Jeffries, D.S., Tørseth, K., Høgåsen, T., Bowman, J., Mannop, J., Monteith, D.T., Mosello, R., Rogara, M., Ryzchon, D., Vesely, J., Wieting, J., Wilander, A. and Worsztynowicz, A. 2005. Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, **137**, 165-176.

Soulsby, C., Turnbull, D., Hirst, D., Langan, S.J. and Owen, R. 1997. Reversibility of stream acidification in the Cairngorm region of Scotland. *Journal of Hydrology*, **195**, 291-311.

Stevens, P.A., Ormerod, S.J. and Reynolds, B. 1997. Final Report on the Acid Waters Survey for Wales. CEH Bangor, Bangor.

Stewart–Oaten, A., Murdoch, W.W. and Parker. 1986. Environmental impact assessment: "pseudoreplication" in time? *Ecology*, **73**, 1396-1404.

Stoddard, J.L., Jeffries, D. S., Lükewille, A., Clair, T. A., Dillon, P. J., Driscoll, C. T., Forsius, M., Johannessen, M., Kahl, J. S., Kellogg, J. H., Kemp, A., Mannio, J., Monteith, D. T., Murdoch, P. S., Patrick, S., Rebsdorf, A., Skjelkvåle, B. L., Stainton, M. P., Traaen, T., van Dam, H., Webster, K. E., Wieting, J. and Wilander, A. 1999. Regional trends in aquatic recovery from acidification in North America and Europe. *Nature,* **401**, 575-578.

Stoner, J.H., Gee, A.S. and Wade, K.R. 1984. The effects of acidification on the ecology of streams in the upper-Tywi catchment in west Wales. *Environmental Pollution*, **35,** 125-157.

Strahler, A.N. 1952. Hypsometric (area-altitude) analysis of erosional topography. *Bulletin of the Geographical Society of America*, **63,** 1117-1142.

Sutcliffe, D.W. and Carrick, T.R. 1973. Studies on mountain streams in the English Lake District. *Freshwater Biology*, **3**, 437-462.

Sutcliffe, D. W. and Hildrew, A. G. 1989. Invertebrate communities in acid streams. (Eds R. Morris, E. W. Taylor, D.J.A. Brown and J.A. Brown). *Acid Toxicity and Aquatic Animals*. Cambridge University Press, Cambridge.

ter Braak, C.J.F. and Šmilauer, P. 1998. *CANOCO Reference Manual and Users Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4)*. Microcomputer Power, Ithaca: New York.

Townsend, C.R., Hildrew, A.G. and Francis, J. 1983. Community structure in some southern English streams: The influence of physicochemical factors. *Freshwater Biology,* **13**, 521-544.

Underwood, A.J. 1993. The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. *Australian Journal of Ecology*, **18**, 99-116.

Wade, K.R., Ormerod, S.J. and Gee, A.S. 1989. Classification and ordination of macroinvertebrate assemblages to predict stream acidity in upland Wales. *Hydrobiologia,* **171**, 59-78.

Wallace, I. D., Wallace, B. and Phillipson, G.N. 2003. *Keys to Case-bearing Caddis Larvae of Britain and Ireland,* Freshwater Biological Association, Scientific Publication **61**.

Weatherley, N.S. and Ormerod, S.J. 1992. The biological response of acidic streams to catchment liming compared to the changes predicted from stream chemistry. *Journal of Environmental Management*, **34**, 105-115.

Wright, J.R., Moss, D., Armitage, P.D. and Furse, M.T. 1984. A preliminary classification of running water sites in Great Britain based on macroinvertebrate species and the prediction of community type using environmental data. *Freshwater Biology*, **14**, 221-256.

5.7 List of tables

Table 1. Significant sources of variation in abundance of macroinvertebrates and species richness, assessed using repeated measures ANOVA. Model included effects of treatment (L = limed streams; A = acid control sites; C = circumneutral streams), time (before/after treatment) and time x treatment interactions. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; DF = degrees of freedom)

Variable	Source of variation	F	DF
Total abundance	Treatment (L vs A)	8.9 **	1,22
Species richness	Treatment (L vs A)	5.8 *	1,22
Total abundance	Treatment (L vs C)	32.3 ***	1, 29
Species richness	Treatment (L vs C)	29.2 ***	1, 29
Richness of acid-sensitive species	Treatment (L vs A)	25.9 ***	1,22
Richness of acid-sensitive species	Time (L vs A)	4.0 *	9, 22

Table 2. Acid-sensitive species within the upper Wye and Irfon catchments, identified by Detrended Correspondence Analysis. Before and After columns indicate the number of limed sites at which these species occurred before liming (April 2003) and in the first two years following treatment (April 2004 and 2005).

Species	Before	After (2004)	After (2005)
Apatania spp.	0	0	0
Baetis muticus	1	0	0
Baetis rhodani	0	3	3
Baetis scambus	0	0	0
Chloroperla tripunctata	1	2	2
Diura bicaudata	1	0	0
Diplectrona felix	1	0	0
Gammarus pulex	0	0	0
Hydropsyche instabilis	0	0	0
Heptagenia lateralis	3	1	3
Hydropsyche siltalai	2	4	4
Leuctra fusca	1	0	0
Limnius volckmari	1	3	3
Odontocerum albicorne	2	2	3
Oulimnius spp.	1	1	0
Pisidium spp.	1	0	0
Perlodes microcephala	1	0	0
Potamophylax rotundipennis	0	1	2
Rhabdiopteryx acuminata	0	2	1
Rhithrogena germanica	0	1	0
Rhithrogena semicolorata	0	0	1
Rhyacophila obliterata	0	0	0
Wormaldia spp.	1	0	0

5.8 List of Figures

Figure 1. Location of sites studied in experimental liming programme, with 23 sites on the upper reaches of the Wye and 19 on the river Irfon. Stars indicate locations of lime application in 2003; D = Location of discharge gauge; black symbol = acid control survey site; grey = limed survey site; white = circumneutral reference site. (WY = upper-Wye study sites; IF/AC = Irfon sites).

Figure 2. (a) pH (mean ± SD) and (b) Ca (mean ± SD at sites on upper Wye and Irfon. Bars represent means at acid, limed and circumneutral sites over 10 quarterly sampling points in 2003 and 2004.

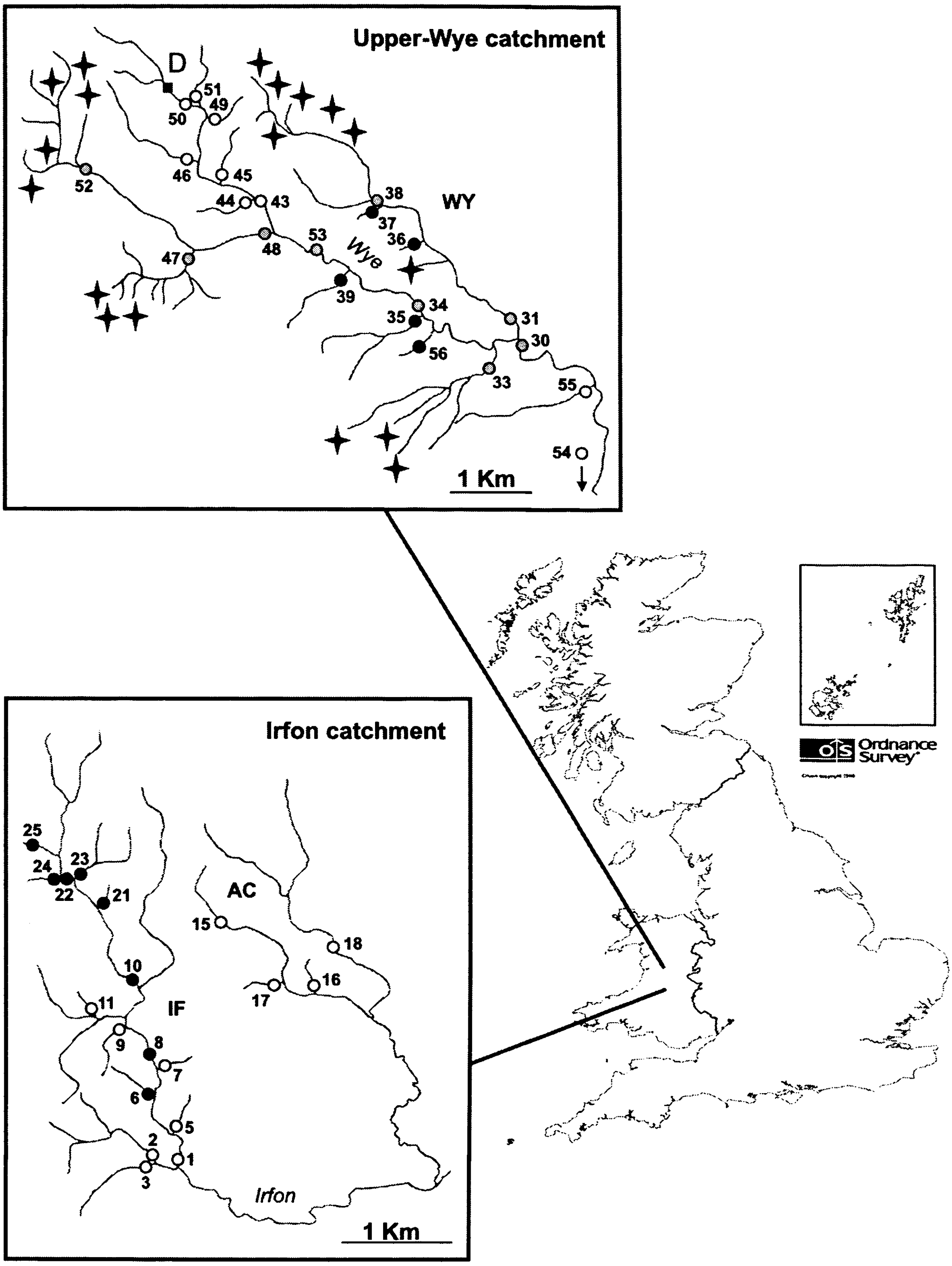
Figure 3. Discharge ($m^3\ s^{-1}$) (mean ± SD) of River Wye at Plynlimon monitoring station over 10 sampling occasions.

Figure 4. (a) Mean abundance (mean ± SD) and (b) mean species richness (mean ± SD) of macroinvertebrates at acid, limed and circumneutral streams in April, one year before lime treatment (2003) and in the first two years following treatment (2004, 2005).

Figure 5. (a) Sites (open circles = acid sites; grey triangles = limed sites; black squares = circumneutral sites) and (b) species scores, along the first two ordination axes of a Detrended Correspondence Analysis for macroinvertebrates collected at sites within the Wye catchment. Common taxa are labelled, abundant species are in bold (*Br = Baetis rhodani*; *Ig = Isoperla grammatica*; *Pm = Perlodes microcephala; Ra = Rhabdiopteryx acuminata*).

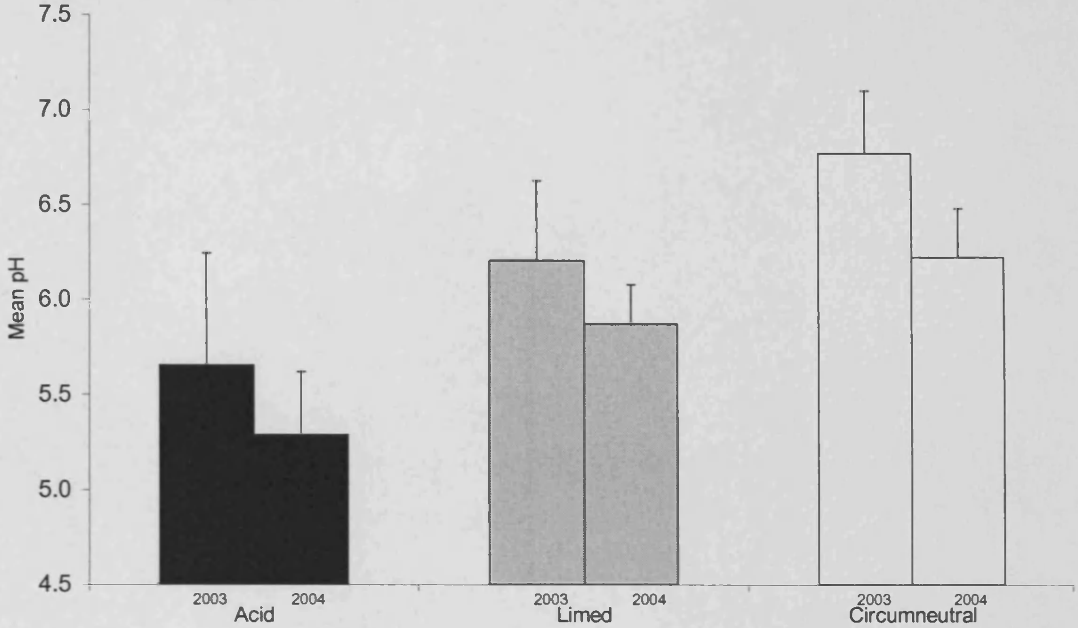
Figure 6. (a) Abundance of acid-sensitive macroinvertebrates against mean pH (2003) and (b) mean species richness (mean ± SD) of acid-sensitive species at acid control, limed and circumneutral reference streams one year before lime treatment (2003) and in the first two years following treatment (2004, 2005).

Figure 7. Mean pH and mean calcium concentration of upper Wye and Irfon sites in (a) 2003 and (b) 2004. Filled symbols = *Baetis rhodani* present; open symbols = *B. rhodani* absent; circles = control and reference sites; triangles = limed sites. Polygons outlines sites at which the acid sensitive mayfly *B. rhodani* was present.

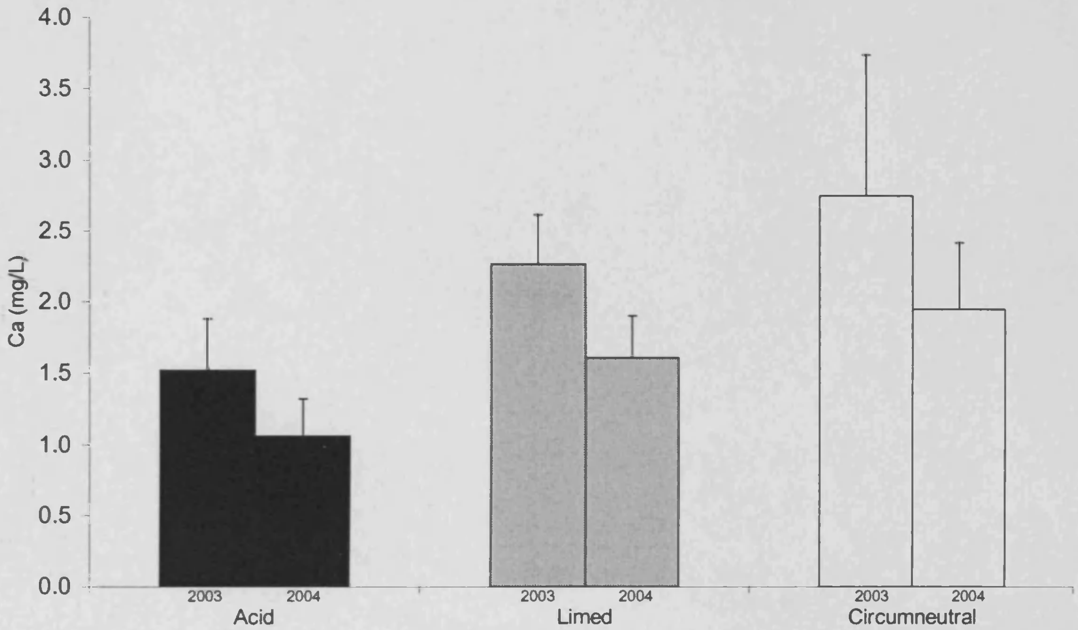


Upper-Wye catchment
D
51
49
50
46
45
44
43
52
38
37
WY
36
53
Wye
48
47
39
34
35
31
30
56
33
55
54
1 Km
Ordnance Survey
Irfon catchment
25
23
24
22
21
AC
15
18
10
17
16
11
IF
9
8
7
6
5
2
1
3
Irfon
1 Km

Figure 1.


7.5
7.0
6.5
6.0
5.5
5.0
4.5
Mean pH
2003
2004
Acid
2003
2004
Limed
2003
2004
Circumneutral

(a)


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Ca (mg/L)
2003
2004
Acid
2003
2004
Limed
2003
2004
Circumneutral

(b)

Figure 2.

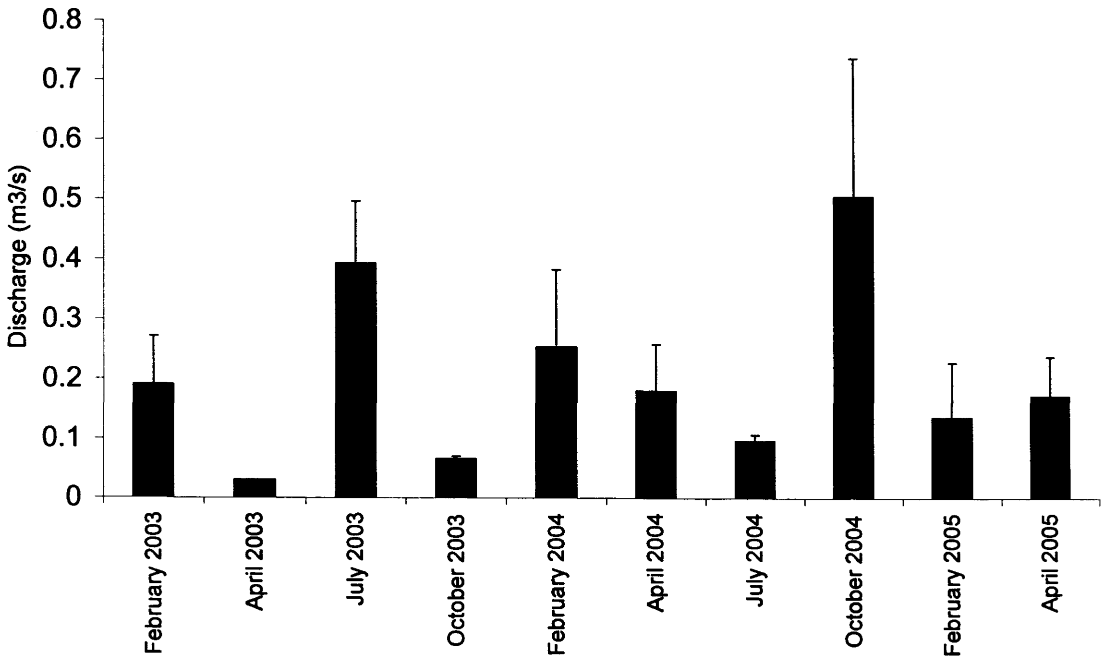
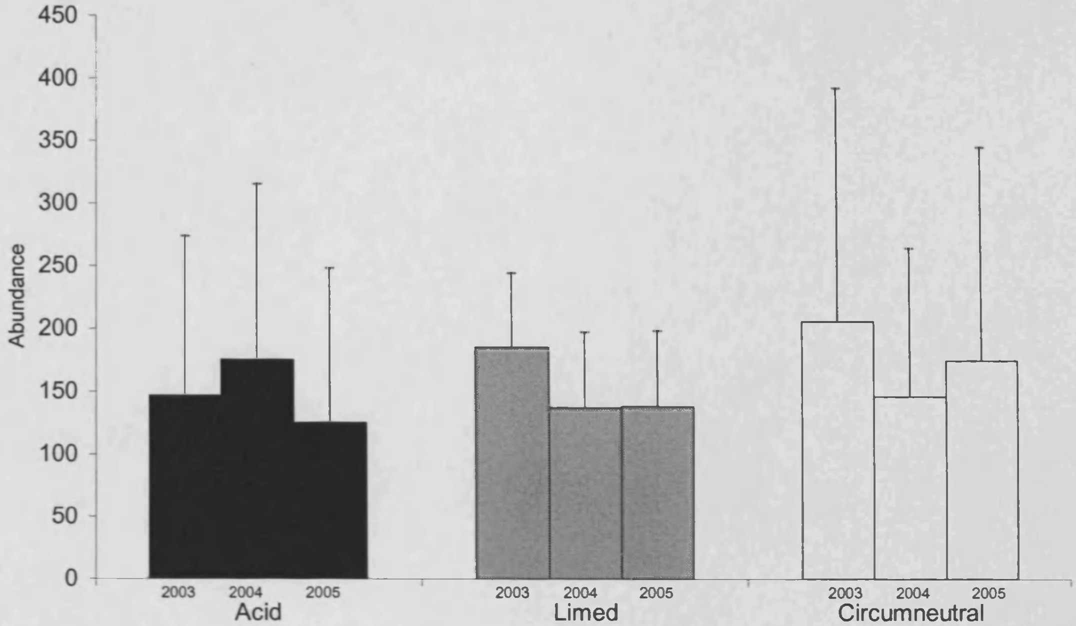
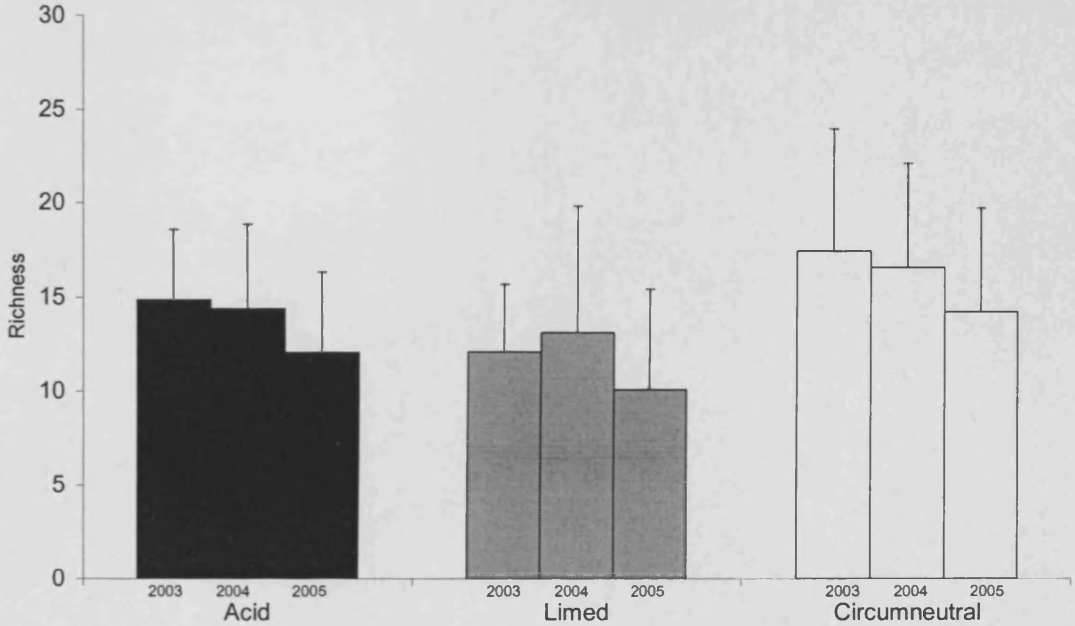

Discharge (m3/s)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
February 2003
April 2003
July 2003
October 2003
February 2004
April 2004
July 2004
October 2004
February 2005
April 2005

Figure 3.



450
400
350
300
250
200
150
100
50
0
Abundance
2003
2004
2005
Acid
2003
2004
2005
Limed
2003
2004
2005
Circumneutral

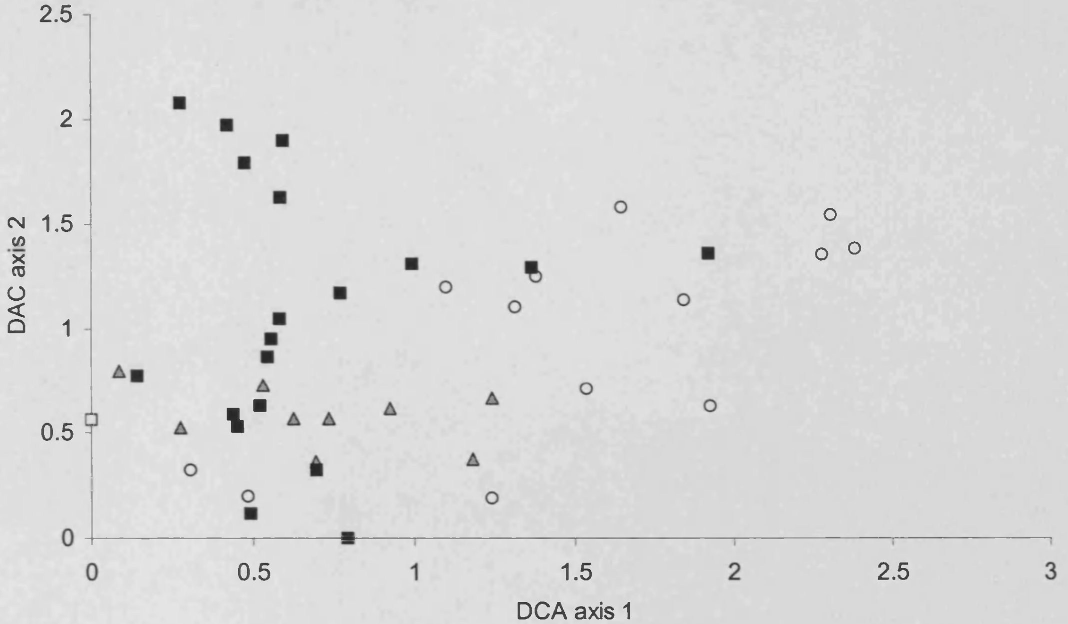
(a)



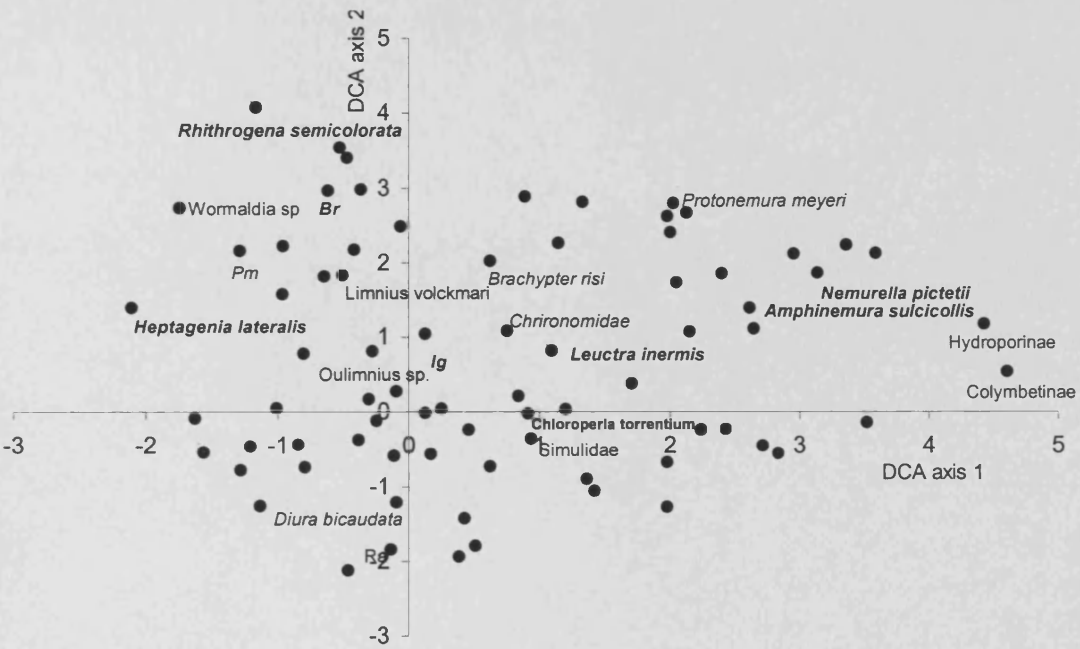
30
25
20
15
10
5
0
Richness
2003
2004
2005
Acid
2003
2004
2005
Limed
2003
2004
2005
Circumneutral

(b)

Figure 4.

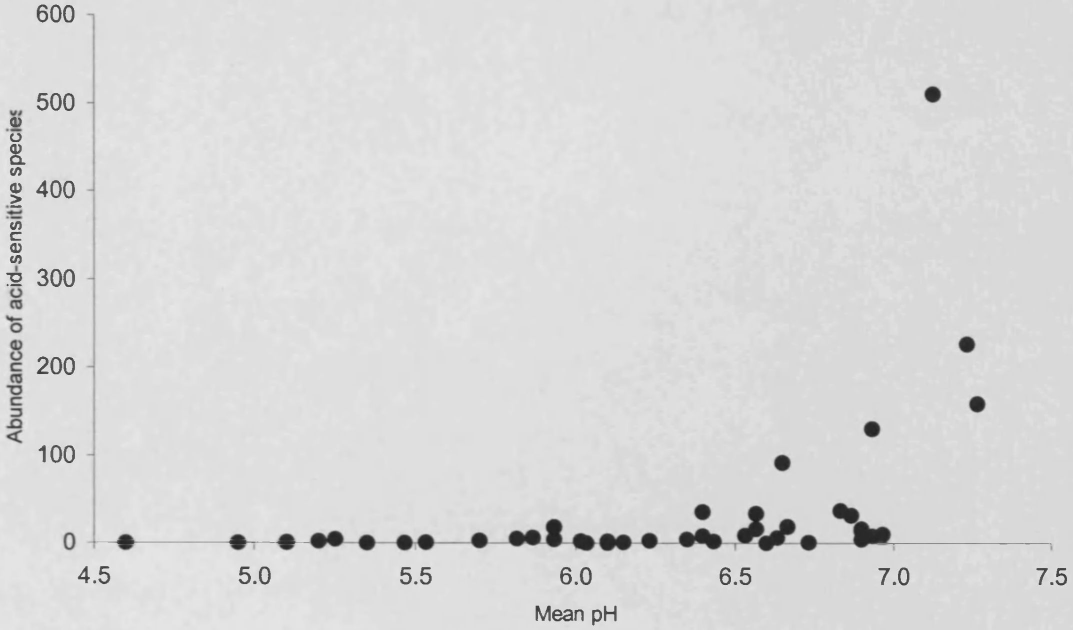

2.5
2
1.5
1
0.5
0
DAC axis 2
0
0.5
1
1.5
2
2.5
3
DCA axis 1

(a)

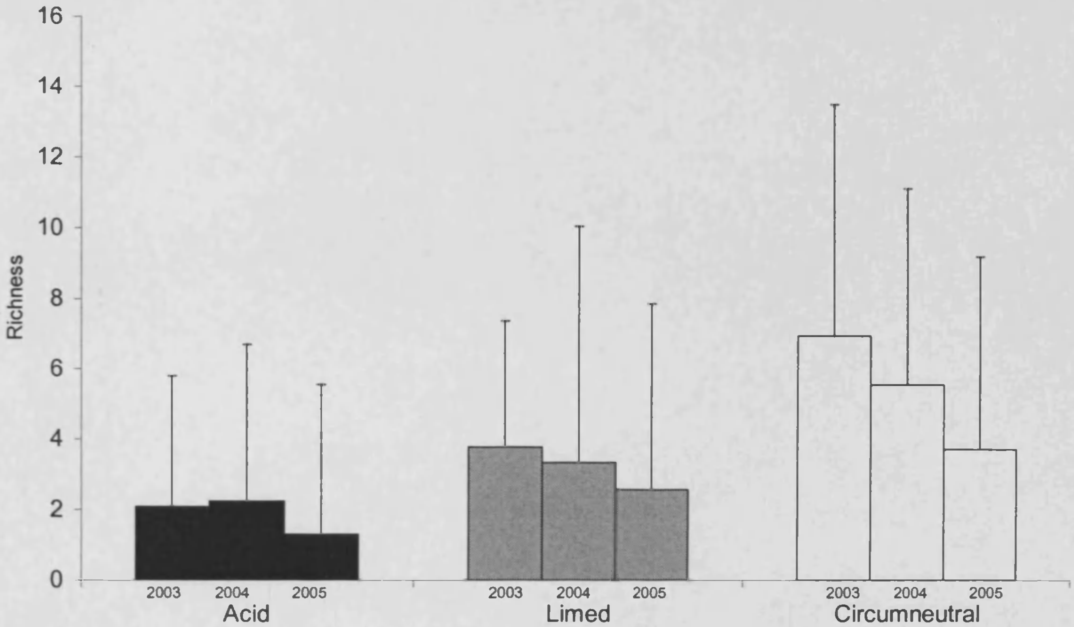

DCA axis 2
DCA axis 1
Rhithrogena semicolorata
Wormaldia sp
Br
Pm
Limnius volckmari
Heptagenia lateralis
Oulimnius sp.
Ig
Diura bicaudata
Re
Protonemura meyeri
Brachypter risi
Chrironomidae
Leuctra inermis
Nemurella pictetii
Amphinemura sulcicollis
Hydroporinae
Colymbetinae
Chloroperla torrentium
Simulidae

(b)

Figure 5.

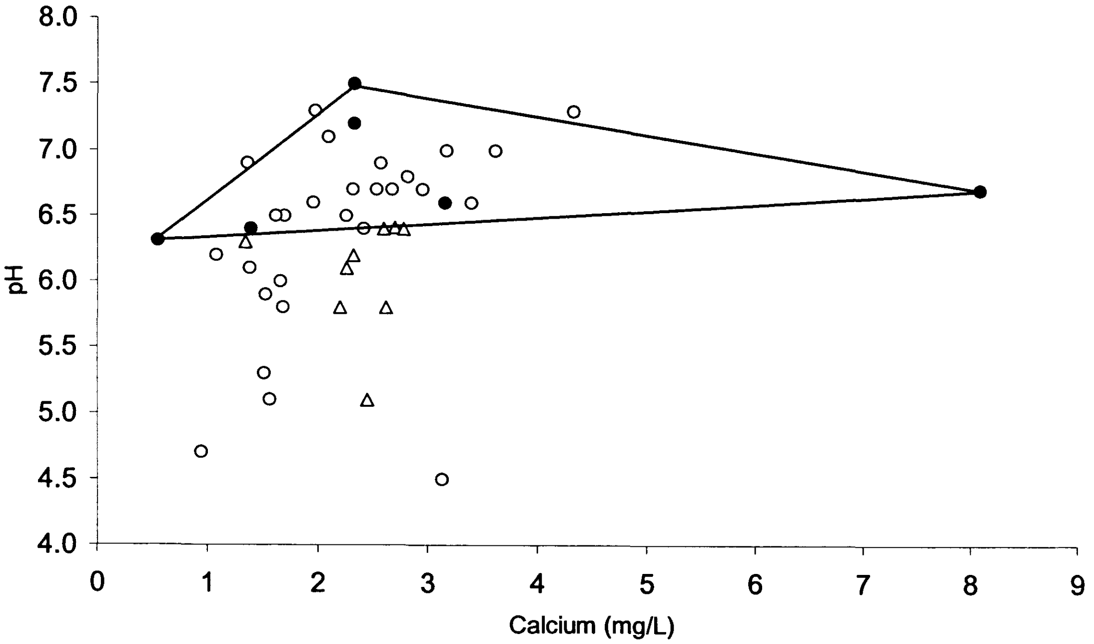

600
500
400
300
200
100
0
Abundance of acid-sensitive species
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Mean pH

(a)

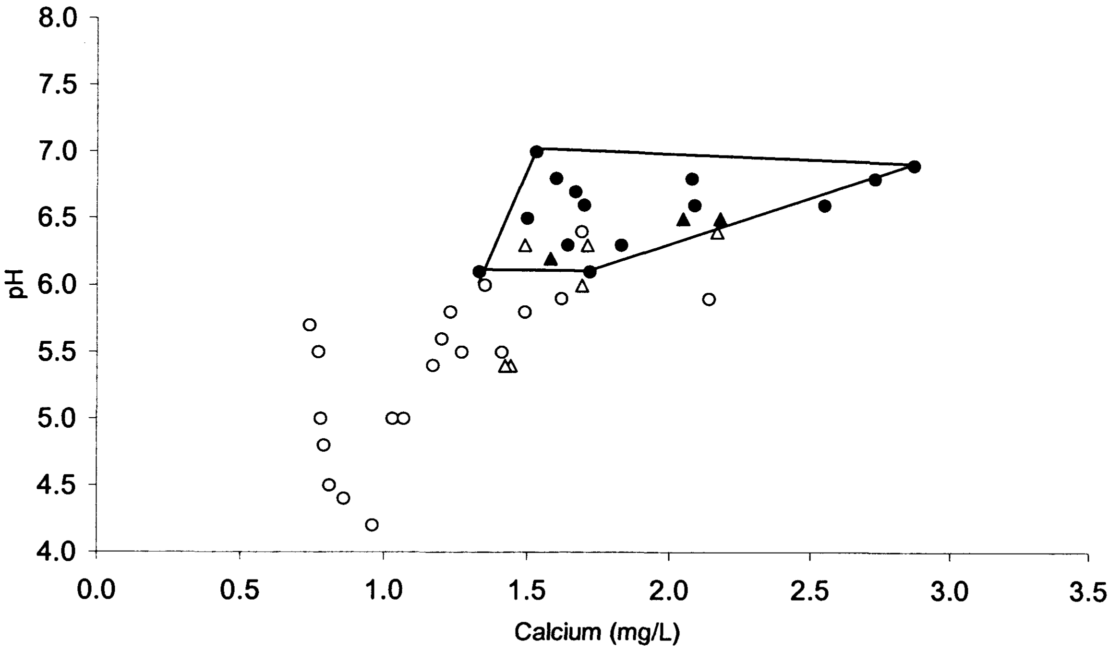

16
14
12
10
8
6
4
2
0
Richness
2003
2004
2005
Acid
2003
2004
2005
Limed
2003
2004
2005
Circumneutral

(b)

Figure 6.


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
pH
0
1
2
3
4
5
6
7
8
9
Calcium (mg/L)

(a)


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
pH
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Calcium (mg/L)

(b)

Figure 7.

Chapter 6

Survival of the acid-sensitive *Baetis rhodani* in streams targeted for liming during episodic and base flows.

6.1 Summary

1. Despite reductions in acidifying emissions and palliative treatment with limestone, many upland Welsh streams have failed to recover fully biologically from acidification. Increasing evidence suggests that acid episodes prevent the colonisation of acid-sensitive species and hence recovery. Here, the conditions in streams targeted for liming during low flow and high flow periods are assessed and the survival of the acid-sensitive mayfly *Baetis rhodani* in limed, acid and adjacent circumneutral reference streams is appraised.
2. During winter high flow, *Baetis* survival among three treatments (replicate limed, acid and circumneutral streams) was measured over 19 days. The experiment was then repeated during varying flows in summer. In addition, during the summer, some *Baetis* individuals were transiently removed from limed and acid streams during periods of higher flow, to assess whether avoidance of episodic conditions would increase survival.
3. During the winter high flow experiment, mean pH was significantly lower in acid (4.4) and limed (4.8) sites relative to circumneutral (5.7) streams. Mean pH was higher in all streams during the summer, when limed sites also had higher pH than acid controls (acid 4.8; limed tributaries 5.8; limed main river 6.1; circumneutral 6.3)
4. During both experiments, *Baetis* mortality during prolonged exposure in limed and acid streams reached 70%, greater than in circumneutral reference streams (25%). Mortality was not significantly lower in transplanted animals exposed only briefly in acid and limed streams. The initial pH to which *B. rhodani* individuals were exposed appeared to influence subsequent survival.

5. These data illustrate that even at low flow, limed streams in the Wye catchment cannot yet support acid-sensitive macroinvertebrate species and that liming has so far been insufficient to allow chemical and biological recovery, particularly at high flow.

Key Words: *acid-sensitive species, acidification, episodes, liming, macroinvertebrates, transplantation, mortality.*

6.2 Introduction

The effects of catchment liming on the recovery from acidification of stream organisms are still incompletely understood. Recovery by comparison with expectations is sometimes modest both in Europe and North America (Stoddard *et al.*, 1999; Fowler *et al.*, 2001; Monteith and Evans, 2005; Skjelkvåle, *et al.*, 2005). Among the hypotheses for limited recovery are: (i) limited dispersal of acid-sensitive taxa may prevent the re-colonisation of previously excluded species (Weatherley and Ormerod, 1990; Rundle *et al.*, 1995); (ii) lack of food sources or habitat types may prevent recolonisation (Rundle *et al.*, 1995); (iii) natural variation may mask changes resulting from liming (Weatherley and Ormerod, 1990; Bradley and Ormerod, 2001); (iv) acid-sensitive species may be competitively excluded from recovering locations (Ledger and Hildrew, 2005) and (v) continued acid episodes may prevent acid-sensitive species from persisting (Bradley and Ormerod, 2002; Kowalik *et al.*, in press). So far, these competing explanations have not been fully evaluated.

The effects of continuing acid episodes on the recovery of acid streams has been particularly emphasised (Bradley and Ormerod, 2002; Lepori *et al.*, 2003; Hirst *et al.*, 2004; Kowalik *et al.*, in press). Davies *et al.* (1992) describe episodes as short-term chemical changes resulting from increases in discharge which result in decreases in acid neutralising capacity (ANC). Although base cation dilution can be sufficient to explain such changes, the addition of strong acid anions can reduce ANC to below zero. Acid conditions may then be toxic to organisms directly through increases in H^+, or through the effects of toxic metals such as aluminium (Ormerod *et al.*, 1987; Merrett *et al.*, 1991). These effects still occur widely and might be sufficient to prevent biological recovery despite increasing mean pH (Lepori *et al.*, 2003; Kowalik and Ormerod, submitted). For example, streams with similar low flow chemistry but different episode chemistry can have marked biological differences (Lepori *et al.*, 2003).

In the case of palliative liming, a further possibility for patchy or incomplete biological recovery is that the effects attained in the small sub-catchments often involved are not fully expressed until they accumulate downstream. This situation is likely in the Wye catchment, where liming has been confined to headwater catchments (Chapter 4) and where many of the most severely impacted streams are small, low order tributaries (Lewis *et al.*, in press). By contrast, larger main river sites are likely to accumulate drainage from several limed sources and also from naturally buffered tributaries.

Although surveys can detect patterns in species assemblages between sites, experimental approaches, such as transplantation and in-situ bioassays survival studies, can be used to link biological processes to chemical conditions (Simonin *et al.*, 1993; Claveri *et al.*, 1995; Masters, 2002; Lepori *et al.*, 2003; Hirst *et al.*, 2004; Kowalik, 2005). In a replicated and balanced, in-situ bioassay, the mortality of *Beatis rhodani* (Pictet) is investigated here under a range of flow conditions in limed tributaries in the Wye catchment and at limed main river sites, acid controls and circumneutral reference streams. The mortality of animals exposed to chronic conditions at each site is measured and survival following transplantation from limed and acid streams to circumneutral streams during higher flows is assessed. As one of the most studied of all Ephemeroptera (Elliot *et al.*, 1988), *Baetis rhodani* is a prime candidate for ecological studies. *B. rhodani* is an acid-sensitive (Fjellheim and

Raddum, 1990), vagile species that sporadically colonises limed locations and has been identified in upland Welsh streams throughout the year (Kowalik and Ormerod, submitted). It was predicted that survival would be higher in limed and circumneutral streams relative to acid controls, and that mortality would be more rapid during high discharge periods due to the acid chemistry associated with these episodes of high flow. Further, mortality was predicted to be lower for *Baetis* individuals which avoided episodic conditions in limed and acid streams. The experiments were carried out in different seasons in order that the survival of *B. rhodani* could be assessed over a range of discharges. To reduce the likelihood of individuals reaching emergence during the experiment, a single pipette was used to collect all animals below a standard size (pipette 40mm diameter). The bivoltine life-cycle of *B. rhodani* (Elliott et al. 1988; Masters 2002), combined with evidence on the risk of episodes during different seasons (Kowalik and Ormerod, 2006), indicate that this experimental design allowed the test animals to be exposed to the large variations in flow that are likely to occur at these study sites throughout the year.

6.3 Methods

6.3.1 Study area

The study sites are located on the upper reaches of the Welsh River Wye and consist of low-order tributaries near the source, on Plynlimon in mid-Wales (52°28'N, 3°45'W; 677m). Here, the geology is predominantly characterised by Lower Palaeozoic (mudstones and shales) and Upper Palaeozoic rocks (marls and sandstone) with mixed land-use of moorland, rough pasture and coniferous forest (Edwards and Brooker, 1982). The area is described in detail elsewhere (Lewis *et al.*, in press; Chapter 3). At the end of July 2003, approximately 750 tonnes of limestone powder (calcium carbonate) were applied to 17 hydrological source areas within the catchment, as part of a large scale river restoration programme (Chapter 4). These applications permitted the current experimental design in which comparisons were made across three replicate acid control streams (WY35, WY39, WY56, mean pH 5.2-5.5), three limed tributaries (WY33, WY47,WY48, mean pH 5.2-5.7), three limed main rivers sites (WY34, WY53, WY60, mean pH 5.9-6.4) and three circumneutral reference streams (WY46, WY49, WY51, mean pH 6.4-6.6) (Figure 1).

6.3.2 Enclosure experiments

During two experiments, one in winter and one in summer, the survival of *B. rhodani* nymphs was assessed in replicate acid, limed and circumneutral tributaries (n = 3), using in-situ enclosures. The winter experiment was undertaken in October 2004 during high flows. The second experiment was carried out in summer, during variables flows in July 2005. The same replicate streams for the three treatment types (acid controls; limed; circumneutral references) were used in both experiments. In addition, limed main river sites were included in the summer experiment. Further, during the summer, in limed and acid tributaries, transient enclosures were set up which were transferred to circumneutral streams during periods of higher flows (days 4-7 and 11-19). When the flow dropped, animals in these enclosures were returned to the stream in which they had initially been placed. The summer experiment thus involved six treatments (acid; limed; circumneutral; limed main river: limed transient; acid transient) whilst the winter experiment included three treatments (acid, limed and circumneutral tributaries).

In all cases, nymphs were collected from one stream (WY51) with abundant *B. rhodani* and where all *Baetis* individuals collected previously belonged to *B. rhodani*. Animals were obtained by gentle disturbance of the substrate into a pond net, from which the contents were transferred to a sorting tray and from there into plastic beakers containing stream water. Once at the target stream (average travel time from source stream to target stream = 15 minutes) the nymphs were placed into cylindrical, mesh experimental enclosures (height 10cm, diameter 16cm, mesh 560μm) anchored into riffle sections of the streams. The same enclosures had been used previously and successfully for similar purposes (Lepori and Ormerod, 2005; Kowalik and Ormerod 2006). Two "replicate" enclosures each containing five *B. rhodani* were fixed in each stream. The upper half of each enclosure protruded above the water level to allow for insect emergence. Pebbles collected locally were placed in each enclosure and replaced regularly to provide a source of algae. Enclosures were checked on Days 2, 6, 8, 10, 14 and 19 of the experiment, when mortality was recorded and dead individuals removed. After completion of each experiment, all nymphs were immediately preserved in industrial methylated spirit and positively identified as *B. rhodani* under a microscope.

6.3.3 Environmental data

For both experiments, pH, conductivity and temperature were measured contemporaneously with survival using a combined pH/EC/TDS meter (HANNA HI 991300). Daily discharge data were recorded using a Campbell Scientific CR10 data logger at a permanent monitoring station near the source of the main river (Figure 1). Water samples were collected on Day 2 of the winter experiment and analysed for alkalinity by Gran titration and base cations (sodium, potassium, calcium, magnesium) by atomic absorption spectrophotometry (AAS, Perkin Elmer). Silicon was measured using the molybdenum blue method, whilst chloride, nitrate (NO_3^--N) and sulphur (SO_4) were analysed by ion chromatography (Dionex). Continuous flow colorimetry with UV digestion (Skalar autoanalyser system) was used to assess Dissolved Organic Carbon (DOC) whilst Aluminium (Al) was determined colorimetrically using the pyrocatechol violet method (Dougan and Wilson, 1974).

6.3.4 Statistical analyses

pH, temperature and conductivity for each treatment were compared between winter and summer data sets using t-tests (MINTAB, version 14). Differences in chemistry across groups of sites in each experiment were assessed using one-way ANOVA, with Tukey's pair-wise comparisons. For each site, Spearman's rank correlation was used to assess the relationship between pH and discharge. Individual r values for each sites were standardised using z scores ((x – mean x)/standard deviation) and mean z scores within site groups were calculated. One-way ANOVA on z transformed r values was performed to examine whether flow affects pH similarly in all groups.

For each exposure treatment, mortality curves were plotted (cumulative mortality over 19 days). Linear regression analysis (MINTAB) was then used to calculate the slope (rate of mortality) of each curve. One-way ANOVA was used to assess differences in slope and final mortality (% of animals dead at the end of the experiment) between treatments. The relationships of slope and final mortality to pH-related variables were examined with multiple linear regression analysis. 'Start pH' (pH on Day 0), 'Initial pH' (mean pH over first four days), 'mean pH' (mean pH between Days 0 and 19) and 'minimum pH' were all initially included in the model. This set of variables was then reduced using "best subsets", with variables selected as those with a combination of low Mallow's C-p values (used to compare the full model to a model with the best subset of predictors) and the highest R-Sq (adj) value (the percentage of total variation in the response that is explained by predictor variables; adjusted for the number of predictors). The final models were checked for significant regression constants ($p < 0.05$), Analysis of Variance (ANOVA; indicating significance of regression equation at $p < 0.05$) and the Variance Inflation Factor (VIF > 5 indicates multicolinearity). Prior to regression calculations final mortality values were probit transformed to linearize the curve.

6.4 Results

6.4.1 Winter experiment

During the winter experimental period, discharge varied between 0.13m^3 s^{-1} and 2.65m^3 s^{-1} (Figure 2 a). Chemical samples collected during the winter experiment revealed that Ca ($F_{2,9} = 5.27, p < 0.05$) and ANC ($F_{2,9} = 11.39, p < 0.05$) were both higher in circumneutral streams than acid and limed streams (Table 1). For the correlation between pH and discharge, z transformed r values were compared between groups. The correlation was significantly higher in circumneutral sites (mean r = -0.97) relative to acid controls (mean r = -0.42) (F = 11.803, $p < 0.05$), but did not differ between limed streams (mean r = -0.70) and the other stream types. Minimum pH at acid, limed and circumneutral streams was 3.5, 3.9, and 5.0 respectively during the winter experiment. pH differed significantly between circumneutral streams (5.7 ± 0.4) and other stream types (acid = 4.4 ± 0.6; limed = 4.8 ± 0.5) ($F_{1,14} = 19.89, p < 0.001$) during the summer, whilst there was no significant difference in the pH at the latter two groups (Figure 3 a). Conductivity was significantly higher at acid streams compared with limed sites ($F_{1, 14} = 4.64, p < 0.05$; Figure 3 c) whilst temperature did not vary between stream types ($F_{2, 21} = 0.41$; Figure 3 e).

The first mortalities were recorded on Day 2 in two of the acid streams and one limed stream. All of the animals held in the acid streams were dead by Day 8 (Figure 4 a) whilst mortality in limed streams was 100% in two of the replicate streams by Day 9. By contrast, mortality remained low throughout the experiment (10-20%) at circumneutral sites. Statistically, final mortality and rate of mortality in circumneutral streams was significantly lower than at acid and limed sites, whilst the latter two did not differ significantly ($F_{2, 9} = 9.19, p < 0.001$; Table 2). The slope of mortality curves were steepest between Days 2 and 8 (Figure 4 a), during a drop in pH, associated with a high flow event (Figure 2 a and 3 a).

6.4.2 Summer experiment

During this experiment, discharge ranged from $0.04 m^3/s$ to $0.27 m^3/s$ (Figure 2 b). There was no significant difference in the strength of the correlation between pH and discharge across stream types with mean r at acid, limed tributary, limed main river and circumneutral sites being -0.29, -0.21, -0.50 and -0.28, respectively. pH and temperature were higher in all stream types during summer sampling compared to winter, whilst conductivity was only higher in acid streams in winter (Table 1). Mean pH of replicate streams was lower at acid sites compared to limed tributaries, limed main river sites and circumneutral streams ($F_{3, 28} = 49.88, p < 0.001$) and also significantly lower at limed sites than circumneutral ($F_{1, 14} = 49.88, p < 0.05$), whist differences between main river sites and limed tributaries or circumneutral streams were not apparent (Figure 3 b). Neither conductivity nor temperature was significantly different across streams types (Figure 3 d, f)

For this experiment, the first mortalities were recorded on Day 2 in acid streams, with 100% mortality at two of the sites by Day 19 and 90% in the third (Figure 4 b). In limed tributaries and main river sites, mean final mortality reached a maximum of 80%. Again mortality was low in circumneutral streams, reaching a mean of 20% by the end of the experiment. As with the winter data, final mortality and the mortality rate of animals exposed in limed or acid streams for any length of time were higher than at circumneutral sites ($F_{4, 15}$= 9.19, $p < 0.05$). The mortality of animals which were periodically removed from acid and limed streams was not significantly different from those animals which remained at these sites throughout the experiment, suggesting that animals exposed to limed or acid conditions even only transiently were sufficiently affected for mortality to occur within a short time following exposure. Mortality of animals exposed to limed main river sites did not differ from those in limed tributaries (at $p < 0.05$).

Relationships between mortality and pH were described using multiple linear regression. Whilst all pH variables were incorporated in the stepwise construction of the multiple linear regression models, 'Initial pH' was found to be the best explanatory variable for final mortality and the slope of the mortality curves (Figure 5). In the case of both models, ANOVA tables confirmed the significance of the equations (for final mortality equation, $F_{27, 8} = 24.4$, $p < 0.001$, R sq (adj) = 48.4%; for slope equation, $F_{27, 8} = 10.3$, $p < 0.001$, R sq (adj) = 26.4%) and low Variance Inflation Factors (< 5) indicted that there was no colinearity.

Maximum mortality increased rapidly and linearly as pH approached the lower tolerance limit for the species in this catchment (5.8). The relationship between final mortality and initial pH of exposure indicated that exposure to pH < 5.7-6.0 early in the experiment rapidly decreased *B. rhodani* survival (Figure 5).

6.5 Discussion

Despite decreases in emissions and deposition of acidifying compounds across much of Europe and North America, the chemical and biological response of upland Welsh streams has been less then expected (Stoddard *et al.*, 1999; Fowler *et al.*, 2001: Fowler *et al.*, 2005; Monteith *et al.*, 2005). Although evidence from elsewhere has led some authors to report changes in episode chemistry (Laudon and Hemond, 2002) there is clear evidence that episodes in upland catchments in Wales persist (Kowali and Ormerod, in press). Recent surveys of diatoms and macroinvertebrates in the catchment of the river Wye indicate that many parts of the upper catchment still have reduced pH, particularly at headwater sites (Lewis *et al.*, in press), signifying a need for large-scale liming of the sources areas.

As a common and abundant species at circumneutral sites, *B. rhodani* might be expected to be an early colonist of limed streams. Annual surveys in the first two years following lime treatment revealed that *B. rhodani* and other acid-sensitive macroinvertebrate species appeared at some limed sites where they had previously been absent. Colonisation was not, however, consistent across all limed sites and abundance remained low (Chapter 5). Data from the Wye supports patterns found in the adjacent Llyn Brianne experimental catchment, where acid-sensitive taxa increased in abundance only transiently, and only one mayfly species (*B. rhodani*) and one acid-sensitive stonefly (*Brachyptera risi*) occurred significantly more frequently after liming (Bradley and Ormerod, 2002).

Several hypothesis for limited biological recovery in streams recovering chemically from acidification have been postulated (Rundle *et al.*, 1995; Bradley and Ormerod 2001; Ledger and Hildrew 2005). That most supported is that anthropogenic acid episodes continue to exclude acid-sensitive organisms and hence offset biological recovery (Raddum *et al.*, 2001; Bradley and Ormerod, 2002; Lepori *et al.*, 2003; Kowalik *et al.*, in press). Whilst increased flow can affect benthic invertebrate communities physically (Growns and Davis, 1994; Nelson and Lieberman, 2002), acid chemistry associated with high discharge in acid-sensitive areas has the clearest influence on macroinvertebrates (Lepori *et al.*, 2003; Kowalik *et al.*, in press) with episodes linked to the retarded recovery of sensitive species of both invertebrates and fish (Lacroix and Korman 1996; Raddum *et al.*, 2001; Lepori *et al.*, 2003; Kowalik *et al.*, in press).

Discharge during the two experimental periods in this study varied substantially, with the highest maximum winter discharge approaching two orders of magnitude higher than the lowest summer flows. This was reflected in the chemistry, with higher pH values recorded in summer than winter across all stream types. Significant differences in mean pH between limed sites and acid streams were apparent during the summer, suggesting that the conditions during low flow at treated sites have changed relative to controls. These differences were not consistent across season, with acid and limed streams not having significantly different pH during the winter experiment. Mortality patterns generally reflected differences in pH across streams types with final mortality and rate of mortality of *B. rhodani* in limed and acid streams higher than at circumneutral sites during both experiments. The higher pH at limed sites compared to acid controls during the summer experiment was not, however, reflected in mortality data, with animals in both streams types showing similarly low survival during both experiments.

Examination of the above literature, relating high flow acid episodes to invertebrate mortality, may lead to the supposition that avoidance of such periods could increase the survival of sensitive animals. Thus for *Beatis* individuals that were transiently removed from acid and limed streams, avoiding episodes of higher flow, mortality was predicted to be lower. However, these animals had similar mortality to those exposed to prolonged conditions in acid and limed streams. Therefore avoidance of episodes was insufficient to prevent high mortality. Lepori and Ormerod (2005) found that exposure to only short episodes did not cause significant mortality of *Baetis alpinus,* either during the period of exposure or in the following days, and the authors concluded that the study species may be able to recover from short acid episodes. Regression analysis of these data suggests that if animals were exposed, albeit briefly, to the conditions in limed or acid streams initially, mortality (rate of mortality and final mortality) was high, which supports studies from the Llyn Brianne catchment which suggest that *Baetis* species are sensitive to even transient exposure to low pH (Kowalik and Ormerod 2006). Using the regression equations produced, it is predicted from these data, that an initial exposure to pH 5.8 leads to a mortality of over 60% within 19 days. This is supported by survey data collected from 42 sites across the study catchment (Chapter 5) and during a previous experiment at Llyn

Brianne (Masters, 2002), where *B. rhodani* was only found at sites with mean pH above 5.8. This is further consistent with data which defines the acid-sensitivity of this species during the emergence stage (Bell, 1971). In cases where episodic events have been empirically linked to invertebrate survival, there is further evidence that the life-cycles of sensitive species increase the risk of exposure to episodes (Kowalik and Ormerod, 2006), intensifying the difficulties for this species to colonise such sites.

Acid-sensitive species are often absent from chronically and episodically acidified streams (Sutcliffe and Carrick 1973; Weatherley and Ormerod 1987) and it is often difficult to separate the effects on organisms of chronic and episodic exposure to low pH (Ormerod and Jenkins, 1994). Interestingly, surveys of streams in the Southern European Alps (Lepori and Ormerod, 2005) suggested similar abundance of an acid-sensitive mayfly between acid, episodic and well-buffered sites during stable flows, although during an in situ toxicity assay under episodic flows, survival was low in episodic streams. In contrast Gibbins *et al.* (2001) found that despite the importance of hydrological variation, in particular high flow events, invertebrate communities in Scottish streams remained stable over a long-term study.

Although episode chemistry clearly has important influences on macroinvertebrates in upland Welsh streams, these data suggest that exposure to the conditions in these limed streams under any flow rate is sufficient to prevent survival of acid-sensitive species. Despite some indications that low flow pH at limed streams had improved during the low flows of summer relative to acid controls, examination of these data along with that from quarterly water chemistry surveys and annual macroinvertebrate sampling over two years suggests that consistent chemical changes have not yet been produced and treated sites are currently unable to support acid-sensitive species. Weatherley and Ormerod (1991) compared episode chemistry and mean pH as predictors in models for fish populations, and found that inclusion of episode chemistry did not improve models. It thus appears that chronic problems related to poor acid-base status in the catchment are preventing colonisation by acid-sensitive species.

Whilst larger main river sites may be expected to recover further than tributaries, through accumulation of drainage from limed tributaries upstream and from inputs from naturally well-buffered sources, it is clear that in the upper Wye, substantial inputs from untreated acid streams persist, and appear to override any positive effects of treatment that may have occurred at the main river.

Although the benefits of transplantation and in-situ bioassays survival studies have been used to elucidate links between acid toxicity and aquatic biota (Simonin *et al.*, 1993; Claveri *et al.*, 1995; Masters, 2002; Lepori *et al.*, 2003; Hirst *et al.*, 2004; Kowalik, 2005), the potential influence of experimental artefacts in such studies have also been recognised (Ormerod and Jenkins, 1994). Avoidance behaviour (predators, competition) and recolonisation behaviour is not possible for study organisms confined during experiments and the test population will only represent part of the real population. Moreover, animals suffering sub-lethal effects or animals which are partially affected and then recover, can give misleading results. However, in this study, mortality in circumneutral reference streams remained low, indicating that any detrimental effects of confinement within the enclosures were minimal. The results of *in situ* bioassay studies such as this are not always easily translated to conclusions about the natural population or community as whole. This difficulty in extrapolating results necessitates an integrated approach to impact assessment, such as combining toxicity tests with chemical and biological monitoring surveys. Such an approach over larger-time-scales and with respect to an array of sensitive species, whose varying tolerances may elucidate some of the mechanisms behind biological recovery from acidification, will be required for accurate assessment of the on-going liming programme in the Wye, and the ecological consequences for the catchment. Further, whilst Fjellheim and Raddum (1992) recorded *B. rhodani* in limed streams in Norway, the species only appeared two years after treatment, demonstrating that as an on-going experiment, the Wye liming project is not yet complete and the first two years of data should thus be used in preliminary evaluation.

From a management perspective, it appears that liming has not yet achieved the stable chemical milieu suitable for biological recovery with conditions currently unsuitable for the survival of acid-sensitive macroinvertebrates. More extensive treatment targeted to address periods of high discharge will increase the potential for chemical and biological restoration to be achieved.

6.6 Acknowledgements

BRL was funded by The Wye and Usk Foundation. Water samples were analysed by Brian Reynolds and Annie Britton at CEH Bangor and Simon Grant provided discharge data. Thank you to D. M. Lewis for help in the field, and R. A. Kowalik for advice on the experimental equipment.

6.7 References

Bell, H.L. 1971. Effect of low pH on the survival and emergence of aquatic insects. *Water Research*, **5**, 313-319.

Bradley, D.C. and Ormerod, S.J. 2001. Community persistence among stream invertebrates tracks the North Atlantic Oscillation. *Journal of Animal Ecology*, **70**, 987-996.

Bradley, D.C. and Ormerod, S.J. 2002. Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater biology*, **47**, 161-171.

Claveri, B., Guerold, F. and Pihan, J.C. 1995. Use of transplanted mosses and autochthonous liverworts to monitor trace metals in acidic and non-acidic headwater streams (Vosges mountains, France). *Science of the Total Environment*, **175** (3), 235-244.

Davies, T.D., Tranter, M., Wigington, P.J. and Eshkeman, K.N. 1992. 'Acidic episodes' in surface waters in Europe. *Journal of Hydrology*, **132**. 25-69.

Dougan, W. K. and Wilson, A. L. 1974. The absorptiometric determination of aluminium in water: A comparison of some chromogenic reagents and the development of an improved method. *Analyst*, **99**, 413-430.

Edwards, R.W. and Brooker, M.P. 1982. *The Ecology of the Wye*, Monographiae Biologicae, Volume 50, JUNK Publishers, The Hague.

Elliot, J.M., Humpesch, U.H. and Macan, T.T. 1988. *Larvae of the British Ephemeroptera: a key with ecological notes*. Freshwater Biological Association, Ambleside, UK.

Fjellheim, A. and Raddum, G.G. 1990. Acid precipitation - biological monitoring of streams and lakes . *Science of The Total Environment*, **96**, 57-66.

Fjellheim, A. and Raddum, G. G. 1992. Recovery of acid-sensitive species of Ephemeroptera, Plecoptera and Trichoptera in River Audna after liming. *Environmental Pollution*, **78**, 173-178.

Fjellheim, A. and Raddum G.G. 1995. Benthic animal response after liming of three Southern Norwegian rivers. *Water, Air, and Soil Pollution*, **85**, 931-936.

Fowler, D., Coyle, M., ApSimon, H.M., Ashmore, M.R., Bareham, S.A., Battarbee, R.W., Derwent, R.G., Erisman, J-W., Goodwin, J., Grennfelt, P., Hornung, M., Irwin, J., Jenkins, A., Metcalfe, S.E., Ormerod, S.J., Reynolds, B. and Woodin, S. 2001. Transboundary Air Pollution: acidification, eutrophication and ground-level ozone in the UK, Rep. No. EPG 1/3/153. DEFRA: Edinburgh.

Fowler, D., Smith, R.I., Muller, J.B.A.., Hayman, G. and Vincent, K.J. 2005. Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, **137**, 15-25.

Gibbins, C.N., Dilks, C.F., Malcolm, R., Soulsby, C. and Juggins, S. 2001. Invertebrate communities and hydrological variation in Cairngorm mountain streams. *Hydrobiologia*, **462**, 205-219.

Growns, I.O. and Davis, J.A. 1994. Longitudinal changes in near-bed flows and macroinvertebrate communities in a Western Australian stream. *Journal of the North American Benthological Society*, **13**, 417-438.

Hirst, H., Chaud, F., Delabie, C., Jüttner, I. and Ormerod, S.J. (2004) Assessing the short-term response of stream diatoms to acidity using inter-basin transplantations and chemical diffusing substrates. *Freshwater Biology*, **49**, 1072-1088.

Jenkins, A., Waters, D. and Donald, A. 1991. As assessment of terrestrial liming strategies in upland Wales. *Journal of Hydrology*, **124**, 243-261.

Kowalik, R. A. 2005. The role of acid episodes in offsetting biological recovery of acidified streams. Unpublished. PhD Thesis, Cardiff University, Cardiff.

Kowalik, R.A. and Ormerod, S.J. 2006. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology*, **51**, 180-191.

Kowalik, R.A., Cooper, D.M. and Ormerod, S.J. Acid episodes retard the biological recovery of upland British streams from acidification. *Global Change Biology*, In press.

Kowalik, R.A. and Ormerod, S.J. Stream invertebrate communities and continued episodic acidification in regions of contrasting acid deposition. Manuscript submitted.

Lacroix, G.L. and Korman, J.1996. Timing of episodic acidification in Atlantic salmon rivers influences evaluation of mitigative measures and recovery forecasts. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 589-599.

Laudon, H. and Hemond, H.F. 2002. Recovery of streams from episodic acidification in Northern Sweden. *Environmental Science and Technology*, **36**, 921-928.

Ledger, M.E. and Hildrew, A.G. 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution*, **137**, 103-118.

Lepori, F., Barbieri, A. and Ormerod, S.J. 2003. Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams. *Freshwater Biology*, **48**, 1873-1885.

Lepori, F. and Ormerod, S.J. 2005. Effects of spring acid episodes on macroinvertebrates revealed by population data and in situ toxicity tests. *Freshwater Biology*, **50**, 1568-1577.

Lewis, B.R., Jüttner, I., Reynolds, B. and Ormerod, S.J. In press. Comparative assessment of stream acidity using diatoms and macroinvertebrates: implications for river management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Masters, Z. 2002. *Factors affecting Stream Macroinvertebrate Recovery from Acidification*. Unpublished. PhD Thesis, Cardiff University, Cardiff.

Merrett, W.J., Rutt, G.P., Weatherley , N.S. Thomas, S..P. and Ormerod, S.J. 1991. The response of macroinvertebrates to low pH and increased aluminium concentration in Welsh streams: multiple episodes and chronic exposure. *Archiv für hydrobiologie,* **121**, 115-125.

Monteith, D.T. and Evans, C.D. 2005. The United Kingdom Acid Waters Monitoring Network: a review of the first 15 years and introduction to the special issue. *Environmental Pollution,* **137**, 3-13.

Monteith, D.T., Hildrew, A.G., Flower, R.J., Raven, P.J. Beaumont, W.R.B, Collen, P., Kreiser, A.M., Shilland, E.M. and Winterbottom, J.H. 2005. Biological responses to the chemical recovery of acidified fresh waters in the UK. *Environmental Pollution*, **137**, 83-101.

Nelson, S.M. and Lieberman, D.M. 2002. The influence of flow and other environmental factors on benthic macroinvertebrates in the Sacramento River, U.S.A. *Hydrobiologia*, **489**, 117-129.

Ormerod, S.J., Boole, P., McCahon, C.P., Weatherley, N.S. Pascoe, D. and Edwards, R.W. (1987). Short-term experimental acidification of a Welsh stream: comparing the biological effects of hydrogen ions and aluminium. *Freshwater Biology*, **17**, 341-356.

Ormerod, S.J. and Jenkins, A. 1994. The Biological Effects of Acid Episodes. In *Acidification of Freshwater Ecosystems: Implications for the* future (Eds,C.E.W. Steinberg, R.F. Wright), pp. 259-272. John Wiley and Sons Ltd, Chichester.

Raddum, G.G., Fjellheim, A. and Skjelkvåle, B.L. 2001. Improvements in water quality and aquatic ecosystems due to reduction in sulphur deposition in Norway. *Water, Air, and Soil Pollution*, **130**, 87-98.

Rundle, S. D., Weatherley, N. S. and Ormerod, S.J. 1995. The effects of catchment liming on the chemistry and ecology of upland Welsh streams: testing model predictions. *Freshwater Biology,* **34**, 165-175.

Simonin, H.A. Kretser, W.A., Bath, D.W., Olson, M. and Gallagher, J. 1993. In situ bioassays of brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) in Adirondack streams affected by episodic acidification. *Canadian Journal of Fisheries and Aquatic Sciences,* **50**, 902-912.

Skjelkvåle, B.L., Stoddard, J.L., Jeffries, D.S., Tørseth, K., Høgåsen, T., Bowman, J., Mannop, J., Monteith, D.T., Mosello, R., Rogara, M., Ryzchon, D., Vesely, J., Wieting, J., Wilander, A. and Worsztynowicz, A. 2005. Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, **137**, 165-176.

Stoddard, J.L., Jeffries, D. S., Lükewille, A., Clair, T. A., Dillon, P. J., Driscoll, C. T., Forsius, M., Johannessen, M., Kahl, J. S., Kellogg, J. H., Kemp, A., Mannio, J., Monteith, D. T., Murdoch, P. S., Patrick, S., Rebsdorf, A., Skjelkvåle, B. L., Stainton, M. P., Traaen, T., van Dam, H., Webster, K. E., Wieting, J. and Wilander, A. (1999). Regional trends in aquatic recovery from acidification in North America and Europe. *Nature,* **401**, 575-578.

Sutcliffe, D. W. and Carrick, T. R. 1973. Studies on mountain streams in the English Lake District. I. pH, calcium and the distribution of invertebrates in the River Duddon. *Freshwater Biology*, **3**, 437-462.

Weatherley, N.S. and Ormerod, S.J. 1987. The impact of acidification on macroinvertebrate assemblages in Welsh streams: towards an empirical model. *Environmental Pollution*, **46**, 223-240.

Weatherley, N.S. and Ormerod, S.J. 1990. The constancy of invertebrate assemblages in soft-water streams: implications for the prediction and detection of environmental change. *Journal of Applied Ecology*, **27**, 952-964.

Weatherley, N.S. and Ormerod, S.J. 1991. The importance of acid episodes in determining faunal distributions in Welsh streams. *Freshwater Biology*, **25**, 71-84.

Weatherley, N.S. and Ormerod, S.J. 1992. The biological response of acidic streams to catchment liming compared to the changes predicted from stream chemistry. *Journal of Environmental Management*, **34**, 105-115.

Weatherley, N.S., Jenkins, M.J., Evans, D.M., Rogers, A.P. and Gee, A.S. 1995. Options for liming rivers to ameliorate acidity – a UK perspective. *Water, Air, and Soil Pollution,* **85**, 1009-1014.

6.8 List of tables

Table 1. Mean pH (± standard deviation), temperature and conductivity of acid, limed and circumneutral streams during winter and summer transplantation experiments and Ca and ANC measured during winter study. Results of T-tests of winter against summer samples are shown for each group (** $p < 0.01$; *** $p < 0.001$; ns = not significant).

Stream Type	Variable	Winter	Summer	T statistic
Acid	pH	4.4 ± 0.6	4.8 ± 0.7	-3.27 **
	Temperature (°C)	9.0 ± 0.6	13.3 ± 1.6	-10.86 ***
	Ca (mg L^{-1})	1.08		
	ANC	-7.83		
	Conductivity (μS/cm)	46.0 ± 4.8	40.0 ± 2.6	5.20***
Limed Tributary	pH	4.8 ± 0.5	5.8 ± 0.5	-6.60***
	Temperature (°C)	8.9 ± 0.6	14.9 ± 3.6	-7.80 ***
	Ca (mg L^{-1})	1.38		
	ANC	21.3		
	Conductivity (μS/cm)	39.1 ± 3.0	40.0 ± 5.8	-0.71 ^{ns}
Circumneutral	pH	5.7 ± 0.4	6.3 ± 0.2	-6.51 ***
	Temperature (°C)	8.7 ± 0.4	15.2 ± 3.1	-8.41 ***
	Ca (mg L^{-1})	2.78		
	ANC	129.27		
	Conductivity (μS/cm)	40.3 ± 8.5	44.4 ± 9.9	-1.40 ^{ns}

Table 2. Final mortality and slope of mortality curves for *B. rhodani* during two survival experiments. Figures represent means (± standard deviation) of three replicates for each stream treatment type.

Experiment 1: Winter

Stream type	Final mortality (%)	Slope of mortality curve
Acid	100 (± 0.0)	5.46 (± 0.7)
Limed	83 (± 28.9)	5.25 (± 2.1)
Circumneutral	13 (± 5.7)	0.83 (± 0.3)

Experiment 2: Summer

Stream type	Final mortality	Slope of mortality curve
Acid	97 (± 5.8)	4.53 (± 0.4)
Limed	70 (± 10.0)	4.00 (± 0.3)
Limed Main river	77 (± 53.8)	3.93 (± 0.4)
Circumneutral	27 (± 20.8)	1.23 (± 1.1)
Acid transient	100 (± 0.0)	5.00 (± 0.4)
Limed transient	80 (± 20.0)	4.36 (± 1.2)

6.9 List of figures

= 12.7 – 1.22 (Initial pH); slope = 10.9 - 1.27 (Initial pH). Final mortality percentages were probit transformed prior to analysis.

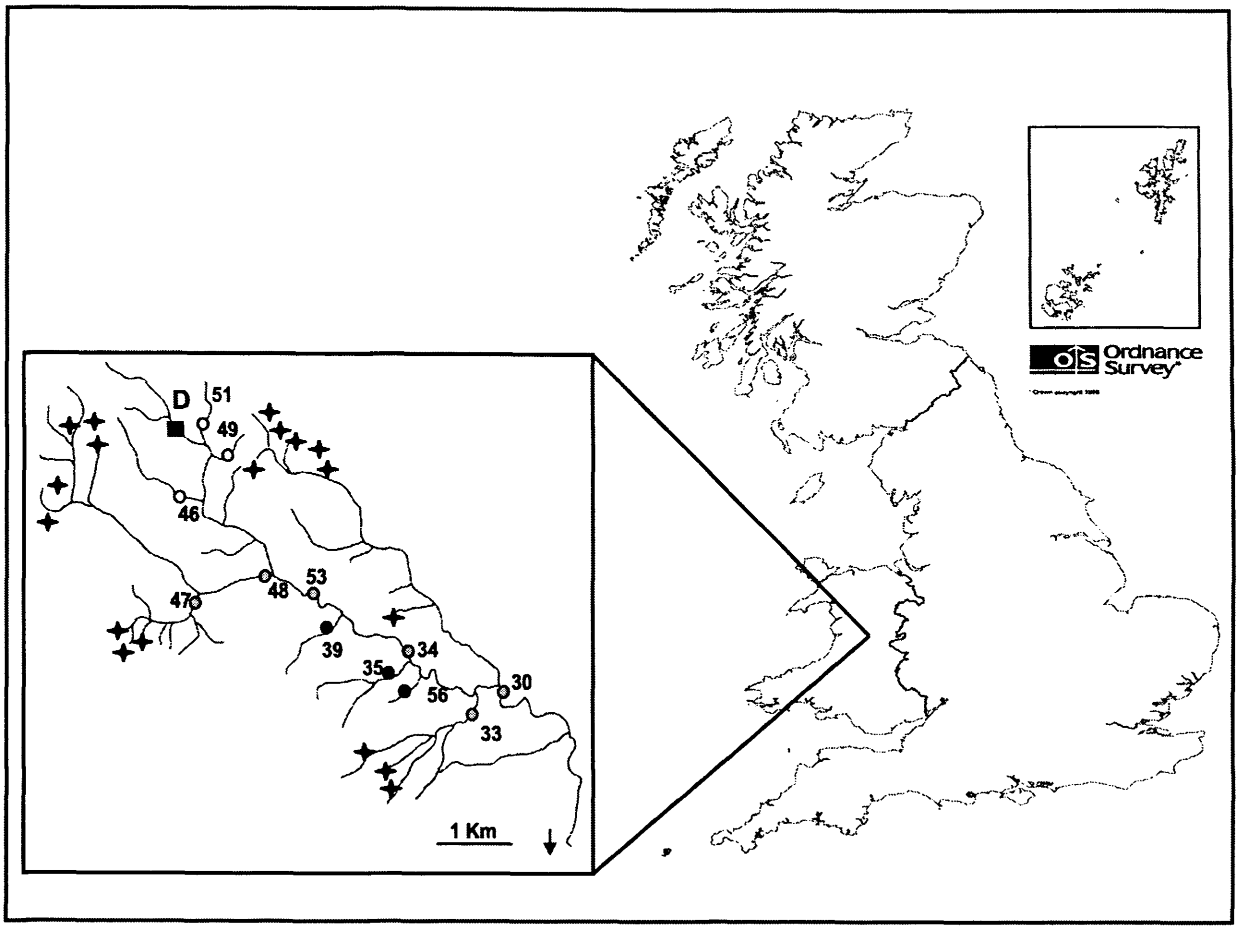
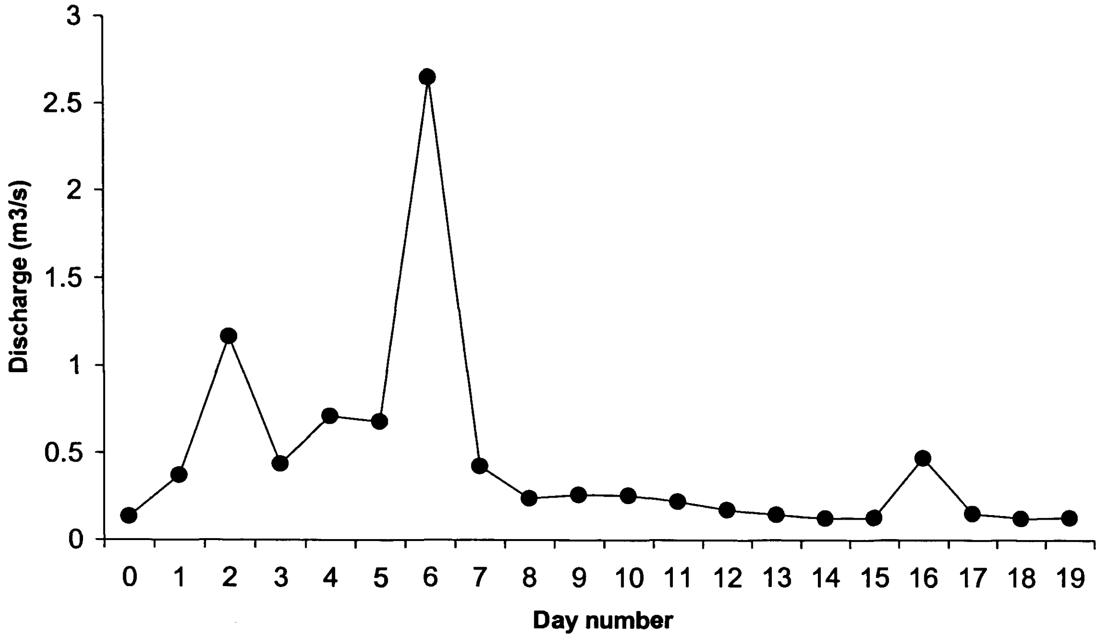
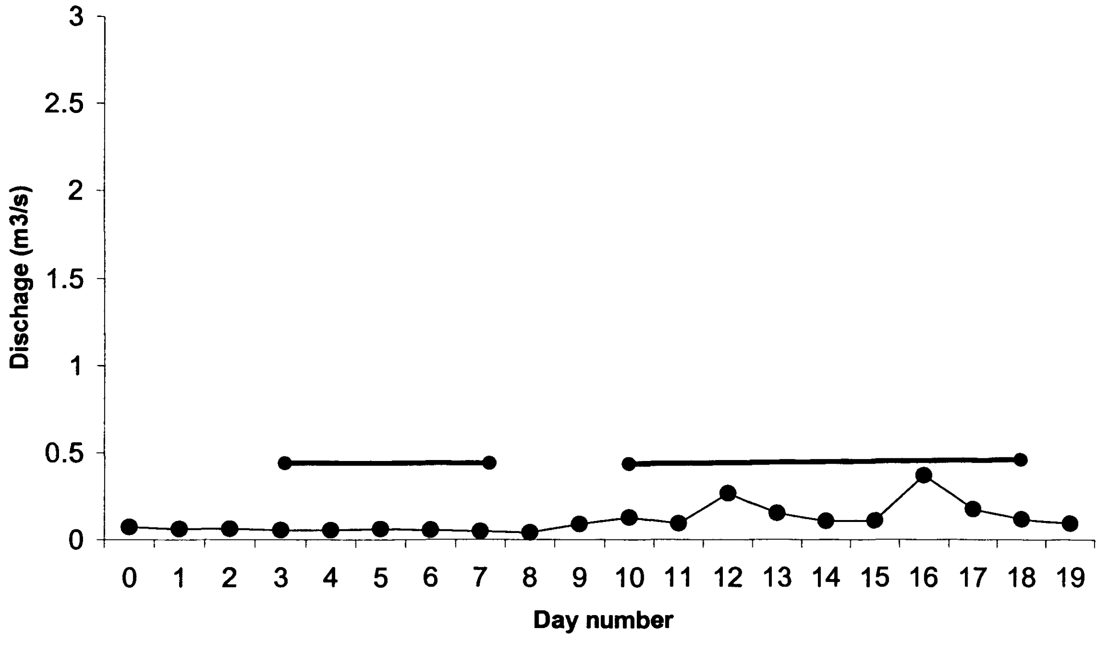


Figure 1.



3
2.5
2
1.5
1
0.5
0
Discharge (m3/s)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Day number

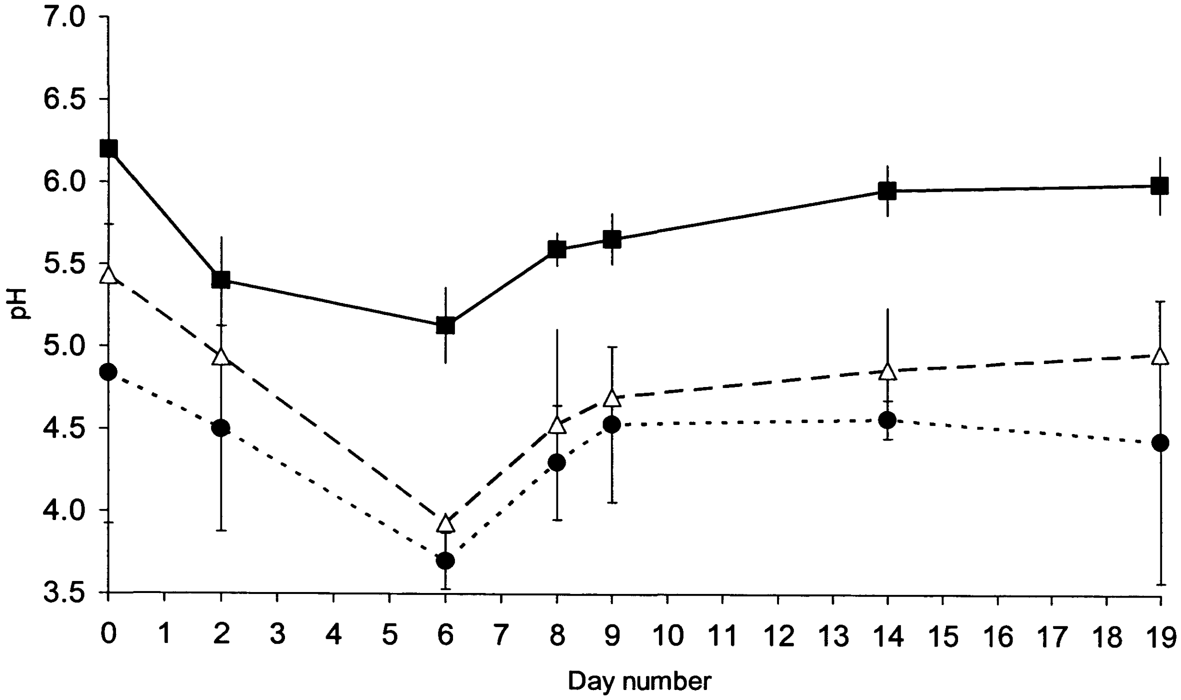
(a)



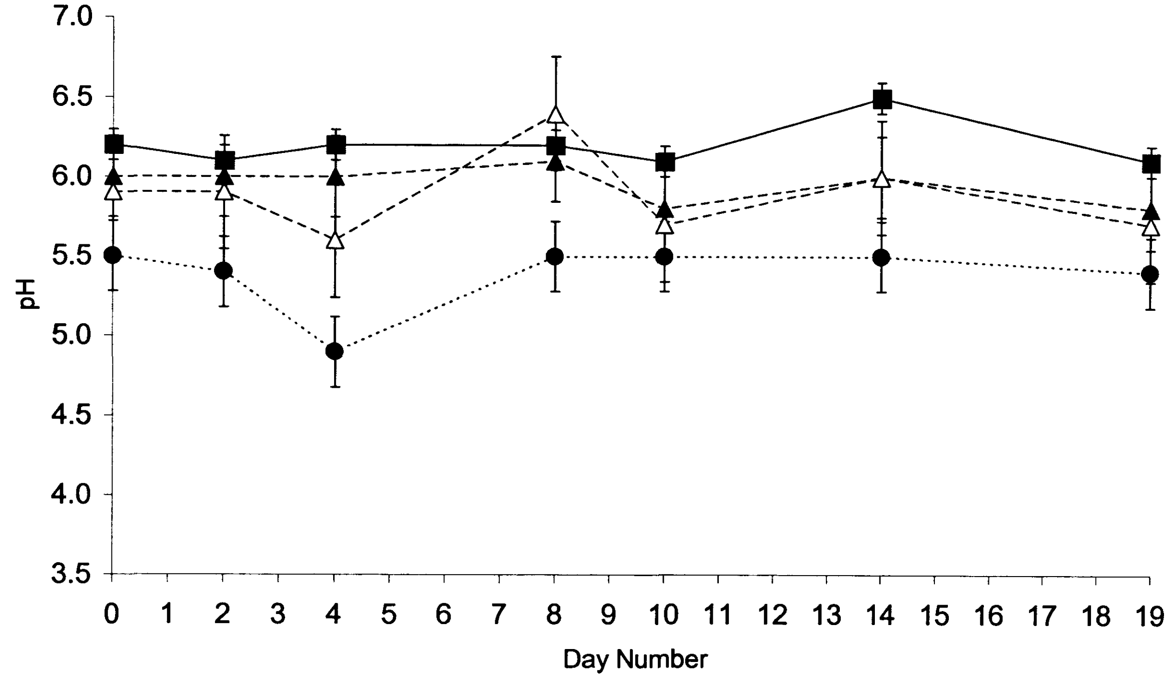
3
2.5
2
1.5
1
0.5
0
Dischage (m3/s)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Day number

(b)

Figure 2.


7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
pH
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Day number

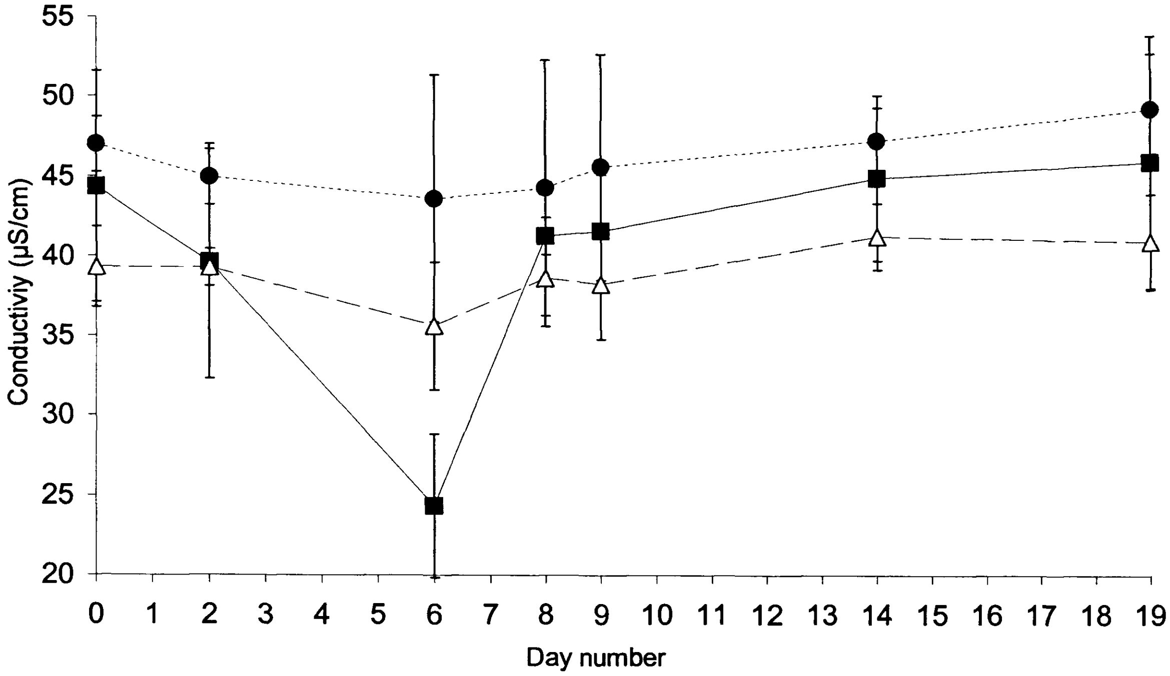
(a)


7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
pH
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Day Number

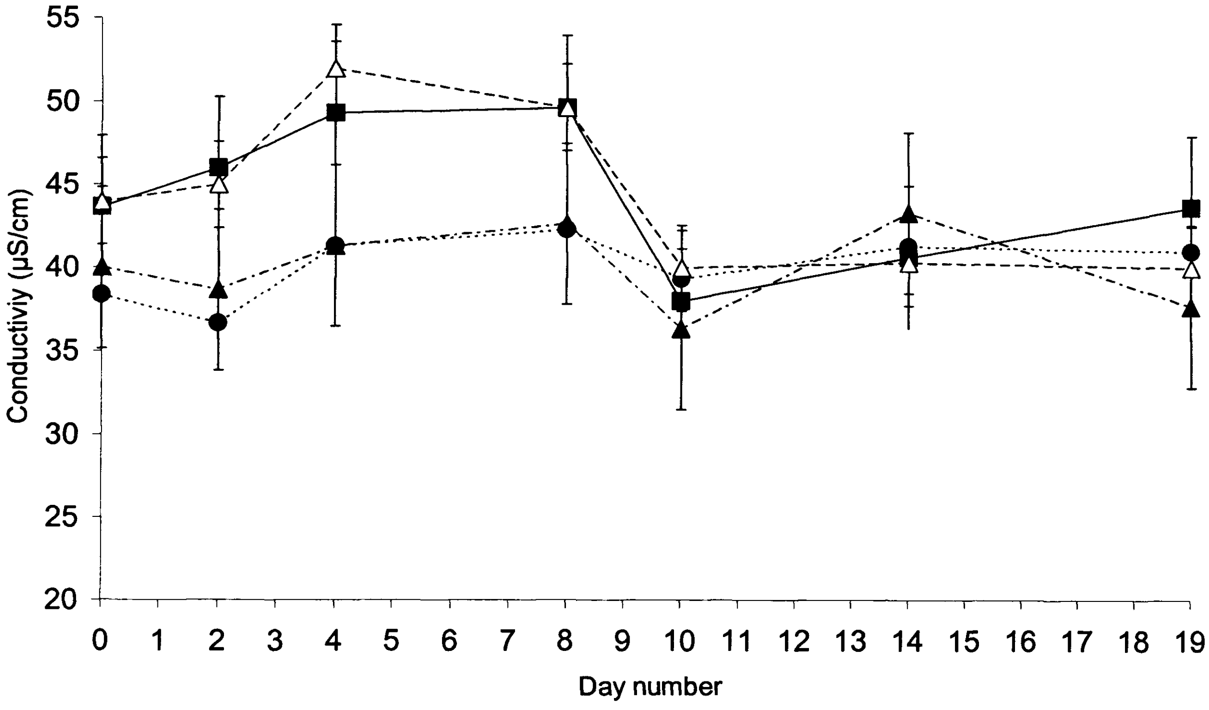
(b)

Figure 3.

Figure 3. cont.

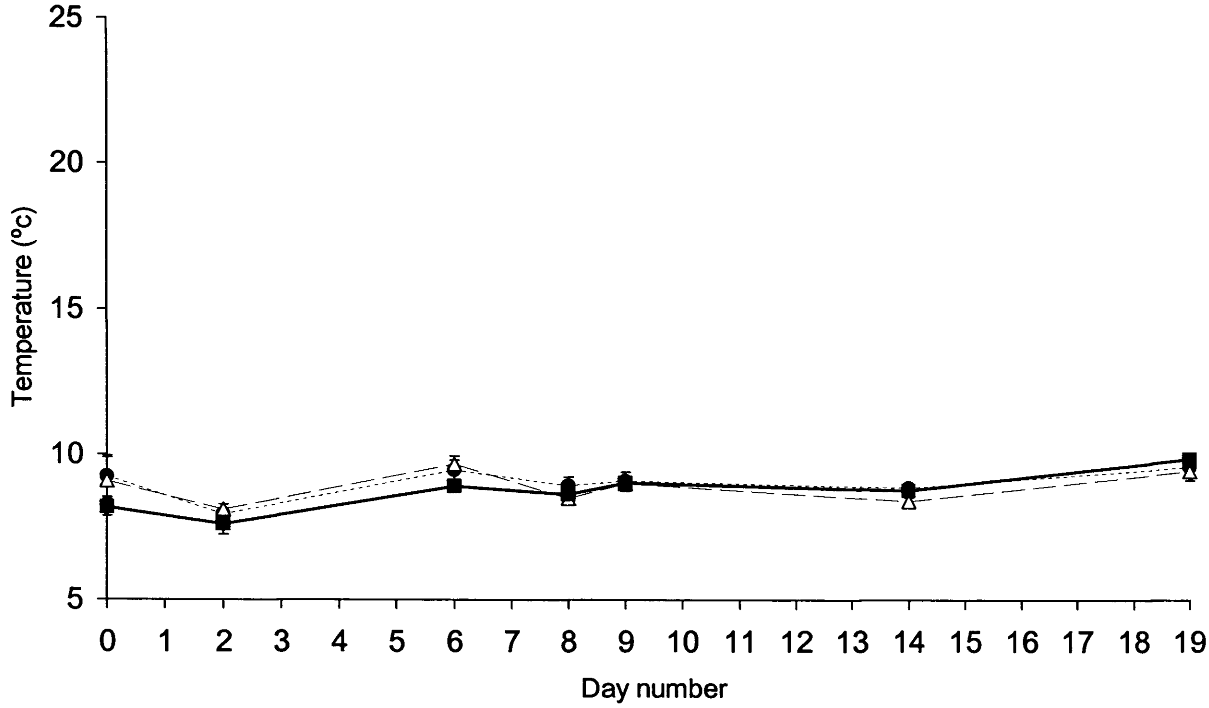


(c)



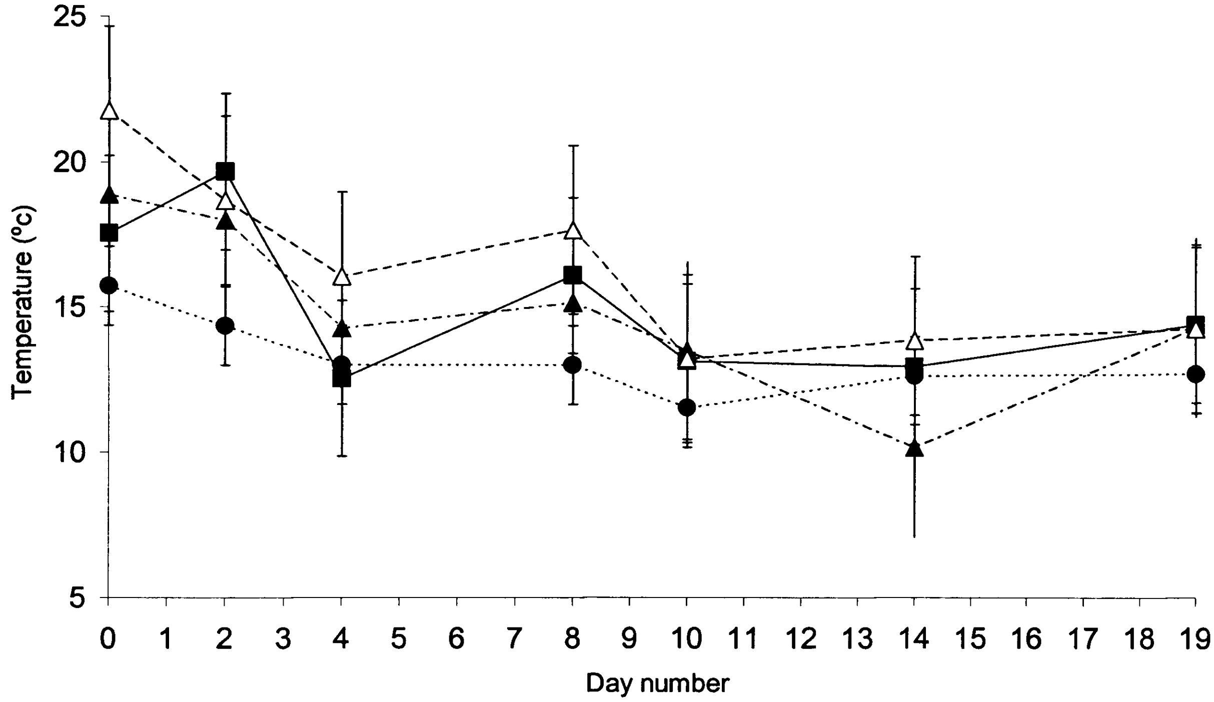
(d)

Figure 3. cont.



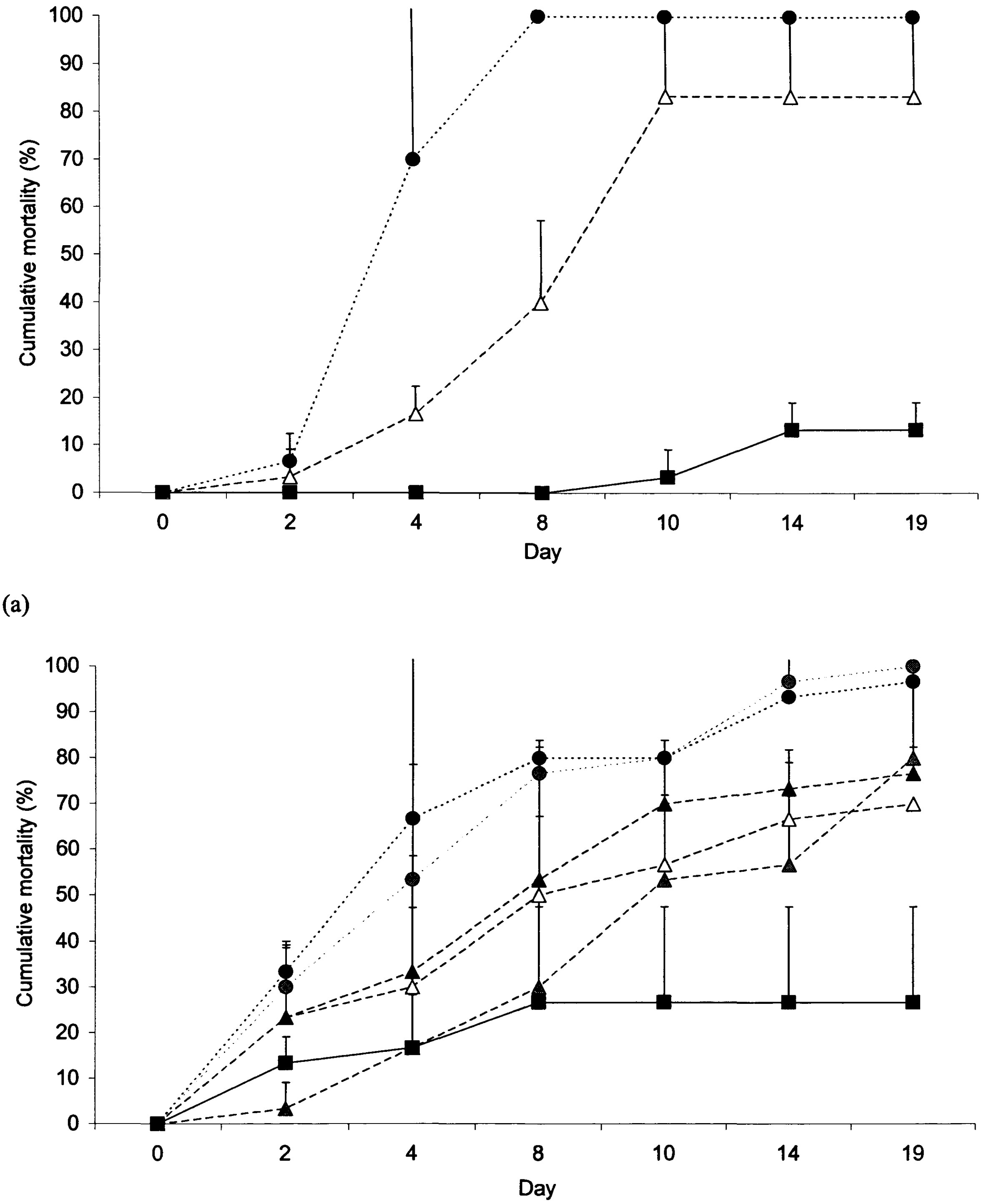
25
20
15
10
5
Temperature (ºc)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Day number

(e)



25
20
15
10
5
Temperature (ºc)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Day number

(f)



Cumulative mortality (%)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
8
10
14
19
Day

(a)

Cumulative mortality (%)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
8
10
14
19
Day

(b)

Figure 4.

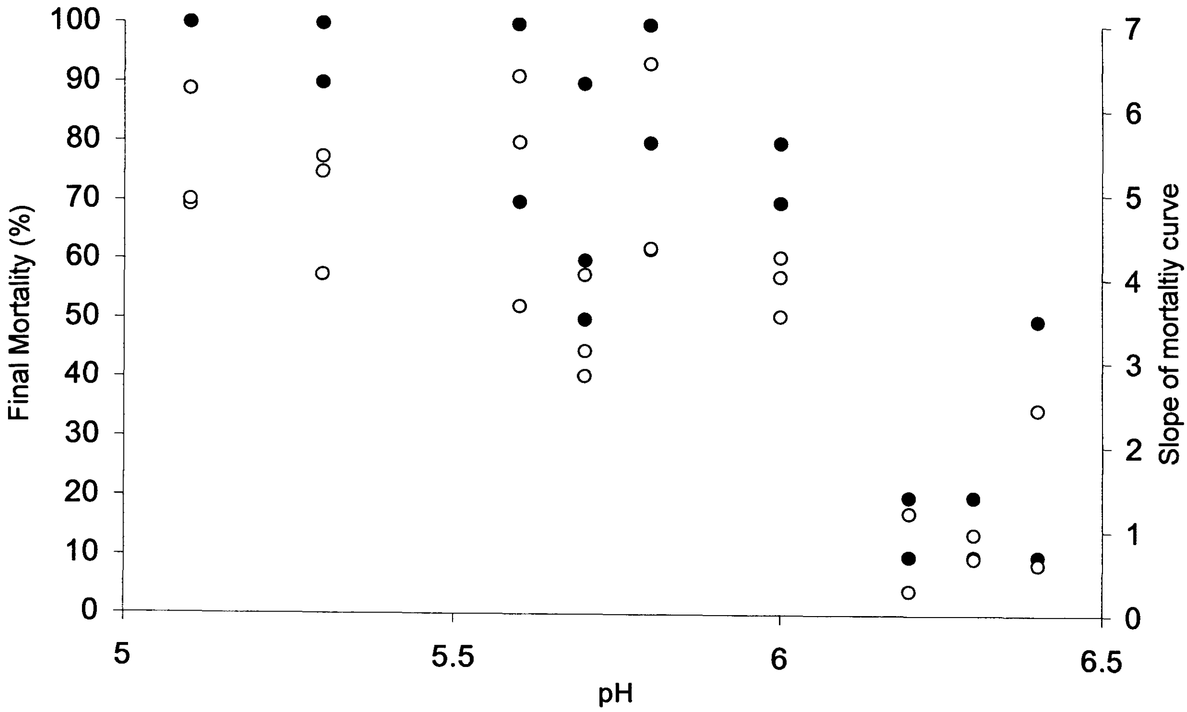

100
90
80
70
60
50
40
30
20
10
0
Final Mortality (%)
7
6
5
4
3
2
1
0
Slope of mortaltiy curve
5
5.5
6
6.5
pH

Figure 5.

Chapter 7

General Discussion

7.1 Introduction

During recent decades, the effects of acidification have been well recognised and extensively described, particularly with regard to the impacts on aquatic biota (Dahl, 1927; Rosseland *et al.,* 1986; Sutcliffe and Hildrew, 1989; Ormerod and Wade, 1990; Jüttner *et al.,* 1997; Orendt, 1998). Due to the transboundary nature of acidification, there have been a number of national and inter-national programmes aimed at reducing emissions of oxides of sulphur and nitrogen (e.g. National Expert Group on Transboundary Air Pollution; ECE International Cooperative Programme on Assessment and Monitoring of Acidification; Canada-U.S. Air Quality Agreement). These efforts have led to reduced deposition of anthropogenically generated acidifying compounds across much of Europe and North America (Pawlowski, 1997; Stoddard *et al.*, 1999; Fowler *et al.*, 2001; Monteith and Evans, 2005; Skjelkvåle *et al.*, 2005). Reduction of emissions into the atmosphere is slow, and chemical and biological responses of aquatic systems have been less than expected (Fjellheim and Raddum, 1992; Weatherley and Ormerod, 1992; Fowler *et al.*, 2005).

This thesis focused on the river Wye in mid-Wales. This has been considered, historically, one of the most productive salmonid fishing rivers in Europe and has high socio-economic importance. Much of the Wye is also significant in terms of biodiversity, providing such important habitats for salmonids, native crayfish and other aquatic species that it is recognised as a candidate Special Area of Conservation under the EU Habitat and Species Directive (Crisp *et al.*, 1996; Smith *et al.*, 1996). In this study, macroinvertebrates and phytobenthos (diatoms) were evaluated as bio-indicators of water quality in the upper reaches of the Wye. Both varied highly significantly with pH, alkalinity and calcium concentrations, and indicated that within the upper reaches of the Wye, significant water quality problems still exist. Water chemistry data, collected prior to treatment, confirmed the poor acid-base status at sites indicated by diatoms and macroinvertebrates to be acidified, and supported the prediction that acidification continues to affect upland Welsh streams, with many stream sites having mean and minimum pH below 5.7. In combination, this information supports other recent evidence of continued chronic and episodic acidification of upland areas of Wales (Kowalik *et al.*, in press).

With environmental monitoring programmes for the European Water Framework Directive (WFD; Directive 2000/60/EC) to be established by the end of 2006, bio-indicators, and integrated approaches to catchment monitoring and restoration are particularly relevant. The ability of diatoms and macroinvertebrates to indicate acid-base status and to act as mutual bio-indicators for each other, confirm the value of each group in environmental monitoring. With WFD monitoring likely to focus on invertebrates as the groups perceived by the Environment Agency to be most sensitive to acidification, this evidence of parallel response across indicators is important. Within the Wye catchment, the data from both groups show that acidification is generated from headwaters at the lower size range of sites likely to be monitored for the WFD (i.e. with catchment area $<10km^2$). Targeted water quality surveys like this one, or the Welsh Acid Waters Survey, therefore will be key options for detecting the true extent of acidification and recovery.

Pre-treatment water chemistry data revealed that the chemical character of the study sites was linked to physical attributes of the catchment. Sites with high NO_3^- were associated with conifer plantations, whilst sites with high conductivity were associated improved/semi-improved grassland and broadleaf/mixed woodland. This latter result is encouraging for those wishing to use land-use practices to address water quality problems and shows how some land treatments – when sufficiently widespread – can increase stream pH. By contrast, afforestation with conifer trees can intensify the problem of acidification through elevated loading of acidic anions, changes in soil drainage efficiency and increased surface area available for scavenging of atmospheric pollutants (Ormerod and Edwards, 1985; Neal *et al.,* 1986; Ormerod *et al.*, 1989; Donald and Gee, 1992; Goulding and Blake, 1998; Ormerod *et al.*, 2004).

Short-falls in recovery from acidification have led to the development, testing and implementation of remedial techniques, including liming, to aid and accelerate recovery. Examination of the relevant literature reveals mixed results from liming experiments. Experimental testing of streambed liming and stream dosing has resulted in significant chemical changes in many cases (Gunn and Keller, 1984; Lacroix, 1992; Menendez *et al.*, 1996; Simmons and Cieslewicz, 1996). However toxic precipitates (e.g. aluminium, iron) sometimes occur downstream following streambed treatment (Gunn and Keller, 1984; Keener and Sharpe, 2005) and direct-dosing does not allow treatment of small upstream tributaries which can contribute substantially to the acid load of the main river. Liming of headwaters and other hydrologically active wetland areas therefore compares favourably against other methods of lime application (Jenkins *et al.*, 1991; Weatherley *et al.*, 1995) despite financial cost and logistical challenge. Not only might increased pH and Ca persist over years and even decades in some cases (Rundle *et* al., 1995; Bradley and Ormerod, 2002), but this technique avoids the costs of repeated applications at least in the short term. However, this method does not target specifically high-flow periods when acidity is likely to be most pronounced (Davies *et al.*, 1992; Soulsby *et al.*, 1995). Careful further considerations of where to lime, and in what quantities, are required to resolve this problem. Whilst some liming experiments have reported increases in the density or abundance of sensitive species following catchment liming, changes are sometimes patchy and partial for reasons that are still being evaluated (Rundle *et al.*, 1995; Soulsby *et al.*, 1997; Bradley and Ormerod, 2002). This study has provided an opportunity for catchment-scale responses to lime treatment to be assessed over multiple indicators, location, seasons and years. In addition, this work complements two long-term data sets acquired from the Llyn Brianne catchment and the UK Acid Waters Monitoring Network sites.

7.2 Liming in the Wye catchment

As part of a large-scale restoration programme, limestone was applied experimentally to upper parts of the catchment of the Wye, in an attempt to alter the water chemistry and improve spawning habitats for salmonids. This study assessed the pre-and post-treatment situation at treated, control and reference sites in the Wye catchment using water chemistry, invertebrate and diatom data. Many experiments to investigate the impacts of treatments have been based on a Before-After-Control-Impact (BACI) design (Stewart-Oaten *et al.*, 1986; Basset *et al.*, 2001; Bradley and Ormerod, 2002), used here to compare temporal trends in limed streams with unmanipulated controls. Control locations vary over time and between sites, and it is therefore important to include more than one control and impact site. The experimental design implemented here involved replicate treatment, control and reference study sites (totalling 42) and multiple sampling periods, allowing a multiple BACI study using repeated measures

ANOVA (Underwood, 1993; Paine, 1996; Niemi *et al*., 1999). One possible drawback in this approach is that trends at individual sites were not considered separately relative to control locations, and this may be required as the experiment proceeds into the future: a range of site-specific factors might affect the outcomes of liming (e.g. application rates, locations, catchment size etc), so that understanding individual site effects could be valuable.

Following treatment, there was no evidence to support the prediction that liming induced changes in mean pH and alkalinity, or the concentration of calcium and aluminium. In other catchments, liming has been successful as a palliative technique in raising pH and Ca concentrations, and reducing the mobilisation of toxic metals (Howells *et al*., 1992; Bradley and Ormerod, 2002). A relatively common limitation however, is that high flow pH is not sufficiently changed following treatment, with difference between treated and control sections reduced at large discharge (Diamond *et al*., 1992; Lacroix, 1992; Miller *et al*., 1995). In the first year after treatment of the Wye, discharge was higher during sampling than in the pre-treatment period and pH was correspondingly lower in all stream types. During base flows, bedrock geology is the dominant influence on chemistry, with water increasingly derived from upper soil horizons as discharge increases (Hornung, *et al*., 1995), with high discharge usually associated with lower pH and ANC, and lower concentration of base cations (Davies *et al*., 1992; Diamond *et al*., 1992; Soulsby, 1995). This was confirmed by the strong negative correlation between pH and discharge.

One possibility from the chemical results is that the lack of large liming effects were an artefact of the small number of pre-treatment data points (n = 3) coupled with lower discharge when these data were collected. In these circumstances, the integrating (i.e. bio-indicating) effect of biota could be important in revealing whether significant liming effects occurred. In this study, the effects of liming on aquatic biota were assessed through changes in macroinvertebrate assemblages and from *in situ* bioassays with sensitive species. In all years, assemblages were dominated by Trichoptera (caddisfly), Plectoptera (stonefly) and Ephemeroptera (mayfly) larvae. Sites with high pH had abundant acid-sensitive mayflies (e.g. *Heptagenia lateralis*, *B. rhodani* and *R. semicolorata*) and caddis-larvae (e.g. *Diplectrona felix*), whilst sites with lower pH were dominated by acid-tolerant stoneflies (e.g. *Amphinemura sulcicollis* and *Nemurella pictetii*), typical of other UK sites (Rutt *et al.*, 1990; Kowalik and Ormerod, submitted). These patterns reflect direct physiological effects, alterations in food resources, predator-prey interactions, competition, growth, respiration, and loss through drift and avoidance behaviour (Sutcliffe and Carrick 1973; Fiance, 1978; Hall *et al.*, 1980; Herrmann and Andersson 1986; Ormerod *et al.*, 1987; Sutcliffe and Hildrew, 1989). In the Wye, acid-sensitive species were absent from all sites with pH below 5.8, which has been suggested as a biologically important threshold, with abundance and richness often significantly lower below this point and many macroinvertebrate species disappearing completely (Sutcliffe and Carrick, 1973; Weatherley and Ormerod, 1991; Feldman and Connor, 1992). Consequently, this level has sometimes been set as the target 'minimum pH' for liming programmes (Fjellheim and Raddum, 2001). One hypothesis of this study was that liming would lead to changes in biota, including increases in abundance and species richness of macroinvertebrates, and the occurrence of previously excluded acid-sensitive species. Following liming, there was no significant overall change in the abundance or richness of macroinvertebrate assemblages at treated sites on the

Wye, by comparison with acid reference streams. The occurrence of some acid-sensitive species at limed sites where they had previously been absent (e.g. *Baetis rhodani*) are so far too sporadic to discount chance effects, and more years' data are required to assess longer-term changes. Other studies have recorded increases in abundance of acid-sensitive species but these changes are often partial or patchy (Rundle *et al.*, 1995; Soulsby *et al.*, 1997; Bradley and Ormerod, 2002; Tipping *et al.*, 2002).

To investigate the possible factors limiting recovery at stream sites on the Wye, survival experiments were conducted over a range of flows using an acid-sensitive species, *Baetis rhodani*. The direct toxicological effects of acidity have been demonstrated previously for many groups, including macroinvertebrates (Bell, 1971; Simonin *et al.*, 1993; Claveri *et al.*, 1995; Masters, 2002; Lepori and Ormerod, 2005; Hirst *et al.*, 2004; Kowalik and Ormerod, 2006). During the survival experiments in winter and summer, the mortality of *B. rhodani* was greater in acid and limed streams than circumneutral reference streams. Mortality at limed and acid streams was particularly rapid during winter high flows. Although pH was higher in limed streams than acid controls during the summer experiment, survival in both stream types was still low, indicating that even at low flow the conditions are not yet suitable for survival. The mortality of animals which were periodically removed from acid and limed streams during periods of higher flow in the summer experiment were predicted to be lower than those animals which remained at these sites throughout the experiment, but were not found to be significantly different. It therefore appears that conditions in limed streams remain insufficient to support *Baetis* survival, even following transient exposure. Other studies have also shown that transient exposure to low pH can increase *Baetis* mortality compared to circumneutral controls (Kowalik and Ormerod, 2006).

It has been suggested that liming is not likely to produce the identical chemical or invertebrate characteristics that prevailed prior to acidification. Hasselrot and Hultberg (1984) suggested that a neutralised stream is not a restored ecosystem, but instead describe a 'new phase in the environment, where colonisation can occur, but is limited by the stress of acid episodes and changing chemical conditions'. Any palliative treatment should thus be applied with consideration of the pre-treatment conditions and awareness of any potential negative effects on non-target organisms and other local conservation issues (Howells *et al.*, 1992; Weiher and Boylen 1994; Buckton and Ormerod, 1997). The impact of liming on non-target ecosystems may be as strong as the effect of acidification, an issue that has implications for the suitability of liming as a conservation method. Liming may pose a particular risk when application involves terrestrial ecosystems (Ormerod and Rundle, 1998), for example, liming of soils can alter existing microbiological conditions, elemental turnover, levels of inorganic carbon and ammonia concentration (Grahn *et al.*, 1974; Traaen, 1980, cited in Weatherly, 1988; Raulund-Rasmussen, 1989; Brandrud, 2002). In the Wye, these negative effects were avoided by appropriate consultation with the Countryside Council for Wales, and similar protocols should be followed in similar future studies: catchment liming should be undertaken with awareness and consideration of possible effects on non-target species and effects on soil systems.

7.3 Conclusions

This research has described the chemistry and benthic biota of the upper Wye, and appraised the response to palliative liming. Two years following treatment, recovery has been limited and it appears that liming has not yet been unable to induce chemical conditions necessary to support colonisation by acid-sensitive macroinvertebrate species or assemblages similar to those in naturally circumneutral reference sites. Liming may have produced conditions allowing *B. rhodani* to occur sporadically at treated sites, but these animals cannot yet survive for prolonged periods in these streams. In other studies, even in cases where chemical recovery has been achieved through palliative treatment, biological change has often only been modest and patchy (Weatherley and Ormerod, 1992; Rundle *et al.*, 1995; Bradley and Ormerod 2002). Acid episodes can be an important factor limiting recovery, in particular at recently investigated sites in the Llyn Brianne catchment (Bradley and Ormerod, 2002; Kowalik and Ormerod, 2006) and modelling of the effects of base-flow and storm-flow pH on invertebrates has led to the predication that such episodes could delay recovery by decades (Kowalik, 2005). Several other explanations for limited biological recovery have been proposed including limited dispersal of acid-sensitive taxa, lack of food sources or habitat types, masking of changes by natural background variation and competitive exclusion of acid-sensitive species by acid tolerant species which have filled the grazing niche occupied by the former (Weatherley and Ormerod, 1990; Rundle *et al.*, 1995; Bradley and Ormerod, 2001; Ledger and Hildrew, 2005). In this study however, the lack of biological recovery is most likely linked with the lack of significant changes in water chemistry. Possible reasons for the latter include insufficient dosage and rate of lime application, the contribution of untreated acid tributaries to the acid load of the main river, and local factors, for example afforestation, which has been linked with acid chemistry and depauperate macroinvertebrate assemblages (Tierney *et al.*, 1998).

Across the UK, aquatic acid-base status is assessed annually by the UK Acid Water Monitoring Network (UKAWMN), and the experimental design and methods employed here will complement the UKAWMN data set and allow for comparisons to be made. This data set also complements records from over 20 years of investigation at the Llyn Brianne catchment. For the Wye, additional and more intensive liming of the previously treated sites, and inclusion of further acidic headwaters is advocated for significant changes in chemistry to be achieved. It has been suggested that testing the impact of acidification or de-acidification over only a few years provides insufficient evidence of the effects of acid deposition over a number of decades (Ormerod and Rundle, 1998). Therefore monitoring of the next stage of lime treatment during several post-treatment years will be essential for thorough evaluation of liming in this catchment.

7.4 References

Basset, Y., Charles, E., Hammond, D.S. and Brown, V.K. 2001. Short-term effects of canopy openness on insect herbivores in a rain forest in Guyana. *Journal of Applied Ecology*, **38**, 1045-1058.

Bradley, D.C. and Ormerod, S.J. 2001. Community persistence among stream invertebrates tracks the North Atlantic Oscillation. *Journal of Animal Ecology*, **70**, 987-996.

Bradley, D.C. and Ormerod, S.J. 2002. Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater Biology*, **47**, 161-171.

Brandrud, T.E. 2002. Effects of liming on aquatic macrophytes, with emphasis on Scandinavia. *Aquatic Botany*, **73**, 395-404.

Buckton, S. T. and S. J. Ormerod. 1997. Effects of liming on the Coleoptera, Hemiptera, Araneae and Opiliones of catchment wetlands in Wales. *Biological Conservation*, **79,** 43-57.

Claveri, B., Guerold, F. and Pihan, J.C. 1995. Use of transplanted mosses and autochthonous liverworts to monitor trace metals in acidic and non-acidic headwater streams (Vosges mountains, France). *Science of the Total Environment*, **175**, 235-244.

Clayton, J.L., Dannaway, E.S., Menendez, R., Rauch, H.W., Renton, J.J., Sherlock, S.M. and Zurbuch, P.E. 1998. Application of limestone to restore fish communities in acidified Streams. *North American Journal of Fisheries Management*, **18**, 347-360.

Council of the European Communities. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* **L327**: 1-72.

Crisp, D.T. and Beaumont, W.R.C. 1996. The trout (*Salmo truffa* L) populations of the headwaters of the rivers Severn and Wye, mid-Wales, UK. *Science of the Total Environment*, **177**, 113-123.

Dahl, K. 1927. The effects of acid water on trout fry. *Salmon and Trout Magazine,* **46**, 35-43.

Davies, T.D., Tranter, M., Wigington Jr., P.J. and Eshleman, K.N. 1992. 'Acidic episodes' in surface waters in Europe. *Journal of Hydrology*, **132,** 25-69.

Diamond, M., Hirst, D., Winder, L., Crawshaw, D.H. and Prigg, R.F. 1992. The effect of liming agricultural land on the chemistry and biology of the river Esk, north-west England. *Environmental pollution,* **78**, 179-185.

Donald, A. P. and Gee, A. S. 1992. Acid waters in upland Wales: causes, effects and remedies. *Environmental Pollution*, **78,** 141-148.

Downes, B. J., Barmuta, L. A., Fairweather, P.G., Faith, D.P., Keough, M.J., Lake, P.S., Mapstone, B.D. and Quinn, G.P. 2002. *Monitoring Ecological Impacts.* Cambridge University Press, Cambridge.

Fjellheim, A. and Raddum, G.G. 1992. Recovery of acid-sensitive species of Ephemeroptera, Plecoptera and Trichoptera in River Audna after liming. *Environmental Pollution*, **78**, 173-178.

Fjellheim, A. and Raddum, G.G. 2001. Acidification and liming of River Vikedal, western Norway. A 20 year study of responses in the benthic invertebrate fauna. *Water, Air, and Soil Pollution*, **130**, 1379-1384.

Fowler, D., Coyle, M., ApSimon, H.M., Ashmore, M.R., Bareham, S.A., Battarbee, R.W., Derwent, R.G., Erisman, J.-W., Goodwin, J., Grennfelt, P., Hornung, M., Irwin, J., Jenkins, A., Metcalfe, S.E., Ormerod, S.J., Reynolds, B., and Woodin, S. 2001. Transboundary Air Pollution: acidification, eutrophication and ground-level ozone in the UK, Report. No. EPG 1/3/153. DEFRA, Edinburgh.

Feldman, R.S. and Connor, E.F. 1992. The relationship between pH and community structure of invertebrates in streams of the Shenandoah-national-park, Virginia, USA. *Freshwater Biology*, **27**, 261-276.

Fowler, D., Smith, R.I., Muller, J.B.A., Hayman, G. and Vincent, K.J. 2005. Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, **137**, 15-25.

Goulding, K.W.T and Blake, L. 1998. Land use, liming and the mobilization of potentially toxic metals. *Agriculture, Ecosystems and Environment,* **67,** 135-144.

Grahn, O., Hultberg, H. and Landner, L. 1974. Oligotrophication – a self accelerating process in lakes subjected to excessive supply of acid substances. *Ambio*, **3**, 93-94.

Gunn, J. M. and Keller, W. 1984. In situ manipulation of water chemistry using crushed limestone and observed effects on fish. *Fisheries*, **9,** 19-24.

Hasselrot, B. and Hultberg, H. 1984. Liming of Swedish lakes and streams and its consequences for aquatic ecosystems. *Fisheries*, **9,** 4-9.

Hirst, H., Chaud, F., Delabie, C., Jüttner, I., and Ormerod, S.J. 2004. Assessing the short-term response of stream diatoms to acidity using inter-basin transplantations and chemical diffusing substrates. *Freshwater Biology*, **49**, 1072-1088.

Hornung, M., Brown, S.J. and Ranson, A. 1990. Amelioration of surface water acidity by catchment management. In *Acid Waters in Wales* (Eds R. W. Edwards, A. S. Gee. and J. H. Stoner). Kluwer Academic Publishers, Dordrecht, pp311-33-.

Hornung, M., Bull, K.R., Cresser, M., Ullyett, J., Hall, J.R. Langan, S., Loveland, P.J. and Wilson, M.J. 1995. The sensitivity of surface waters of Great Britain to acidification predicted from catchment characteristics. E*nvironmental Pollution*, **87**, 207-214.

Howells, G., Dalziel, T.R.K., and Turnpenny, A.W.H. 1992. Loch Fleet: liming to restore a brown trout fishery. *Environmental Pollution*, **78**, 131-139.

Jenkins, A., Waters, D. and Donald, A. 1991. An assessment of terrestrial liming strategies in upland Wales. *Journal of Hydrology*, **124**, 243-261.

Jüttner, I., Lintelmann, J., Michalke, B., Winkler, R., Steinberg, C.E.W., Kettrup, A. 1997. The acidification of the Herrenwieser See, Black Forest, Germany, before and during industrialisation. *Water Research*, **31,** 1194-1206.

Keener, A.L. and Sharpe, W.E. 2005. The effects of doubling limestone sand applications in two acidic Southwestern Pennsylvania streams. *Restoration ecology*, **13**, 108-119. .

Kowalik, R. A. 2005. The role of acid episodes in offsetting biological recovery of acidified streams. Unpublished. PhD Thesis, Cardiff University, Cardiff.

Kowalik, R.A. and Ormerod, S.J. 2006. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology*, **51**, 180-191.

Kowalik, R.A., Cooper, D.M. and Ormerod, S.J. Acid episodes retard the biological recovery of upland British streams from acidification. *Global Change Biology*. In press.

Kowalik, R.A. and Ormerod, S.J. Stream invertebrate communities and continued episodic acidification in regions of contrasting acid deposition. Manuscript submitted.

Kullberg, A., Bishop, K.H., Hargeby, A., Jansson, M. and Petersen, R.C. 1993. The ecological significance of dissolved organic-carbon in acidified waters. *Ambio*, **22**, 331-337.

Lacroix, G. L. 1992. Mitigation of low stream pH and its effects on salmonids. *Environmental Pollution*, **78,** 157-164.

Lepori, F. and Ormerod, S.J. 2005. Effects of spring acid episodes on macroinvertebrates revealed by population data and in situ toxicity tests. *Freshwater Biology*, **50**, 1568-1577.

Masters, Z. 2002. *Factors affecting stream macroinvertebrate recovery from acidification.* Unpublished. PhD Thesis, Cardiff University, Cardiff.

McCartney, A.G., Harriman, R., Watt, A.W., Moore, D.W., Taylor, E.M., Collen, P. and Keay, E.J. 2003. Long-term trends in pH, aluminium and dissolved organic carbon in Scottish fresh waters; implications for brown trout (*Salmo trutta*) survival. *Science of the Total Environment*, **31,** 133-141.

Menendez, R., Clayton, J. L. and Zurbuch, P. E. 1996. Chemical and fishery responses to mitigative liming of an acidic stream, Dogway Fork, West Virginia. *Restoration Ecology*, **4,** 220-233.

Merrix, F.L., Lewis B.R. and Ormerod, S.J. Experimental liming offsets the impact of low pH on Beech litter decomposition in acid-sensitive streams. Submitted.

Miller, J.D. 1995. The consequences of liming a highly acidified catchment in central Scotland. *Water, Air, and Soil Pollution*, **85,** 1015-1020.

Monteith, D.T. and Evans, C.D. 2005. The United Kingdom Acid Waters Monitoring Network: a review of the first 15 years and introduction to the special issue. *Environmental Pollution,* **137**, 3-13.

Neal, C., Whitehead, P.G., Neal, R., Crosby, B.J. 1986. Modelling the effects of acid deposition and conifer afforestation on stream acidity in the British Uplands. *Journal of Hydrology*, **86**, 15-26.

Neal, C., Robson, A.J., Neal, M. and Reynolds, B. 2005. Dissolved organic carbon for upland acidic and acid sensitive catchments in mid-Wales. *Journal of Hydrology*, **304**, 203-220.

Niemi, G.J., Hershey, A.R., Shannon, L., Hanowski, J.M., Lima, A., Axler, R.P. and Regal, R.R. 1999. Ecological effects of mosquito control on zooplankton, insects and birds. *Environmental Toxicology and Chemistry*, **18**, 549-559.

Orendt, C. 1998. Macroinvertebrates and diatoms as indicators of acidification in forest spring brooks in a region of eastern Germany (Leipzig-Halle-Bitterfeld) highly impacted by industrial activities. *Archiv für Hydrobiologie*, **143**, 435-467.

Ormerod, S.J. and Edwards, R.W. 1985. Stream acidity in some areas of Wales in relation to historical trends in afforestation and the usage of agricultural limestone. *Journal of Environmental Management*, **20**, 189-197.

Ormerod, S.J., Donald, A.P. and Brown, S.J. 1989. The influence of plantation forestry on the pH and Aluminium concentration of upland welsh streams - a re-examination. *Environmental Pollution,* **62**, 47-62.

Ormerod, S. J. and Wade, K. R. 1990. The role of acidity in the ecology of Welsh lakes and streams. In *Acid Waters in Wales* (Eds R. W. Edwards, J. H. Stoner and A. S. Gee). Kluwer Academic Publishers, Dordrecht, pp. 93-120.

Ormerod, S.J., Weatherley, N.S., Merrett, W.J., Gee, A.S. and Whitehead, P.G. 1990. Restoring acidified streams in upland Wales - a modelling comparison of the chemical and biological effects of liming and reduced sulphate deposition. *Environmental Pollution*, **64**, 67-85.

Ormerod, S.J. and Rundle, S.D. 1998. Effects of experimental acidification and liming on terrestrial invertebrates: implications for calcium availability to vertebrates. *Environmental Pollution*, **103**, 183-191.

Ormerod, S.J., Jones, M.E., Jones, M.C., Phillips, D.R. 2004. The effects of riparian forestry on invertebrate drift and brown trout in upland streams of contrasting acidity. *Hydrology and Earth System Science*, **8**, 578-588.

Paine, M.D. 1996. Repeated measures designs. *Environmental Toxicology and Chemistry*, **15**, 1439-1441.

Pawlowski, L. 1997. Acidification: its impact on the environment and mitigation strategies. *Ecological Engineering*, **8**, 271-288.

Raulund-Rasmussen, K. 1989. Effect of artificial acid rain and liming on the base status in an acid forest (*Picea abies* (L.), Karst.) soil (Typic Haplohumod). *Scandinavian Journal of Forest Research*, **4**, 417-425.

Resh, V.H. and Rosenberg, D.M. 1984. *The Ecology of Aquatic Insects*. Praeger, New York.

Rosseland, B.O., Skogheim, O.K., Abrahamsen, H. and Matzow, D. 1986. Limestone slurry reduces physiological stress and increases survival of Atlantic salmon (*Salmo *salar*) in an acidic Norwegian river. *Canadian Journal of Fisheries and Aquatic Sciences*, **43,** 1888-1893.

Rundle, S. D., Weatherley, N. S. and Ormerod, S.J. 1995. The effects of catchment liming on the chemistry and ecology of upland Welsh streams: testing model predictions. *Freshwater Biology,* **34**, 165-175.

Rutt GP, Weatherley NS, Ormerod SJ. 1990. Relationships between the physiochemistry and macroinvertebrates of British upland streams: the development of modelling and indicator systems for predicting fauna and detecting acidity. *Freshwater Biology* **24**: 463-480

Sciera, K.L., Isely, J.J., Tomasso, J.R., Klaine, S.J. 2004. Influence of multiple water-quality characteristics on copper toxicity to fathead minnows (*Pimephales promelas*). *Environmental Toxicology and Chemistry*, **23**, 2900-2905.

Simmons, K.R. and Cieslewicz, P.G. Limestone treatment of Whetstone Brook, Massachusetts. 1. Treatment methodology and water-chemistry changes during treatment. 1996. *Restoration Ecology*, **4**, 264-272.

Simonin, H.A., Kretser, W.A., Bath, D.W., Olson, M. and Gallagher, J. 1993. In situ bioassays of brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) in Adirondack streams affected by episodic acidification. *Canadian Journal of Fisheries and Aquatic Sciences,* **50**, 902-912.

Skjelkvåle, B.L., Stoddard, J.L., Jeffries, D.S., Tørseth, K., Høgåsen, T., Bowman, J., Mannop, J., Monteith, D.T., Mosello, R., Rogara, M., Ryzchon, D., Vesely, J., Wieting, J., Wilander, A. and Worsztynowicz, A. 2005. Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, **137**, 165-176.

Smith, G. R. T., Learner, M. A., Slater, F. M. and Foster, J. 1996. Habitat features important for the conservation of the native crayfish *Austropotamobius pallipes* in Britain. *Biological Conservation*, **75**, 239-246.

Soulsby, C. 1995. Contrasts in storm event hydrochemistry in an acidic afforested catchment in upland Wales. *Journal of Hydrology*, **170**, 159-179.

Soulsby, C., Turnbull, D., Hirst, D., Langan, S.J. and Owen, R. 1997. Reversibility of stream acidification in the Cairngorm region of Scotland. *Journal of Hydrology*, **195**, 291-311.

Stewart–Oaten, A., Murdoch, W.W. and Parker, K.R. 1986. Environmental impact assessment: "pseudoreplication" in time? *Ecology*, **73**, 1396-1404.

Stoddard, J.L., Jeffries, D. S., Lükewille, A., Clair, T. A., Dillon, P. J., Driscoll, C. T., Forsius, M., Johannessen, M., Kahl, J. S., Kellogg, J. H., Kemp, A., Mannio, J., Monteith, D. T., Murdoch, P. S., Patrick, S., Rebsdorf, A., Skjelkvåle, B. L., Stainton, M. P., Traaen, T., van Dam, H., Webster, K. E., Wieting, J.

and Wilander, A. 1999. Regional trends in aquatic recovery from acidification in North America and Europe. *Nature,* **401**, 575-578.

Sutcliffe, D. W. and Hildrew, A. G. 1989. Invertebrate communities in acid streams. In *Acid Toxicity and Aquatic Animals* (Eds R. Morris, E. W. Taylor, D.J.A. Brown and J.A. Brown). Cambridge University Press, Cambridge.

Swedish Ministry of Agriculture, Environment '82 Committee. 1982. *Acidification Today and Tomorrow*. Riebergs Tryckeri AB, Uddevalla, Sweden.

Tierney, D., Kelly-Quinn, M. and Bracken, J.J. 1998. The faunal communities of upland streams in the eastern region of Ireland with reference to afforestation impacts. *Hydrobiologia*, **389**, 115-130.

Tipping, E., Bass, J.A.B., Hardie, D., Haworth, E.Y., Hurley, M.A., and Wills, G. 2002. Biological responses to the reversal of acidification in surface waters of the English Lake District. *Environmental Pollution*, **116**, 137-146.

Traaen, T.S. 1980. Effect of acidity on the decomposition of organic matter in aquatic environments. In *Ecological Impact of Acid Precipitation* (Eds D. Drablos and T. Tollan). SNSF Project, Oslo.

Underwood, A.J. 1993. The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. *Australian Journal of Ecology*, **18**, 99-116.

Waters, C.J., Back, R.C., Halvorsen, S., Hudson, R.J.M., Morrison, K.A. and Wente, S.P. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Science of the Total Environment*, **219**, 183-208.

Weatherley, N.S. 1988. Liming to mitigate acidification in freshwater ecosystems: a review of the biological consequences. *Water, Air and Soil Pollution*, **39**, 421-437.

Weatherley, N.S. and Ormerod, S.J. 1992. The biological response of acidic streams to catchment liming compared to the changes predicted from stream chemistry. *Journal of Environmental Management*, **34**, 105-115.

Weiher, E. and Boylen, C.W. 1994. Patterns and prediction of alpha-diversity and beta-diversity of aquatic plants in Adirondack (New-York) lakes. *Canadian Journal of Botany*, **72**, 1797-1804.

Appendix 1

Principal Components Analysis loading values for (a) land-use, (b) bank character and (c) channel character variables, for data collected from a River Habitat Survey of the river Wye.

Land-use	PC1	PC2
Artificial open water	-0.0608	0.2255
Broadleaf/mixed woodland	-0.5435	-0.2337
Coniferous woodland	-0.1393	0.9494
Improved/semi-improved grassland/pasture	-0.7374	-0.4902
Moorland/Heath	0.1208	0.0986
Natural open water	-0.0528	0.1694
Rock, scree or sand dunes	0.0523	-0.1578
Rough/unimproved grassland/pasture	0.8864	-0.2709
Scrub and shrubs	-0.3044	-0.105
Tall herb/rank vegetation	-0.2081	0.1491
Wetland	0.7054	-0.1229

(a)

Bank character	PC1	PC2
Bank Height	-0.2024	-0.0468
Bank Width	0.0379	-0.1554
Bedrock as bank material	0.001	-0.5162
Boulders as bank material	0.1354	-0.1523
Cobbles as bank material	0.031	-0.4953
Composite bank	0.0158	0.3403
Earth as bank material	-0.3336	0.922
Eroding cliffs	0.1769	0.0872
Exposed boulders	-0.3129	-0.0189
Exposed bedrock	0.0819	-0.3967
Fallen Trees	-0.4001	-0.0478
Gentle bank	0.2841	0.4859
Overhanging boughs	-0.8224	-0.2362
Reinforced bank	-0.1541	0.0347
Roots	-0.5559	0.0881
Shaded	-0.7824	-0.0697
Stable cliffs	0.1234	-0.3996
Steep bank	-0.191	-0.3321
Trees	-0.9608	-0.228
Un-vegetated point bars	0.051	0.0133
Un-vegetated side bars	-0.0703	-0.2997
Vegetated bedrock/boulders	-0.119	-0.1028
Vegetated mid-channel bars	0.0694	-0.1044
Vegetated point bars	-0.1462	0.4113
Vegetated sides bars	-0.0405	0.1388
Woody Debris	-0.582	-0.0622

(b)

Channel character	PC1	PC2
Bedrock substrate	0.9128	-0.2813
Boulder substrate	0.2306	-0.1547
Broken waves	0.1504	-0.667
Chute flow	0.4685	-0.5072
Cobble substrate	-0.9815	-0.1605
Gravel substrate	0.3789	0.7179
Rippled flow	-0.1746	0.7363
Smoot flow	-0.1554	0.5849
Unbroken waves	-0.2032	-0.145
Water depth	-0.0642	0.2633
Water width	-0.3439	0.075

(c)

Appendix 2.

Species list of macroinvertebrates found during surveys of the upper-Wye and river Irfon

Taxa	Order
Brachyptera risi	Plecoptera
Brachyptera puata	Plecoptera
Rhabdiopteryx acuminata	Plecoptera
Capnia atra	Plecoptera
Capnia spp.	Plecoptera
Chloroperla torrentium	Plecoptera
Chloroperla tripunctata	Plecoptera
Diura bicaudata	Plecoptera
Isoperla grammatica	Plecoptera
Perlodes microcephala	Plecoptera
Leuctra fusca	Plecoptera
Leuctra hippopus	Plecoptera
Leuctra inermis	Plecoptera
Leuctra moselyi	Plecoptera
Leuctra spp.	Plecoptera
Leuctra nigra	Plecoptera
Nemoura cambrica	Plecoptera
Nemoura cinerea	Plecoptera
Nemoura spp.	Plecoptera
Nemurella pictetii	Plecoptera
Amphinemura sulcicollis	Plecoptera
Amphinemura standfussi	Plecoptera
Protonemura praecox	Plecoptera
Protonemura montana	Plecoptera
Protonemura meyeri	Plecoptera
Baetis muticus	Ephemeroptera
Baetis rhodani	Ephemeroptera
Baetis scambus	Ephemeroptera
Baetis vernus	Ephemeroptera
Heptagenia lateralis	Ephemeroptera
Heptagenia fuscogrisea	Ephemeroptera
Heptagenia spp.	Ephemeroptera
Rhithrogena semicolorata	Ephemeroptera
Rhithrogena germanica	Ephemeroptera
Halesus radiatus	Trichoptera
Limnephilus spp.	Trichoptera
Potamophylax rotundipennis	Trichoptera
Odontocerum albicorne	Trichoptera
Lepidostoma hirtum	Trichoptera
Hydroptila sp.	Trichoptera
Hydropsyche instabilis	Trichoptera
Diplectrona felix	Trichoptera
Plectrocnemia conspersa	Trichoptera
Plectrocnemia geniculata	Trichoptera
Polycentropus flavomaculatus	Trichoptera
Polycentropus kingi	Trichoptera

Rhyacophila dorsalis	Trichoptera
Rhyacophila obliterata	Trichoptera
Wormaldia sp.	Trichoptera
Holocentropus dubius	Trichoptera
Apatania spp.	Trichoptera
Chironomidae	Diptera
Simuliidae	Diptera
Tipulidae	Diptera
Colymbetinae	Coleoptera
Hydroporu spp.	Coleoptera
Hydroporinae	Coleoptera
Cercyon	Coleoptera
Esolus parallelepipedus	Coleoptera
Oulimnius tuberculatus	Coleoptera
Limnius volckmari	Coleoptera
Coleoptera larvae	Coleoptera
Gammarus pulex	Amphipoda
Corixidae	Hemiptera
Velia spp.	Hemiptera
Pisidium sp.	Bivalvia
Oligochaeta	Oligochaeta
Glossiphoniidae	Hirundinia
Erpobdellidae	Hirundinia
Ancylidae	Gastropoda
Pyralidae	Ptycoptera
Oligochaeta	Oligochaeta