Evidence of recovery from acidification in the macroinvertebrate assemblages of UK fresh waters: a 20-year time series.

John F. Murphy[a], Julie H. Winterbottom[a], Stuart Orton[a], Gavin L. Simpson[b], Ewan M. Shilland[b], Alan G. Hildrew[a]

[a] *School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK.*

[b] *ENSIS-ECRC, Department of Geography, University College London, Pearson Building, Gower Street, London WC1E 6BT, UK.*

ABSTRACT

This paper deals with the 20-year (1988-2008) record of macroinvertebrate sampling from the UK's Acid Waters Monitoring Network. At 12 of the 22 sites a significant temporal trend in the macroinvertebrate community is now evident. Indices of acidification suggest biological recovery at five of the 11 streams sites and at five of the lakes. All 10 sites indicating biological recovery from acidification also showed an increase in acid-neutralising capacity (ANC), although a further seven sites showed chemical (ANC) recovery but no evident biological recovery. On a site-by-site basis eight (four streams and four lakes) of the 20 sites investigated had significant relationships between biotic indices of acidification and chemical measures of acidity, the latter explaining between 30% and 72% of the biotic variation. Thus, the match between chemical and biological recovery is incomplete, with biological recovery lagging improvements in chemistry, modest community changes and most sites still showing signs of acid stress. The sluggish biological recovery may be ascribed to aspects of the chemical environment that are still deleterious and/or to ecological inertia in the reassembly of an acid-sensitive fauna.

Keywords

Acidification, macroinvertebrates, benthos, recovery, monitoring

1. Introduction

By the 1980s there was a broad international consensus that anthropogenic acidification was a considerable threat to freshwater ecosystems (Schindler, 1988). The acidification of streams and lakes resulted in shifts in phytoplankton assemblages, reduced diversity of benthic invertebrates, local extinction of commercially important fish species and reduced fitness of riverine birds (Lacoul et al., 2011; Ormerod and Durance, 2009). Since then, following implementation of more rigorous environmental regulations, there have been substantial reductions (38 -72%) in sulphur emissions in Europe and North America, and this has led in turn to declines in acid deposition (wet sulphate: 10 - 45%, Lynch et al., 2000; Fowler et al., 2005).

Biomonitoring schemes (including the UK's Acid Waters Monitoring Network [AWMN]; Monteith and Evans, 2005) have consequently been set up to detect chemical and biological responses to this reduced acid deposition. The AWMN sites are "sentinel systems", mostly located on resistant and/or base-poor geology of limited acid buffering capacity and are the subject of this Special Issue, although there are several other suites of sites in the UK, Europe and North America where intensive, long-term research into freshwater acidification has been carried out (Johnson, 1999; Hildrew, 2009; Ormerod and Durance, 2009; Civerlo et al., 2011). Together, these research and monitoring initiatives have generated considerable insight into the effects of acidification, and are beginning to reveal the extent of biological recovery of freshwater ecosystems damaged by acid stress (Ledger and Hildrew, 2005; Monteith et al., 2005). So far, evidence for a recovery in lake and stream biological communities has been equivocal (Monteith et al., 2005; Ormerod and Durance, 2009; Gray et al., 2012). The zooplankton in chemically recovering lakes in Ontario, Canada showed a gradual shift over time from an assemblage typical of acid lakes to one more characteristic of circumneutral lakes although, despite decades of emission reductions, recovery is still not complete (Gray et al., 2012). There was no consistent recovery evident in the phytoplankton of Swedish lakes over a 21-year monitoring period, although the assemblage of some acidified lakes did approach that of circumneutral lakes (Johnson and Angeler, 2010). Further, the density of juvenile brown trout densities had recovered at only two of the 13 UK stream and lakes sites with significant chemical recovery trends after 15 years of the AWMN (Montieth et al., 2005). Various reasons have been put forward to explain the patchy biotic recovery despite apparent widespread improving water quality trends (Yan et al., 2003; Monteith et al., 2005). Continued acid episodes (Kowalik et al, 2007), rising concentrations of natural organic acids (Evans et al., 2008) and nitrate deposition (Wright et al., 2001), as well as the over-arching issue of climate change (Johnson and Angeler, 2010), may act partially to negate chemical recovery. Further, biotic factors such as the persistence of acid-tolerant species dominating niche-space (Vinebrooke et al., 2003) or top-down predation pressure (Layer et al., 2011) may impede the re-establishment of acid-sensitive species. It would appear that the relative importance of the different abiotic and biotic mechanisms varies among biological groups (Gray et al., 2012).

Macroinvertebrates constitute one of the main groups of freshwater organisms used for the biological monitoring of streams and rivers worldwide (Bonada et al., 2006; Friberg et al., 2011). They are useful in detecting a variety of forms of pollution, including organic matter and eutrophication, but also acidity and acidification (Simpson et al., 2009; Murphy et al., unpublished). Macroinvertebrates have been collected regularly, systematically and semi-quantitatively at AWMN sites between 1988 and 2008 (sampling every spring at all streams and lakes, with consistent methods and processing). An analysis of the first 15 years of data showed encouraging, though patchy and modest, signs of recovery at several sites (Monteith et al., 2005). The present analysis adds five more years of data and allows an assessment of whether these changes are continuing. Our general objective is to provide the latest available view of the course of recovery from acidification for this important group of organisms (major drivers of patterns in biodiversity and ecological status) across the AWMN.

Specifically we asked:

I. Have there been any directional temporal changes in benthic invertebrates across the network?
II. Do such changes indicate recovery from acidification?
III. Are there any systematic differences between lake and streams in their response to changes in water chemistry?
IV. Has biological recovery in this group of organisms now matched the extent and pace of chemical recovery in the AWMN?

2 Methods

2.1. *Sampling and laboratory analysis*

Acid Waters Monitoring Network sites were chosen to include lake and stream sites in regions of the UK with base-poor geology and, hence, particular susceptibility to acidification from atmospheric deposition. The Network also includes lake and stream sites in areas receiving relatively little acid deposition, such as north-western Scotland (Patrick et al., 1995) (Fig. 1). Sampling began in spring 1988 and has continued at most sites up to 2008, with some sites having missing years due to access restrictions in 2001 during a foot-and-mouth disease outbreak, or to deletion of the site (Kernan et al., 2010). The 'control' (unpolluted) lake site, Loch Coire nan Arr, was affected by damming that increased the water level and hence was formally replaced in 2001 by Loch Fionnaraich. It is, as yet, too early to discuss trends in the macroinvertebrate data at Loch Fionnaraich, so we have presented results for Loch Coire nan Arr, where monitoring continued up to 2008 despite being outside the official Network.

In April-May of each year, five, one-minute kick (streams) or sweep (lakes) samples were taken at each site (beginning in the south of the UKJ) with a 330µm mesh net, with the objective of obtaining consistent replicate samples from the same habitat year after year. Thus, stony riffles were always sampled in streams and lake samples were taken from stony or sandy littoral habitat (0.3 - 0.5m depth) including sweeping through rooted macrophytes, where present . The samples were preserved in the field in 70% Industrial Methylated Spirit, until sorting and identification to the lowest possible taxonomic level (mostly to species), according to standard AWMN protocols (Patrick et al., 1991). Benthic sampling was undertaken in late spring in an attempt to assess the macroinvertebrate community immediately following the months in the annual hydrograph when it is most likely to have been exposed to sustained or brief periods of high stream discharge and associated acid episodes (Weatherley and Ormerod, 1987; Wade et al., 1989).

A suite of water chemistry parameters [including pH, alkalinity, calcium (Ca), labile aluminium (Al_{lab}), acid neutralising capacity (ANC) and dissolved organic carbon (DOC)], was measured at each site, monthly for streams and quarterly for lake outflows, starting in late 1988 for most sites except for Narrator Brook, Afon Gwy, Blue Lough and Coneyglen Burn, where sampling started in 1991. Further details of the field and laboratory methods are provided in Patrick et al. (1995) and Monteith and Evans (2005).

2.2. *Analysis*

Following an initial principal components analysis (PCA) to quantify variation in the biological data over the monitoring period, redundancy analysis (RDA), with sampling year as the sole explanatory variable, was used to test for temporal trends in the macroinvertebrate community at each site. The macroinvertebrate abundance data were first converted to percentage composition to focus the analysis on changes in the relative abundance of different taxa, and then square-root transformed to reduce the influence of dominant taxa on the analysis. All taxa recorded were included in the analysis, including those recorded only once in the time series, since such rare taxa could be important in identifying change. The presence of a trend was determined using a restricted permutation test, in which the ordering of samples (years) was maintained but the 'starting sample' was selected via random cyclic shifts of the time series. This is a conservative significance test, as the maximum number of permutations is equal to the number of samples within each times series (17 - 21), which is at the limit of detection of trends at the 95% level. The extent to which the change in community composition over time was directional, i.e. followed a consistent trajectory moving away from its initial state, was illustrated by plotting the RDA axis 1 sample scores against year of sampling. Directional change does not necessarily indicate biological recovery but it does imply a consistent shift in assemblage composition. A new diagnostic index has been developed (Acid Water Indicator Community, AWIC*sp*) for detecting recovery from acidification in streams, based on macroinvertebrates (Murphy et al., unpublished), and has been applied to AWMN data. It assigns a 'score' to 49 species and genera based on the relative position of their distribution within a training dataset aligned along an acid gradient (higher scores for progressively less acid-tolerant species). An AWIC*sp* score for a site is calculated by averaging the scores assigned to all taxa recorded at a site from a sample taken in spring. AWIC*sp* scores were calculated for each stream site in each year from the pooled list of taxa from the five replicate kick samples.

A further new diagnostic tool has been developed to assess acidification pressure on lake littoral macroinvertebrates (Acid Waters Invertebrate Status Tool, AWIST). AWIST measures deviation in the biological community (considered as the relative abundance of individual taxa, the number of taxa and the proportion of individuals in particular groups) from an expected 'reference state' as Ecological Quality Ratios (EQRs) (Simpson *et al*., 2009). The tool uses ensembles of classification trees (a 'Random Forest') to predict the probability of a site being in each of four a *priori* assigned quality classes; High, Good, Moderate and Poor-Bad, based on the macroinvertebrate data. The final EQR is then the weighted-average of these probabilities, with a lower EQR indicating greater acidification stress (Simpson *et al*., 2009). Mann-Kendall trend tests were run to assess whether there were significant trends in AWIC*sp* and AWIST scores over the monitoring period.

We used χ^2 contingency tests to assess the extent to which sites that exhibited clear chemical recovery trends (in terms of pH, ANC, DOC and Al_{lab},; as determined by Kernan et al., 2010) also showed evidence of biological improvement. Additive models, incorporating both seasonal and inter-annual components, were used to describe and assess the significance of the chemical trends over the same time periods as the biological monitoring at each site (Kernan et al., 2010). For each acidity related chemical variable, we counted the number of sites (out of 22) that exhibited a chemical recovery trend (as determined by the additive models) and the number with a biological recovery trend (as determined by AWIC*sp* and AWIST trends),. We then estimated the number of sites with a) both trends, b) a biological but not a chemical trend, c) a chemical but not a biological trend, and d) with neither trend. The χ^2 test assessed whether the distribution of sites across the four cells in the contingency table was different to that due to chance.

We also used stepwise multiple regression to assess whether variations in AWIC*sp* or AWIST at a given site could be accounted for by year and variation in four descriptors of acid stress, pH, ANC, DOC and Al_{lab} expressed as the average of values recorded in March-May for streams and March-June for lakes (the latter two variables were log-transformed). Year was entered into the stepwise selection process only after the four acid chemistry variables had been considered, so that we could primarily identify the aspects of acid chemistry best associated with the biological variation, rather than just the temporal trend. Alternative models were also explored without this restriction. There were insufficient chemical data to allow analysis of temporal relationships at Narrator Brook, Afon Gwy and Loch Fionnaraich.

Multivariate and trend analyses were carried using the Vegan (Oksanen et al., 2011) and Kendall (McLeod, 2011) packages within R (R Development Core Team, 2011). Minitab 16 was used to carry out χ^2 contingency tests and stepwise multiple regressions.

3. Results

Over the course of the 20 years of monitoring, 186 different taxa have been recorded across the 23 stream and lake sites, with between 25 (Bencrom River) and 91 (Loch Chon) taxa recorded at individual sites (see Table S1 in supplementary material). Half the recorded taxa were water beetles (Coleoptera) and caddis flies (Trichoptera), with water bugs (Hemiptera), stoneflies (Plecoptera) and mayflies (Ephemeroptera) also contributing to the total recorded taxa. Among the most frequently occurring taxa were Chironomidae (non-biting midges), Oligochaeta, Tipulidae (crane flies), Empididae (dagger flies), *Leuctra inermis* and *Nemoura* sp. (stoneflies), *Plectrocnemia* sp. and *Polycentropus* sp. (caddis flies) (Table S1). In general, the macroinvertebrate fauna ranged from one characteristic of oligotrophic soft waters to profoundly acidified. A significant proportion of the variation in the macroinvertebrate community data was explained by the temporal trend at 12 of the 22 sites, comprising five streams and seven lakes (Table 1). Less certain ($P \leq 0.1$) temporal trends were apparent at five further sites (Bencrom River, Dargall Lane, Llyn Llagi, Round Loch of Glenhead and Scoat Tarn). At six of the 12 sites (Allt a'Mharcaidh, Narrator Brook, Burnmoor Tarn, Loch Chon, River Etherow, Allt na Coire nan Con) the trend was broadly linear, indicating a consistent directional change in the assemblage (Fig. 2). A more sigmoidal relationship was evident at Coneyglen Burn, suggesting a period of directional change restricted to the mid-1990s, while an asymptotic relationship was apparent at a further four of the 12 sites with a significant temporal trend (Loch Grannoch, Loch Tinker, Llyn Cwm Mynach, Blue Lough), suggesting a period of directional change up to the late 1990s but not subsequently (Fig. 2). At the remaining site (Afon Hafron) there was little change in RDA axis 1 scores up to 2000 but marked directional shift in assemblage composition thereafter (Fig. 2).

AWIC*sp* index values increased through time (i.e. an indication of biological recovery) at five (Allt na Coire nan Con, Dargall Lane, R. Etherow, Narrator Brook, Afon Hafren) of the 11 stream sites (Fig. 3); at no site was there a decrease in AWIC*sp* values. A further two sites (Allt a'Mharcaidh and Beagh's Burn) had less certain increasing trends at $P = 0.06$. Of the five sites with detectable trends, River Etherow had the most pronounced increase in values from 3.4 in 1989 to 5.2 in 2006. In contrast, at Allt na Coire nan Con which also had a significant trend but is less impacted by acid deposition, values ranged from 5.2 in 1992 to 6.4 in 2005. The LOESS smoother lines suggest that at some sites there may be non-linear biological responses to changes in acid stress status. For instance, at Allt a'Mharcaidh, a series of pronounced peaks in Al_{lab} in 1992-1995 (cross reference to hydrochemical paper) may account for the evident dip in AWIC*sp* values during the same period (Fig. 3). Similarly at Beaghs Burn a distinct plateau in the increasing trend in AWICsp values after 2000 may be related to intermittent peaks in Al_{lab} during 2000-2008. Four of the five stream sites showing significant evidence of biological recovery (from AWIC*sp* results) also had a significant community change trend (from the RDA), as did the marginally significant Allt a'Mharcaidh but not Beagh's Burn. Thus, around half the network's stream sites show evidence of both temporal community change and biological recovery from acidification.

Temporal variation in AWIST scores for the lakes suggest that five of the 11 sites had significant increasing trends, indicating biological recovery from acidification (Round Loch of Glenhead, Loch Tinker, Llyn Llagi, Burmoor Tarn, Loch Chon), while Llyn Cwm Mynach had a significantly decreasing trend (Fig. 4). At this last site, water chemistry has not responded to the decline in acid deposition and pH continues to hover around 5.5, with Al_{lab} concentrations at around 55 μeql^{-1} and ANC around 10 μeql^{1}. Four of these six sites also had significant temporal trends in community change over the monitoring period (Table 1). Two sites had significant trends in the community over time but no apparent biological recovery from acidification (Loch Grannoch and Blue Lough) (Fig. 4). Trends at such sites must be associated with other environmental or biological drivers, or biological recovery is occurring but is not yet detectable with the tools available. At Scoat Tarn and Blue Lough, AWIST EQR values have remained <0.6 throughout the 20-year monitoring period, indicating continued moderate acidification stress at these sites. In contrast, at Burnmoor Tarn and Loch Tinker, AWIST EQR values suggest that there has been a substantial biological recovery, with both lakes now classified as of good ecological status (> 0.6), whereas previously they were of moderate ecological status with respect to acidification. In Burnmoor Tarn the improving trend is such that the AWIST EQRs are now approaching the boundary between good and high status (0.8) (Fig. 4).

Most sites with a trend of increasing ANC over the 20-year period also had increasing diagnostic index values, implying a change in the biological community towards one dominated more by acid-sensitive taxa (Table 2). This may also be the case for sites with increasing pH, although in this case the association was somewhat less certain (P = 0.056). No other significant spatial associations were found between chemical and biological trends. However, this analysis needs to be interpreted with caution given that the χ^2 contingency tests where based on only 22 sites. On a site-by-site basis we found that eight (four streams and four lakes) of the 20 sites investigated had significant relationships between biotic indices and acidity, explaining between 30% and 72% of the biotic variation (Table 3). The explanatory variables most commonly included in the regression models were DOC (seven sites) and ANC (five). At six of these eight sites there was evidence of a significant increasing temporal trend in AWIST/AWIC*sp* values (Figs 3 and 4). Interestingly, at Burnmoor Tarn and the River Etherow, two sites with very distinct evidence of biological recovery, the variation in AWIST/AWIC*sp* values was unrelated to the preceding spring mean acid chemistry values, but was strongly related to Year (Table 3).

Overall, community change and diagnostic index results confirm that the extent of biological recovery from acidification is less widespread than that reported for chemistry (cross reference to hydrochemical paper). Faunal change at most sites remains fairly modest and biological recovery, while now clearly apparent at 10 of the 22 active network sites, is still in the early stages and does not seem to be consistently related to the improvements in acid chemistry.

4. Discussion

4.1 Overall patterns in the network

There has been directional change in assemblages at just over half of the network (12 of the 22 sites; five streams and seven lakes), and such changes have been shown to be consistent with biological recovery from acidification (as determined by diagnostic indices) at eight of these 12 sites [Specific questions i) and ii)]. There was some evidence that sites with significantly increasing ANC and pH also have showed recovery in their macroinvertebrate community. However, on a site-by-site basis most (12 of 20) showed no relationship between inter-annual variation in diagnostic indices and the mean acid chemistry of the preceding spring. We found no evidence for any systematic differences between lake and streams in their response to changes in water chemistry [Specific question iii)].

After the first 15 years of AWMN monitoring, significant shifts in macroinvertebrates assemblage structure related to year of sampling were also observed at 12 of the 22 sites, comprising seven lakes and five streams (Monteith et al., 2005). A similar proportion of sites were found to have significant time trends after 20 years, though only seven of the 12 sites with trends after 15 years retained their temporal trend in the current analysis. It is worth noting that the RDA Monte Carlo permutations used to test for trend significance were quite conservative, given that we had at most only 21 sampling occasions in time. At a more relaxed significance threshold ($P \leq 0.1$) 13 of the 22 sites had temporal trends in assemblage change after 15 years, while this figure rose to 15 after 20 years with 10 sites having trends over both time periods.

A new feature of the current analysis, not available in the 15-year assessment, is the application of diagnostic indices (AWIC*sp* and AWIST) to the dataset; both indices being designed to infer the acid status of a site on the basis of samples of benthic/littoral macroinvertebrates. These revealed that the significant temporal change at 12 sites could be ascribed to biological recovery from acidification at eight of them. Both indices have been developed based on comprehensive calibration datasets from which variations in the frequency of occurrence of taxa across the known gradient of acid conditions have been quantified (Simpson *et al*., 2009; Murphy *et al*., unpublished). As such they provide a robust indication of whether the assemblage is accruing a greater range of acid-sensitive taxa (e.g. *Baetis* and Heptageniidae mayflies) relative to more tolerant taxa (e.g. Nemouridae stoneflies). The index scores also suggest that the 22 biological communities were not all equally impacted by acidification at the beginning of the monitoring period. Assemblages at Allt na Coire nan Con and Loch Chon were not greatly impaired at the start of the study, as expected in the former, but they both did still show detectable increases in index values over the course of the 20 years. On the other hand, Dargall Lane and Llyn Llagi both supported communities indicating substantial acid stress in the late 1980s. By the end of the study both assemblages had improved significantly to a condition similar to that found at Allt na Coire nan Con and Loch Chon in 1988.

After 20 years of monitoring there was a significant association between sites showing biological recovery and increasing ANC. When variation in biotic indices was related to chemistry at each site individually, ANC and DOC were found to be selected consistently as explanatory variables. Previous studies have highlighted the usefulness of ANC as a composite descriptor of acidification status, as it is correlated well to acid deposition, pH, Al_{lab} and Ca (Wright and Cosby, 2004). Eight of the 22 sites had a significant relationship between acid chemistry and values of the appropriate biotic index, even though most network sites had clearly improving water chemistry (*cross refer to Hydrochemistry paper*). Clearly, at least based on macroinvertebrates, biological recovery has not matched the extent or pace of chemical recovery [Specific question iv)]. The seemingly modest association between biological and chemical recovery may well be explained by the fact that, for the site-by-site multiple regression analysis, we characterised the chemistry for each sampling year as the mean of values recorded in March-May for streams and March-June for lakes. This was based on the assumption that the spring sample of the biological community at each lake and stream would be most influenced by conditions in the immediately preceding months (Monteith et al., 2005). This will have inevitably led to differences in inter-annual patterns in the various acid determinands, with spring means being more variable.

4.2 Biological changes at the sites

There has been further accrual of evidence over the last five years that biological recovery (based on macroinvertebrates) of surface waters is occurring. Overall, there is substantial agreement between chemical and biological trends; sites showing chemical recovery also show biological recovery, though with a lag in the latter, while at some sites with chemical trends biological recovery is not yet significant. In this context note that the sequence of samples is still relatively short, in terms of time-series analysis, with which to try to detect trends. While such evidence is encouraging, and inspection of the data shows that (at sites with significant trends) species known to be acid-tolerant are often in decline while more acid-sensitive species are gradually appearing, recovery is still fragile and at an early stage. Certainly, no wholesale shift in the core community is evident at any AWMN site, though species replacements seem to be occurring at many.

At Allt na Coire nan Con (stream) increases in ANC and DOC (cross refer to hydrochemistry paper), and a decline in Al_{lab}, were accompanied by an increasing diversity of caddis flies (Trichoptera), even though there was no significant relationship with AWIC*sp*. At Dargall Lane (stream) declines in all four chemical measures of acid stress over the 20 years corresponded with colonisation by acid-sensitive species such as the stonefly *Brachyptera risi*, the caddis fly *Hydropsyche siltalai*, and an increase in overall taxon richness, particularly in the mid 1990s. The benthos of the River Etherow showed very significant recovery from acidification and there was strong evidence of improving chemical conditions (particularly in Al_{lab}), although the biological and chemical signals did not coincide. Both abundance and taxon richness in the River Etherow have increased dramatically over the 20-year monitoring period, with several acid-tolerant taxa having declined (the stoneflies *Leuctra hippopus* and *Nemoura*), while the acid-sensitive stonefly *Brachyptera risi* and the highly sensitive mayflies, *Baetis* and *Electrogena lateralis*, have increased. This site provides one of the clearest indications of biological recovery (from a rather impacted baseline) of any site in the network, although the core assemblage, consisting here of moderately acid-tolerant stoneflies (*Leuctra inermis*, *Amphinemura sulcicollis* and *Siphonoperla torrentium*) and midge larvae (Chironomidae) has persisted and actually increased in abundance. Narrator Brook has a relatively species-rich benthos and is one of the least acidic streams in the network. The core community has persisted and includes some markedly acid-sensitive species, including *Hydropsyche siltalai* (a net-spinning caddis fly) and the predatory stonefly *Isoperla grammatica,* plus a mixture of more tolerant taxa. There has been an overall increase in the number of species at this site [pH has risen above the biologically significant threshold of c 5.5 (Sutcliffe and Hildrew, 1989)], some (though not all) of which are acid-sensitive (*Chloroperla tripunctata, Ecdyonurus* sp). At Afon Hafren (stream) significant improvement in acid chemistry coincided with the appearance and persistence of more acid-sensitive species, such as the stonefly *Isoperla grammatica*, and riffle beetles (Elmidae), though mayflies are still notably rare, with only very sporadic occurrences of Leptophlebiidae in the latter part of the 20 year record.

At Loch Chon, the most species-rich of all the monitoring sites, the marked improvements in acid chemistry resulted in a moderate increase in AWIST EQRs and the establishment of acid-sensitive species in the lake, particularly in the latter half of the monitoring period (e.g. the snail *Radix balthica*, the mayfly *Caenis* and the caddis flies *Cyrnus*, *Athripsodes* and *Mystacides*) and a reduction in the abundance of acid-tolerant species (such as the stonefly *Leuctra nigra* and the water boatman *Sigara scotti*). With considerable species turnover over time at the lake, there has been no detectable change in the overall diversity of taxa or abundance of individuals, despite the improvements in chemistry. Loch Tinker had significant increases in DOC and ANC over the 20-year period, but only moderate increases in pH and no obvious trend in Al_{lab}. A number of acid-sensitive species have increased, including the caddis *Mystacides* and *Tinodes waeneri*, although several moderately acid-tolerant species persist (including the riffle beetle *Oulimnius tuberculatus*). Burnmoor Tarn is the lake with clearest evidence of recovery based on littoral macroinvertebrates.

Never strongly acidified, it has shown modest but significant increases in pH and ANC. Several acid-sensitive taxa showed clear increases near the end of the record, including mayflies of the genus *Caenis*, the caddis *Lepidostoma* and a few snails (*Radix balthica*), while the acid-tolerant beetle *Platambus maculatus* declined. Although there are clear indications of chemical and biological recovery at Llyn Llagi, the lake is still susceptible to episodes of high Al_{lab} concentration and dips in pH, most recently in 2005 and 2007 (cross refer to Hydrochemistry paper). While there have been improvements in acid chemistry at Round Loch of Glenhead, it still has a low ANC (< 20 μeql^{-1}), moderate Al_{lab} concentrations (ca. 25 μeql^{-1}) and pH values regularly below 5.5. As such there has been detectable but modest recovery in the macroinvertebrate community, primarily involving the establishment of small populations of the snail *Radix balthica* and *Pisidium* (pea mussel). Blue Lough is the most acidic of all the monitoring sites with current pH readings regularly < 5.0 (they were < 4.7 in the late 1980s). While there has been a relative chemical recovery in terms of ANC and Al_{lab} the lake still has very much the characteristics of an acid system, with a sparse macroinvertebrate community dominated by acid-tolerant leptophlebiid mayflies, water boatmen (*Callicorixa wollastoni*), polycentropodid caddis flies and midge larvae (Chironomidae).

At four sites (Coneyglen Burn, Loch Grannoch, Allt a Mharcaidh and Llyn Cwm Mynach) there was evidence of directional change in the assemblage not associated with a move towards a more acid-sensitive community. Such apparently anomolous results may be due to the fact the two stream sites were never greatly impacted by acidification; being included in the AWMN as control, low deposition sites. The changes seen in Coneyglen Burn were mainly concentrated between 1994 and 1999, with the 1994 sample being taken following a spate. It had a particularly depauperate fauna with only 42 individuals recorded across eight taxa. In subsequent years the community recovered and in the process gained many new taxa not recorded at the site pre-1994. At Loch Grannoch, acid stress continued to impact the lake right up to the late 1990s and only began to lessen thereafter (cross refer to Hydrochemistry paper). It is still a very acidic lake with pH readings >5 rare, although Al_{lab} and DOC concentrations and ANC indicate substantial chemical improvement since 2000 (cross refer to Hydrochemistry paper). The AWIST EQR temporal pattern did appear to track this non-linear trajectory; something not picked up by the linear trend test applied to the data. The stepwise regression analysis failed to capture this association, partly due to the fact that the chemistry data were presented as a spring average and also because there was an approximate two-year time lag between the beginning of the chemical recovery in 1997-1999 and the biological response in 2000-2001. Llyn Cwm Mynach, like Loch Grannoch, has an afforested catchment and was unusual in that the biological data indicated that the lake was becoming more stressed by acidification, particularly in the period before 2000. Through the 1990s pH did decline at this site and there has been no recovery evident in pH, Al_{lab}, ANC or DOC concentrations over the entire 20 years of monitoring (cross refer to Hydrochemistry paper).

4.3 Perspectives on biological recovery

There has been some debate about why biological recovery from acidification is modest (Yan et al., 2003; Monteith et al., 2005; Hildrew, 2009). These hypotheses essentially refer to: a) the extent and persistence of chemical recovery, b) the difficulty of acid-sensitive species dispersing to acid-sensitive sites, and c) whether there are ecological interactions that resist a straightforward recovery of the community following the same trajectory along which it declined. To this we should add that other environmental changes, particularly to the climate, may well be complicate or obscure any simple recovery from acidification (Rose et al., 2004). In Swedish lakes exhibiting chemical recovery from acidification the associated biological recovery was complicated by inter-annual variability in climate (Johnson and Angeler, 2010). Similarly, in Welsh upland streams affected by acid deposition, Ormerod and Durance (2009) found that wet winters could reverse the biological recovery trajectory by as much as 41% of the total 25-year decreasing trend in acidity.

The biological and chemical threshold of pH 5.5 seems to be important (Sutcliffe and Hildrew, 1989), and sites that have moved across this threshold show the clearest biological recovery. The episodic chemistry of streams also clearly plays a role in their slow biological recovery from acidification, and a good deal of evidence for this has been gathered (Kowalik and Ormerod, 2006; Kowalik et al., 2007). However, biological recovery in lakes is similar to that in streams, and lakes are far less chemically episodic.

The 'dispersal hypothesis' can largely now be rejected, at least for mobile species including most aquatic insects. There is now much direct and indirect evidence of long distance dispersal sufficient to recolonise recovering freshwaters (e.g. Briers et al., 2004, Masters et al., 2007; Hildrew, 2009), with the possible exception of very large areas containing uniformly acidified freshwater systems.

The evidence that ecological interactions limit the recovery of communities and ecosystem processes is circumstantial, and needs more research. A great strength of the AWMN is that it offers a framework of reliable and sustained environmental and ecological data providing a firm basis for further hypothesis-driven research, and such research now offers evidence for the third of our hypotheses to account for muted biological recovery; that there is biological 'resistance' to a simple reversal of acidification. Investigations of herbivore-algal food web linkages in AWMN streams found evidence that generalist herbivores and putative detritivores, tolerant to acid conditions, may inhibit the return of acid-sensitive specialist algal grazers (Ledger and Hildrew, 2005; Layer et al., in press). Additionally, Layer et al. (2010a) found that the littoral food web at the acidic Lochnagar was reticulate and based on external resources of detritus with a community consisting primarily of trophic generalists and omnivores. Together, these characteristics are likely to make the community dynamically stable and resistant to invasions of potential new colonist (and specialist) species. A recent synthesis of studies of zooplankton recovery in lakes from regions severely affected by acidification found that biological recovery was limited by slow chemical recovery, dispersal limitation, and community resistance, though the relative importance of the three factors varied among and within regions (Gray and Arnott, 2009). Gray et al., (2012) detected clear shifts in zooplankton communities towards that typical of circumneutral lakes, with predation pressure from fish and *Chaoborus* playing a central role in the determining the structure of the recovering Cladocera assemblage.

Further, the macroinvertebrate community at Broadstone Stream, close to Old Lodge and with a similar community, has seen a series of changes at the top of the food web since the 1970s, consisting of invasions or irruptions of progressively larger-bodied predators accompanying a decline in acidity (Hildrew, 2009). While there have been marked changes in relative density none of the previously common (acid tolerant) taxa have been lost and thus biological recovery has not seen a simple switch to an acid-sensitive community. This suggests that predation plays a key role in determining the trajectory of recovery, rather than the latter being proximately controlled by water chemistry, and that the top-down effects of the generalist predators spread diffusely through the reticulate food web. Layer et al. (2011) have recently carried out dynamical simulations of the Broadstone stream food web that indicate that it has become less robust over time as pH has risen and larger predators (and particularly fish) have become dominant. In terms of recovery, then, this implies that a period of 'ecological buffering' of community change has to be overcome, via a sustained improvement in environmental conditions.

Finally, Layer et al. (2010b) carried out dynamic modelling across multiple generations of 20 real stream food webs across a wide pH gradient, including AWMN sites, and found that fewer species were lost from the more acid food webs over the course of the modelling period than those at high pH. This supports the suggestion of a negative relationship between stability and increasing pH, and is in agreement with (and potentially accounts for) empirical observations of other forms of stability, including high persistence in acid stream communities and suggestions of inertia in their recovery as pH ameliorates (Townsend et al., 1987; Speirs et al., 2000; Hildrew et al., 2004; Ledger and Hildrew, 2005; Monteith *et al*., 2005). Such biological mechanisms would probably only delay recovery, however, and would not prevent it if the chemical conditions continue to ameliorate. The evidence from the macroinvertebrates, therefore, is encouraging even if recovery is far from complete.

References

Civerlo, K.L., Roy, K.M., Stoddard, J.L., Sistla, G. 2011. A comparison of the temporally integrated monitoring of ecosystems and Adirondack long-term monitoring programs in the Adirondack mountain region of New York. Water Air Soil Pollut., 222, 285-296.

Bonada, N., Prat, N., Resh, V.H., Statzner, B. 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annu. Rev. of Entomol., 51, 495-523.

Briers, R.A., Gee, J.H.R., Cariss, H.M., Geoghegan, R. 2004. Inter-population dispersal by adult stoneflies detected by stable isotope enrichment. Freshwater Biol., 47, 161-171.

Evans, C.D., Monteith,D.T., Reynolds, B., Clark, J.M. 2008. Buffering of recovery from acidification by organic acids. Sci. Total Environ., 404, 316-325.

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

Friberg, N. Bonada, N., Bradley, D.C., Dunbar, M.J., Edwards, F.K., Grey, J., Hayes, R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M., Woodward, G. 2011. Biomonitoring of human impacts in natural ecosystems: the good the bad and the ugly. Adv. .Ecol. Res., 44, 1-68.

Gray, D.K., Arnott, S.E. 2009. Recovery of acid damaged zooplankton communities: measurement, extent, and limiting factors. Environ. Rev., 17, 81-99.

Gray, D.K., Arnott, S.E., Shead, J.A., Derry, A.M. 2012. The recovery of acid-damaged zooplankton communities in Canadian lakes: the relative importance of abiotic, biotic and spatial variables. Freshwater Biol., 57, 741-758.

Hydrochemistry paper

Hildrew, A.G., Woodward, G., Winterbottom, J.H., Orton, S. 2004. Strong density dependence in a predatory insect: large-scale experiments in a stream. J. Anim. Ecol., 73, 448-458.

Hildrew, A.G. 2009. Sustained research on stream communities: a model system and the comparative approach. Adv. .Ecol. Res., 41, 175-312.

Johnson, R.K. 1999. Regional representativeness of Swedish reference lakes. Environ. Manage. 23, 115-124.

Johnson, R.K., Angeler, D.G. 2010. Tracing recovery under changing climate: response of phytoplankton and invertebrate assemblages to decreased acidification. J. N. Am. Benthol. Soc., 2010, 1472-1490.

Kernan M., Battarbee, R.W., Curtis, C.J., Monteith, D.T., Shilland, E.M. (Eds.). 2010. UK acid waters monitoring network 20-year interpretative report. Environmental Change Research Centre, UCL, London, UK. 465 pp.

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

Kowalik, R.A., Cooper, D.M., Evans, C.M., Ormerod, S.J. 2007. Acid episodes retard the biological recovery of upland British streams from acidification. Glob. Change Biol., 13, 2439-2452.

Lacoul, P., Freedman, B., Clair, T. 2011. Effects of acidification on aquatic biota in Atlantic Canada. Environ. Rev., 19, 429-460.

Layer, K., Hildrew, A.G., Monteith, D. ,Woodward, G. 2010a. Long-term variation in the littoral food web of an acidified mountain lake. Glob. Change Biol., 16, 3133-3143.

Layer, K., Riede, J., Hildrew, A.G., Woodward, G. 2010b. Food web structure and stability in 20 streams across a wide pH gradient. Adv. .Ecol. Res., 42, 265-299.

Layer, K., Hildrew, A.G., Jenkins, G.B., Riede, J.O., Rossiter, S.J., Townsend, C.R., Woodward, G. 2011. Long-term dynamics of a well-characterized food web: four decades of acidification and recovery in the Broadstone Stream model system. Adv. Ecol. Res., 44. 69-117.

Layer, K., Hildrew, A.G., Woodward, G. (in press) Grazing and detritivory in 20 stream food webs across a broad pH gradient. Oecologia.

Lynch, J.A., Bowersox, V.C., Grimm, J.W. 2000. Changes in sulfate deposition in eastern USA following implementation of Phase I of Title IV of the Clean Air Act Amendments of 1990. Atmos. Environ., 2000, 34, 1665-1680.

Ledger, M., Hildrew, A.G. 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a pH gradient in streams. Environ. Pollut., 137, 103-118.

Masters, Z., Petersen, I., Hildrew, A.G., Ormerod, S.J. 2007. Insect dispersal does not limit the biological recovery of streams from acidification. Aquat. Conserv., 17, 375-383.

McLeod, A.I. 2011. Kendall: Kendall rank correlation and Mann-Kendall trend test. R package version 2.2. http://CRAN.R-project.org/package=Kendall.

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. Environ. Pollut., 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., Winterbottom, J.H 2005. Biological responses to the chemical recovery of acidified fresh waters in the UK. Environ. Pollut., 137, 83-101.

Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H. 2011. vegan: Community Ecology Package. R package version 2.0-0. http://CRAN.R-project.org/package=vegan.

Ormerod, S.J., Durance, I. 2009. Restoration and recovery from acidification in upland Welsh streams over 25 years. J. Appl. Ecol., 46, 164-174.

Patrick, S., Waters, D., Juggins, S., Jenkins, A. (Eds.) 1991. The United Kingdom Acid Waters Monitoring Network: site descriptions and methodology report. Report to the Department of the Environment and Department of the Environment Northern Ireland , ENSIS Ltd., London. ISBN 1 871275 04 0.

Patrick, S., Monteith, D.T., Jenkins, A. (Eds.) 1995. UK Acid Waters Monitoring Network: the first five years. Analysis and interpretation of results, April 1988-March 1993. ENSIS Ltd, London, ISBN1 871275 25 3.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rose, N., Monteith, D.T., Kettle, H., Thompson, R., Yang, H. and Muir, D. 2004. A consideration of potential confounding factors limiting chemical and biological recovery at Lochnagar, a remote mountain loch in Scotland. J. Limnol., 63, 63–76.

Schindler, D.W. 1988. Effects of acid rain on freshwater ecosystems. Science, 239, 149-157.

Simpson, G.L., Turner, S., Brooks, S., Greenwood, M., Yang, H., Monteith, D.T., Patrick, S. 2009. Acid waters macroinvertebrate status tool. Report to Scottish Environment Protection Agency Project WFD60a . Environmental Change Research Centre, UCL, London, UK. 77 pp.

Speirs, D.C., Gurney, W.S.C., Hildrew, A.G., Winterbottom, J.H. 2000. Long-term demographic balance in the Broadstone stream insect community. J. Anim. Ecol., 69, 45-58.

Sutcliffe, D.W., Hildrew, A.G. 1989. Invertebrate communities in acid streams. In: Morris, R., Taylor, E.W., Brown, D.J.A., Brown, J.A. (Eds.) Acid toxicity and aquatic animals. Seminar Series of the Society for Experimental Biology, Cambridge University Press, Cambridge, pp 13-29.

Townsend, C.R., Hildrew, A.G., Schofield, K. 1987. Persistence of stream invertebrate communities in relation to environmental variability. J. Anim. Ecol., 56, 597-613.

Vinebrooke, R.D., Graham, M.D., Findlay, D.L., Turner, M.A. 2003. Resilience of epilithic algal assemblages in atmospherically and experimentally acidified boreal lakes. Ambio, 32, 521-544.

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

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

Wright, R.F., Alewell, C., Cullen, J.M., Evans, C.D., Marchetto, A., Moldan, F., Prechtel, A., Rogora., M. 2001. Trends in nitrogen deposition and leaching in acid-sensitive streams in Europe. Hydrology and Earth Systems Sciences, 5, 299–310

Wright, R.F., Cosby, B.J. 2004. Recovery of acidified mountain lakes in Norway as predicted by the MAGIC model. J. Limnol., 63, 101-110.

Yan N.D., Leung B., Keller W., Arnott S.E., Gunn J.M., Raddum G.G. 2003. Developing conceptual frameworks for the recovery of aquatic biota from acidification. Ambio, 32, 165-169.

Fig. 1. Location of the 11 stream (circles) and 12 lake (squares) AWMN sites across the UK. See Table 1 for key to abbreviated site names.

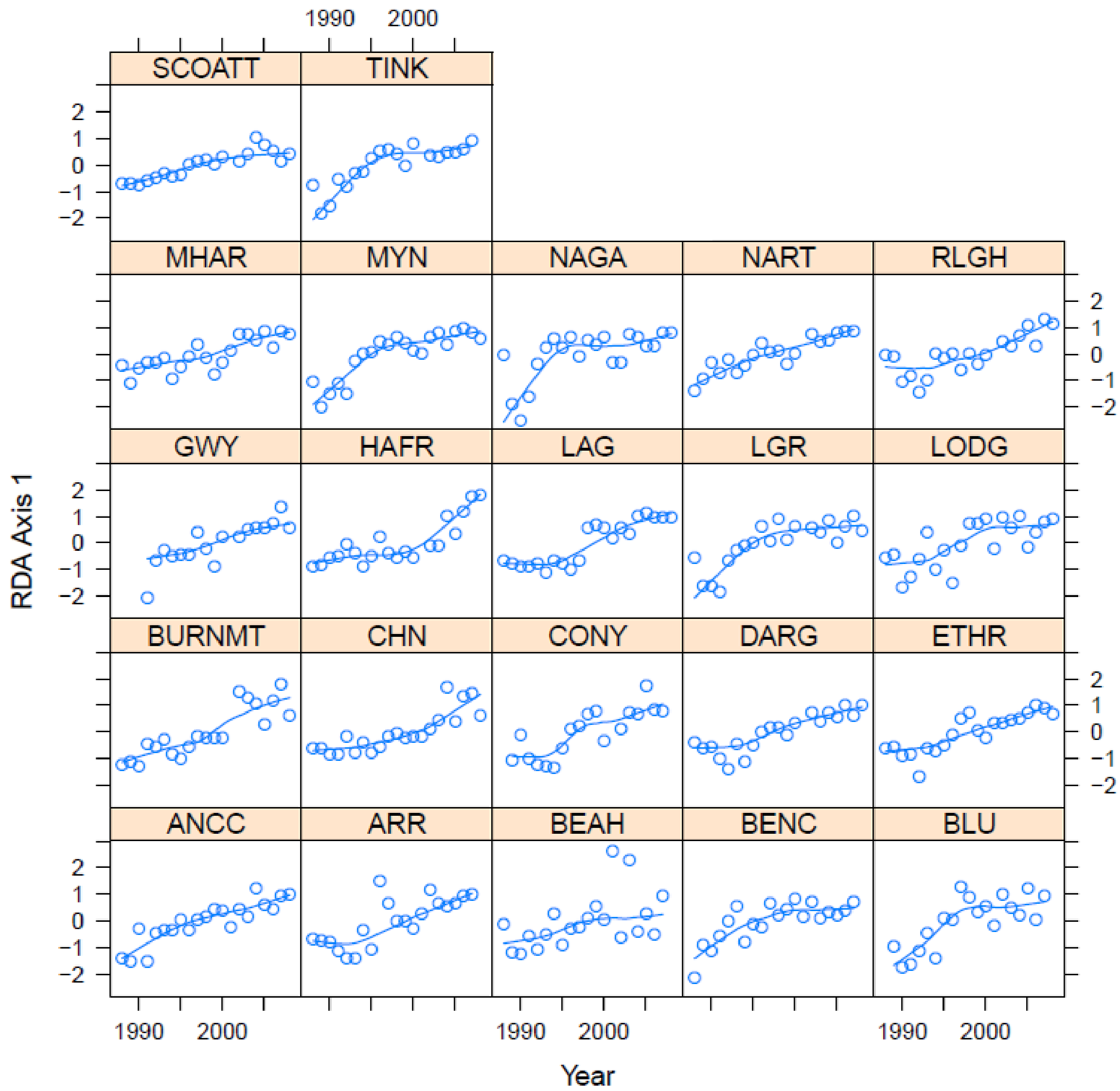

Fig. 2. Axis 1 scores from Redundancy Analysis fitted to macroinvertebrate data from each of the 22 AWMN sites, constrained by sampling year. The solid line in each plot is a LOESS smoother. See Table 1 for key to abbreviated site names.

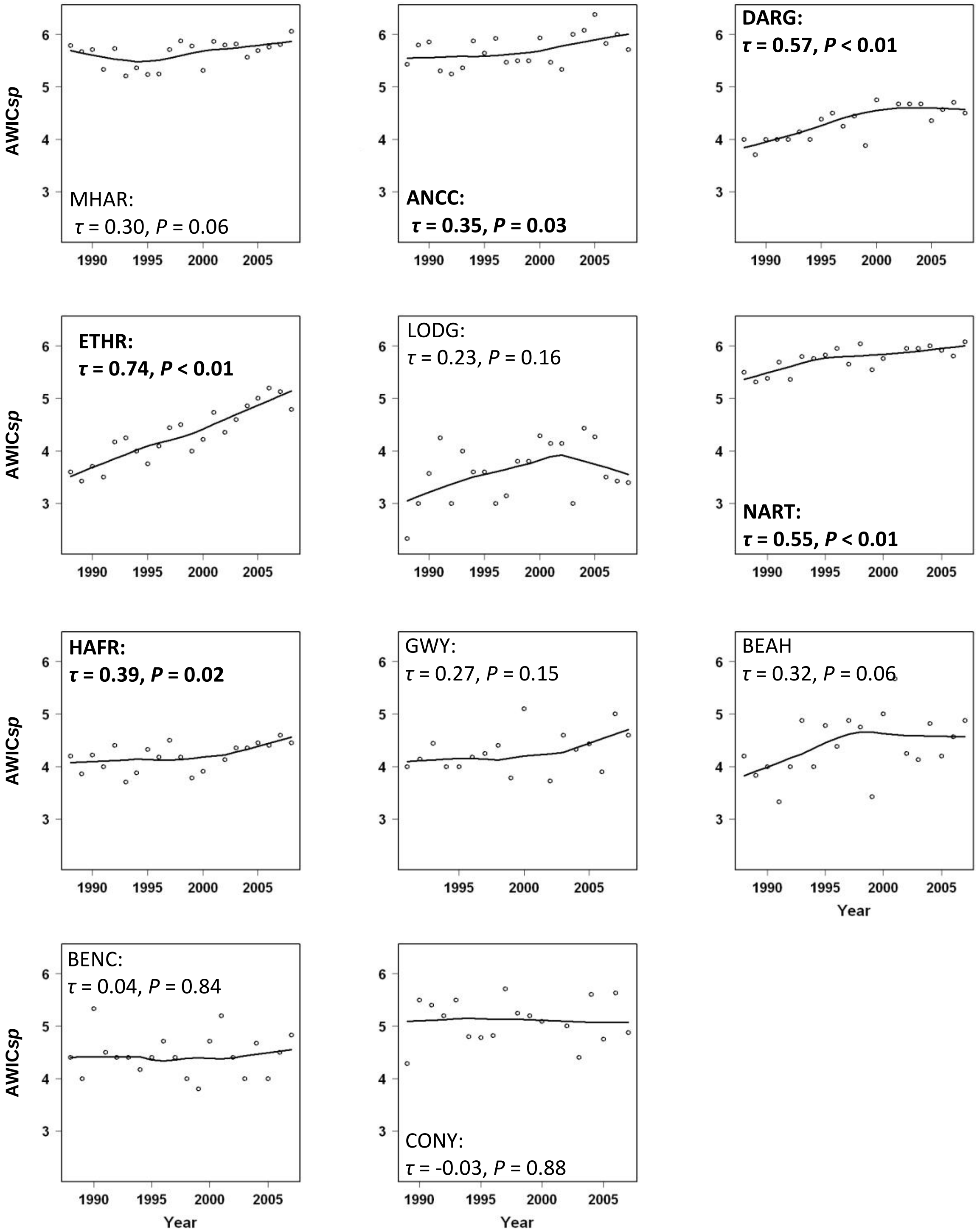

Fig. 3. Variation in AWIC*sp* scores at each of the 11 UKAWMN stream sites. A higher AWIC*sp* indicates a macroinvertebrate community containing more acid-sensitive taxa. The black line is a LOESS smoother through the data to indicate the dominant trend over time. The Mann-Kendall linear trend test τ value and associated significance level are also provided. Codes and statistics of sites with a significant increase are in bold. See Table 1 for key to abbreviated site names.

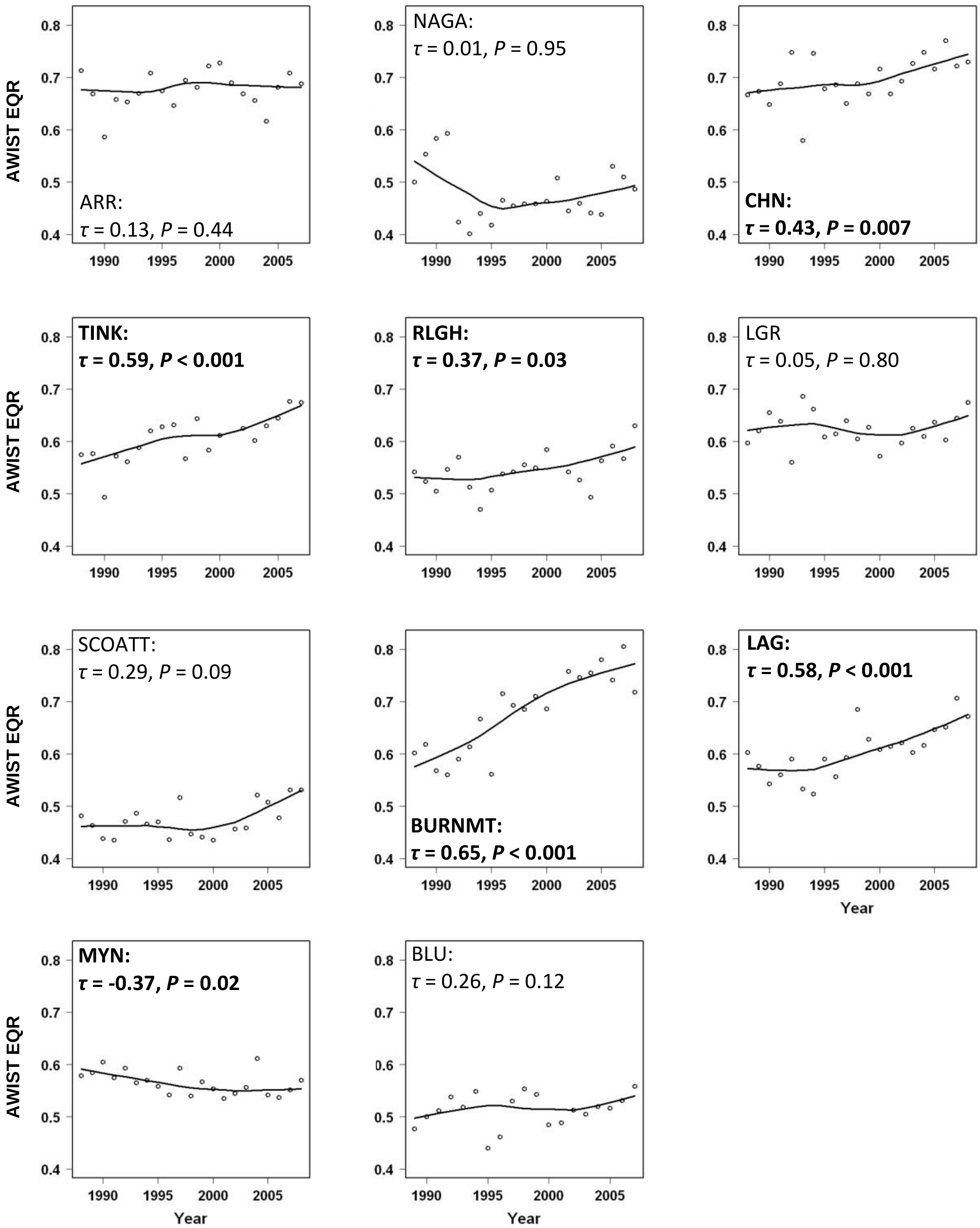

Fig. 4. Variation in AWIST EQRs at each of the 11 UKAWMN lake sites. A higher EQR value (varying from 0 to 1) indicates a macroinvertebrate community closer to that expected were the lake not impacted by acidification stress. The black line is a LOESS smoother through the data to indicate the dominant trend over time. The Mann-Kendall linear trend test τ value and associated significance level are also provided. Codes and statistics for sites with a significant increase are in bold. See Table 1 for key to abbreviated site names.

Table 1

Results of the RDA trend analysis for the AWMN samples. $PCA_{\lambda 1}$ is the Eigenvalue of the first PCA axis; $RDA_{\lambda time}$ is the Eigenvalue of the RDA axis; % PCA_1 and % RDA_1 are the variances in the species data explained by PCA axis 1 and time (RDA); *F* is the pseudo-*F* statistic; *n* is the number of samples in the series; min *p* is the minimum achievable *p*-value, and *p* is the exact permutation *p*. Sites with significant time trends are in bold.

Site (abbreviation)	$PCA_{\lambda 1}$	$RDA_{\lambda time}$	$\%PCA_1$	$\% RDA_1$	F	n	min p	p
Allt na Coire nan Con (ANCC)	9.115	4.095	30	13.5	2.957	21	0.048	**< 0.048**
Loch Coire nan Arr (ARR)	8.752	4.028	33.1	15.2	3.236	20	0.05	0.15
Beagh’s Burn (BEAH)	8.553	1.425	54.1	9	1.783	20	0.05	0.6
Bencrom River (BENC)	4.672	1.737	32.4	12.1	2.467	20	0.05	0.1
Blue Lough (BLU)	9.031	4.821	53.9	28.8	6.869	19	0.053	**< 0.053**
Burnmoor Tarn (BURNMT)	12.25	8.89	40.1	29.1	7.396	20	0.05	**< 0.05**
Loch Chon (CHN)	5.179	3.273	28.2	17.8	4.111	21	0.048	**0.04762**
Coneyglen Burn (CONY)	8.185	5.156	38.3	24.1	5.08	18	0.056	**0.05556**
Dargall Lane (DARG)	4.161	2.749	32.9	21.8	5.006	20	0.05	0.1
River Etherow (ETHR)	5.042	2.907	28.6	16.5	3.756	21	0.048	**0.04762**
Afon Gwy (GWY)	5.999	3.018	37	18.6	3.428	17	0.059	0.1176
Afon Hafren (HAFR)	6.297	3.843	42.9	26.2	6.374	20	0.05	**0.05**
Llyn Llagi (LAG)	7.873	5.872	39.7	29.6	7.998	21	0.048	0.09524
Loch Grannoch (LGR)	7.32	3.673	37	18.6	4.108	20	0.05	**0.05**
Old Lodge (LODG)	6.928	2.532	45.1	16.5	3.744	21	0.048	0.1429
Allt a’Mharcaidh (MHAR)	2.442	1.099	33.9	15.2	3.416	21	0.048	**0.04762**
Llyn Cwm Mynach (MYN)	8.835	6.169	50.3	35.1	10.27	21	0.048	**0.04762**
Lochnagar (NAGA)	11.73	2.176	46.6	8.6	1.796	21	0.048	0.2857
Narrator Brook (NART)	2.929	2.112	25.2	18.2	3.782	19	0.053	**< 0.053**
Round Loch of Glenhead (RLGH)	3.954	2.129	43.4	23.3	5.479	20	0.05	0.1
Scoat Tarn (SCOATT)	1.344	0.838	40.2	25	6.008	20	0.05	0.1
Loch Tinker (TINK)	5.152	2.899	33.9	19.1	4.01	19	0.053	**< 0.053**

Table 2

Assessment of spatial coherence between sites exhibiting chemical and biological recovery. The number of sites (out of 22) that exhibited biological recovery, chemical recovery, both trends, one but not the other or neither, is presented and the associated χ^2 contingency test result to assess whether the pattern was different to that due to chance.

		Biological recovery trend	Chemical recovery trend	Biological and chemical recovery trend	Biological but no chemical recovery trend	Chemical but no biological recovery trend	No biological or chemical recovery trend	χ^2 statistic, $P < 0.05$ in bold
Diagnostic Index (AWICsp/AWIST) trend	pH	11	16	10	1	6	5	3.667 (P = 0.056)
	ANC	11	18	11	0	7	4	**4.889**
	DOC	11	20	10	1	10	1	0
	Al_{lab}	11	15	8	3	7	4	0.210
Community (RDA) trend	pH	12	16	8	4	8	2	0.489
	ANC	12	18	10	2	8	2	0.041
	DOC	12	20	10	2	10	0	1.833
	Al_{lab}	12	15	7	5	8	2	1.180

Table 3

Stepwise regression of variation in diagnostic index values (AWIC*sp* for streams and AWIST for lakes) against variation in four descriptors of acid stress (average of preceding spring levels) and Year, with Year being entered into the stepwise selection process only after the other four acid chemistry variables have been considered. Sites with significant models in bold; alternative significant models (in italics), where Year was not restricted as above, are shown where significant.

Site	pH	log Al_{lab}	ANC	log DOC	Year	n	MSE	R^2	*P*
Allt na Coire nan Con (ANCC)									
Loch Coire nan Arr (ARR)									
Beagh's Burn (BEAH)									
Bencrom River (BENC)			0.011	-1.580		19	0.34	30.8	0.022
	1.100				*-0.057*		*0.29*	*48.6*	*0.006*
Blue Lough (BLU)									
Burnmoor Tarn (BURNMT)					0.011	20	0.04	75.2	0.001
Loch Chon (CHN)			0.005	-0.300		20	0.03	45.3	0.031
	0.097						*0.04*	*36.6*	*0.003*
Coneyglen Burn (CONY)									
Dargall Lane (DARG)			0.026			19	0.18	67.4	0.001
River Etherow (ETHR)					0.076	18	0.25	72.1	0.001
Afon Hafren (HAFR)	0.840	0.970		0.640		18	0.21	40.5	0.036
					0.024		*0.24*	*22.3*	*0.028*
Llyn Llagi (LAG)	0.186		-0.005	0.217		19	0.03	72.0	0.002
					0.006		*0.03*	*55.2*	*0.001*
Loch Grannoch (LGR)									
Old Lodge (LODG)									
Allt a'Mharcaidh (MHAR)				0.780		20	0.21	29.7	0.008
Llyn Cwm Mynach (MYN)					-0.002	19	0.02	19.8	0.032
Lochnagar (NAGA)									
Round Loch of Glenhead (RLGH)		0.139		0.343		19	0.02	58.8	0.022
Scoat Tarn (SCOATT)					0.002	19	0.03	17.5	0.042
Loch Tinker (TINK)	0.182		-0.003	0.269		18	0.04	38.9	0.047
					0.006		*0.03*	*53.5*	*0.001*

Supplementary Material

	SITE CODE	001	002	003	004	005	006	007	008	009	010	011	012	013
	SITE NAME	Loch Coire nan Arr	Allt a Mharcaidh	Allt na Coire nan Con	Lochnagar	Loch Chon	Loch Tinker	Round Loch of Glenhead	Loch Grannoch	Dargall Lane	Scoat Tarn	Burnmoor Tarn	River Etherow	Old Lodge
	COUNTRY	SCO	SCO	SCO	SCO	SCO	SCO	SCO	SCO	SCO	ENG	ENG	ENG	ENG
	SITE TYPE	Lake	Stream	Stream	Lake	Lake	Lake	Lake	Lake	Stream	Lake	Lake	Stream	Stream
Major Group	TAXON	ARR	MHAR	ANCC	NAGA	CHN	TINK	RLGH	LGR	DARG	SCOATT	BURNMT	ETHR	LODG
Tricladida	*Polycelis* sp.													
Nematoda	NEMATODA					1			1					
Mollusca	*Radix balthica*	1				1		1				1		1
	Acroloxus lacustris											1		
	Ancylus fluviatilis											1		
	Pisidium sp.	1				1	1	1	1			1		1
Annelida	OLIGOCHAETA	1	1	1	1	1	1	1	1	1	1	1	1	1
	Glossiphonia complanata	1				1						1		
	Helobdella stagnalis	1				1	1		1			1		
	Haemopsis sanguisuga													
	Erpobdella octoculata											1		
	Dina lineata													
Crustacea	*Crangonyx pseudogracilis*					1								1
	Gammarus lacustris											1		
	Niphargus aquilex													1
Ephemeroptera	*Siphlonurus lacustris*	1	1	1		1	1	1	1	1		1	1	
	Ameletus inopinatus	1	1	1		1	1		1				1	
	Baetis sp.	1	1	1		1			1	1	1	1	1	1
	Centroptilum luteolum	1				1								
	Centroptilum pennulatum	1												
	Cloeon dipterum					1								
	Procloeon bifidium	1										1		
	Rhithrogena semicolorata	1	1	1										
	Heptagenia sulphurea		1											

Major Group	TAXON	ARR	MHAR	ANCC	NAGA	CHN	TINK	RLGH	LGR	DARG	SCOATT	BURNMT	ETHR	LODG
	Heptagenia fuscogrisea													
	Heptagenia lateralis	1	1	1		1						1	1	
	Ecdyonurus sp.		1	1		1						1		
	LEPTOPHLEBIIDAE	1		1	1	1	1	1	1	1		1	1	
	Serratella ignita										1			
	Caenis horaria					1						1		
	Caenis luctuosa					1						1		
Plecoptera	*Brachyptera risi*		1	1	1					1			1	1
	Protonemura sp.		1	1	1					1			1	
	Amphinemura sulcicollis	1	1	1		1			1	1	1		1	1
	Nemurella picteti				1	1			1				1	1
	Nemoura sp.	1	1	1	1	1	1	1	1	1	1		1	1
	Leuctra geniculata					1								
	Leuctra inermis	1	1	1	1	1	1	1	1	1	1	1	1	1
	Leuctra hippopus		1	1	1	1	1	1	1	1	1		1	1
	Leuctra nigra		1	1	1	1	1	1	1	1	1		1	1
	Leuctra fusca				1									
	Leuctra moselyii													
	Capnia sp.				1				1		1			
	Perlodes microcephala		1	1						1			1	
	Diura bicaudata	1	1	1	1									
	Isoperla grammatica	1	1	1	1	1	1			1		1	1	1
	Siphonoperla torrentium	1	1	1	1	1		1		1	1	1	1	1
	Chloroperla tripunctata	1	1	1	1	1								
Odonata	*Pyrrhosoma nymphula*	1				1	1				1			1
	Ischnura elegans	1				1						1		
	Enallagma cyathigerum					1		1	1			1		
	Coenagrion puella	1												
	Caleopteryx virgo													
	Cordulegaster boltonii			1		1				1				1
	Aeshna sp.										1	1		

Major Group	TAXON	ARR	MHAR	ANCC	NAGA	CHN	TINK	RLGH	LGR	DARG	SCOATT	BURNMT	ETHR	LODG
	Cordulia aenea					1						1		
	Libellula sp.					1								
Hemiptera	*Velia* sp.													1
	Gerris lacustris													
	Notonecta glauca													
	Notonecta obliqua													
	Cymatia bonsdorffi					1			1		1			
	Glaenocorisa propinqua					1	1		1					
	Callicorixa praeusta					1		1			1			
	Callicorixa wollastoni	1				1			1		1			
	Corixa dentipes					1								
	Hesperocorixa sahlbergi					1		1	1					
	Hesperocorixa castanea					1	1				1			
	Hesperocorixa moesta										1			
	Arctocorisa germari	1				1			1					
	Sigara dorsalis						1							
	Sigara distincta				1	1			1					
	Sigara scotti	1				1	1		1		1			
	Sigara lateralis													
	Sigara nigrolineata					1					1			
	Sigara concinna								1					
	Sigara limitata					1								
	Sigara semistriata										1			
	Sigara venusta	1												
Coleoptera	*Haliplus* sp.					1						1		
	Dytiscidae undet. (larvae)				1	1					1	1	1	1
	Coelambus novemlineatus					1					1			
	Nebrioporus assimilis					1		1	1					
	Nebrioporus depressus	1			1	1	1	1	1			1		
	Nebrioporus elegans	1				1		1				1		
	Nebrioporus griseostriatus	1			1	1		1	1		1			

Major Group	TAXON	ARR	MHAR	ANCC	NAGA	CHN	TINK	RLGH	LGR	DARG	SCOATT	BURNMT	ETHR	LODG
	Stictotarsus duodecimpustulatus						1	1				1		
	Oreodytes davisii				1				1				1	
	Oreodytes septentrionalis								1					
	Oreodytes sanmarkii		1	1	1	1		1	1	1			1	
	Hydroporus palustris	1						1	1		1			1
	Hydroporus longulus				1									
	Hydroporus nigrita			1										
	Hydroporus pubescens										1			
	Hydroporus tesselatus	1												
	Hydroporus ferrugineus				1									
	Laccornis oblongus	1												
	Agabus guttatus									1			1	
	Agabus unguicularis					1								
	Agabus didymus												1	
	Agabus arcticus	1				1					1			
	Agabus chalconatus													
	Agabus bipustulatus										1			
	Platambus maculatus	1										1		1
	Ilybius ater													
	Rhantus exsoletus					1								
	Rhantus frontalis	1												
	Gyrinus bicolor										1			
	Gyrinus caspius						1		1					
	Gyrinus aeratus					1								
	Hydraena palustris			1										
	Hydraena gracilis		1	1										
	Helophorus sp.				1				1					1
	Paracymus scutellaris											1		
	Anacaena globulus									1		1		
	Laccobius minutus										1			
	Enochrus sp.											1		

Major Group	TAXON	ARR	MHAR	ANCC	NAGA	CHN	TINK	RLGH	LGR	DARG	SCOATT	BURNMT	ETHR	LODG
	SCIRTIDAE									1				
	Elmis aenea		1	1		1						1	1	
	Esolus parallelepipedus			1			1		1				1	
	Limnius volckmari	1	1	1		1			1	1		1	1	
	Oulimnius tuberculatus	1	1	1	1	1	1	1	1	1		1	1	
Neuroptera	*Sialis lutaria*				1	1	1	1	1		1	1		1
	Sialis fuliginosa					1	1		1				1	1
Trichoptera	*Rhyacophila* sp.		1	1						1	1		1	1
	Rhyacophila dorsalis		1	1						1			1	
	Rhyacophila septentrionis													
	Rhyacophila munda													
	Glossosoma sp.		1											
	Agapetus sp.													
	Philopotamus montanus		1											
	Wormaldia sp.													1
	Plectrocnemia sp.	1	1	1	1	1	1	1	1	1	1	1	1	1
	Polycentropus sp.	1	1	1	1	1	1	1	1	1	1	1	1	
	Holocentropus sp.	1				1		1						
	Cyrnus sp.	1				1	1		1		1	1		
	Tinodes waeneri	1		1		1	1					1		
	Lype sp.											1		
	Metalype fragilis			1			1							
	Hydropsyche pellucidula													
	Hydropsyche angustipennis													
	Hydropsyche siltalai			1		1				1			1	
	Diplectrona felix													1
	Hydroptila sp.						1					1		
	Oxyethira sp.	1					1			1			1	
	Phryganea grandis						1							
	Phryganea bipunctata													
	Agrypnia varia					1	1	1	1		1	1		

Major Group	TAXON	ARR	MHAR	ANCC	NAGA	CHN	TINK	RLGH	LGR	DARG	SCOATT	BURNMT	ETHR	LODG
	Agrypnia obsoleta					1	1	1	1		1	1		
	Drusus annulatus		1	1	1								1	
	Ecclisopteryx guttulata		1											
	Limnephilus sp.	1			1	1	1	1	1	1		1	1	
	Limnephilus rhombicus	1												
	Limnephilus marmoratus	1				1						1		
	Limnephilus lunatus	1				1	1							
	Limnephilus centralis													
	Limnephilus vittatus	1												
	Anabolia nervosa	1			1	1	1		1			1		
	Potamophylax sp.		1	1				1	1	1			1	1
	Halesus sp.	1	1	1	1	1	1	1	1	1	1	1	1	1
	Micropterna sp.								1					1
	Mesophylax impunctatus											1		
	Chaetopteryx villosa		1	1	1	1			1		1			
	Beraea maurus												1	
	Odontocerum albicorne					1								
	Athripsodes sp.	1				1	1	1				1		
	Mystacides sp.					1	1	1	1		1	1		
	Triaenodes bicolor					1								
	Adicella reducta					1								1
	Oecetis ochracea								1					
	Oecetis testacea													
	Silo pallipes			1		1								
	Crunoecia irrorata													1
	Lepidostoma hirtum	1		1		1						1		
	Sericostoma personatum	1	1	1		1	1		1			1		1
Diptera	TIPULIDAE	1	1	1	1	1	1	1	1	1	1	1	1	1
	PEDICIIDAE		1	1	1			1	1	1	1		1	
	LIMONIIDAE					1		1						1
	PSYCHODIDAE													

Major Group	TAXON	ARR	MHAR	ANCC	NAGA	CHN	TINK	RLGH	LGR	DARG	SCOATT	BURNMT	ETHR	LODG
	Dixa sp.		1											
	CHAOBORIDAE		1					1						
	CULICIDAE	1	1	1		1	1	1	1		1	1		1
	CERATOPOGONIDAE	1		1	1	1	1		1		1	1	1	1
	CHIRONOMIDAE	1	1	1	1	1	1	1	1	1	1	1	1	1
	SIMULIIDAE	1	1	1	1	1		1	1	1			1	1
	EMPIDIDAE	1	1	1	1	1	1	1	1	1	1	1	1	1

	SITE CODE	014	015	016	017	018	019	020	021	022	025
	SITE NAME	Narrator Brook	Llyn Llagi	Llyn Cwm Mynach	Afon Hafren	Afon Gwy	Beaghs Burn	Bencrom River	Blue Lough	Coneyglen Burn	Loch Coire Fionnaraich
	COUNTRY	ENG	WAL	WAL	WAL	WAL	NI	NI	NI	NI	SCO
	SITE TYPE	Stream	Lake	Lake	Stream	Stream	Stream	Stream	Lake	Stream	Lake
Major Group	TAXON	NART	LAG	MYN	HAFR	GWY	BEAH	BENC	BLU	CONY	VNG9402
Tricladida	*Polycelis* sp.	1					1				
Nematoda	NEMATODA										1
Mollusca	*Radix balthica*										1
	Acroloxus lacustris										
	Ancylus fluviatilis										
	Pisidium sp.	1	1	1	1	1	1				1
Annelida	OLIGOCHAETA	1	1	1	1	1	1	1	1	1	1
	Glossiphonia complanata										
	Helobdella stagnalis		1								1
	Haemopsis sanguisuga	1									
	Erpobdella octoculata		1	1							
	Dina lineata		1								
Crustacea	*Crangonyx pseudogracilis*										
	Gammarus lacustris		1								
	Niphargus aquilex		1			1					
Ephemeroptera	*Siphlonurus lacustris*	1	1	1		1		1			1
	Ameletus inopinatus		1			1		1	1	1	1
	Baetis sp.	1		1		1	1	1	1	1	
	Centroptilum luteolum										1
	Centroptilum pennulatum										
	Cloeon dipterum										
	Procloeon bifidium										1
	Rhithrogena semicolorata					1					
	Heptagenia sulphurea										

Major Group	TAXON	NART	LAG	MYN	HAFR	GWY	BEAH	BENC	BLU	CONY	VNG9402
	Heptagenia fuscogrisea									1	
	Heptagenia lateralis	1								1	1
	Ecdyonurus sp.	1									
	LEPTOPHLEBIIDAE	1	1	1	1	1			1	1	1
	Serratella ignita										
	Caenis horaria										
	Caenis luctuosa										
Plecoptera	*Brachyptera risi*	1			1	1	1			1	
	Protonemura sp.	1			1	1	1	1		1	
	Amphinemura sulcicollis	1	1		1	1	1	1		1	
	Nemurella picteti			1	1	1	1	1			
	Nemoura sp.	1	1	1	1	1	1	1		1	1
	Leuctra geniculata										
	Leuctra inermis	1	1	1	1	1	1	1		1	
	Leuctra hippopus	1	1		1	1		1	1		
	Leuctra nigra	1		1	1	1	1				
	Leuctra fusca										
	Leuctra moselyii				1						
	Capnia sp.					1					
	Perlodes microcephala	1									
	Diura bicaudata				1	1				1	
	Isoperla grammatica	1			1	1	1	1		1	1
	Siphonoperla torrentium	1	1		1	1	1	1		1	1
	Chloroperla tripunctata	1			1	1		1		1	
Odonata	*Pyrrhosoma nymphula*		1	1							
	Ischnura elegans			1							
	Enallagma cyathigerum			1							1
	Coenagrion puella										
	Caleopteryx virgo	1									
	Cordulegaster boltonii	1	1								
	Aeshna sp.		1	1					1		

Major Group	TAXON	NART	LAG	MYN	HAFR	GWY	BEAH	BENC	BLU	CONY	VNG9402
	Cordulia aenea			1							
	Libellula sp.			1							
Hemiptera	*Velia* sp.										
	Gerris lacustris			1							
	Notonecta glauca								1		
	Notonecta obliqua			1							
	Cymatia bonsdorffi			1					1		
	Glaenocorisa propinqua								1		
	Callicorixa praeusta			1					1		
	Callicorixa wollastoni			1					1		
	Corixa dentipes										
	Hesperocorixa sahlbergi		1	1					1		
	Hesperocorixa castanea		1	1					1		
	Hesperocorixa moesta			1					1		
	Arctocorisa germari								1		1
	Sigara dorsalis	1							1		
	Sigara distincta										1
	Sigara scotti	1	1	1					1		1
	Sigara lateralis								1		
	Sigara nigrolineata										
	Sigara concinna										
	Sigara limitata										
	Sigara semistriata										
	Sigara venusta		1								
Coleoptera	*Haliplus* sp.										1
	Dytiscidae undet. (larvae)			1					1		
	Coelambus novemlineatus										1
	Nebrioporus assimilis										1
	Nebrioporus depressus		1								1
	Nebrioporus elegans										1
	Nebrioporus griseostriatus						1		1	1	1

Major Group	TAXON	NART	LAG	MYN	HAFR	GWY	BEAH	BENC	BLU	CONY	VNG9402
	Stictotarsus duodecimpustulatus			1						1	
	Oreodytes davisii					1				1	
	Oreodytes septentrionalis									1	
	Oreodytes sanmarkii	1			1	1	1			1	
	Hydroporus palustris				1						1
	Hydroporus longulus										
	Hydroporus nigrita							1		1	1
	Hydroporus pubescens						1				
	Hydroporus tesselatus										
	Hydroporus ferrugineus										
	Laccornis oblongus										
	Agabus guttatus										
	Agabus unguicularis										
	Agabus didymus										
	Agabus arcticus										
	Agabus chalconatus								1		
	Agabus bipustulatus								1		
	Platambus maculatus										
	Ilybius ater				1						
	Rhantus exsoletus										
	Rhantus frontalis										
	Gyrinus bicolor										
	Gyrinus caspius										
	Gyrinus aeratus										
	Hydraena palustris										
	Hydraena gracilis	1								1	
	Helophorus sp.					1	1				
	Paracymus scutellaris	1									
	Anacaena globulus				1						
	Laccobius minutus										
	Enochrus sp.										

Major Group	TAXON	NART	LAG	MYN	HAFR	GWY	BEAH	BENC	BLU	CONY	VNG9402
	SCIRTIDAE										
	Elmis aenea	1	1		1	1	1			1	
	Esolus parallelepipedus	1									
	Limnius volckmari	1		1	1	1	1	1		1	1
	Oulimnius tuberculatus	1	1	1	1					1	1
Neuroptera	*Sialis lutaria*		1	1							
	Sialis fuliginosa	1									
Trichoptera	*Rhyacophila* sp.	1			1	1	1	1		1	
	Rhyacophila dorsalis	1			1	1	1				
	Rhyacophila septentrionis	1									
	Rhyacophila munda					1					
	Glossosoma sp.	1									
	Agapetus sp.	1	1								
	Philopotamus montanus	1				1					
	Wormaldia sp.	1									
	Plectrocnemia sp.	1	1	1	1	1	1	1	1	1	1
	Polycentropus sp.	1	1	1	1	1		1	1		1
	Holocentropus sp.		1	1							
	Cyrnus sp.		1	1							1
	Tinodes waeneri	1	1	1		1	1			1	1
	Lype sp.				1						
	Metalype fragilis	1	1			1					
	Hydropsyche pellucidula						1			1	
	Hydropsyche angustipennis	1					1				
	Hydropsyche siltalai	1			1	1	1	1		1	
	Diplectrona felix	1			1		1				
	Hydroptila sp.										1
	Oxyethira sp.	1	1	1	1	1		1			1
	Phryganea grandis										
	Phryganea bipunctata			1							
	Agrypnia varia		1	1					1		

Major Group	TAXON	NART	LAG	MYN	HAFR	GWY	BEAH	BENC	BLU	CONY	VNG9402
	Agrypnia obsoleta		1	1							
	Drusus annulatus	1			1	1	1			1	
	Ecclisopteryx guttulata				1					1	
	Limnephilus sp.	1	1	1			1		1	1	1
	Limnephilus rhombicus										
	Limnephilus marmoratus										
	Limnephilus lunatus										1
	Limnephilus centralis			1							
	Limnephilus vittatus		1								1
	Anabolia nervosa		1	1							
	Potamophylax sp.	1			1	1	1	1		1	
	Halesus sp.	1		1	1	1	1			1	1
	Micropterna sp.	1					1				
	Mesophylax impunctatus			1							
	Chaetopteryx villosa	1	1				1			1	
	Beraea maurus										
	Odontocerum albicorne	1									
	Athripsodes sp.		1								
	Mystacides sp.		1	1							1
	Triaenodes bicolor			1							
	Adicella reducta	1	1								
	Oecetis ochracea										
	Oecetis testacea	1									
	Silo pallipes	1					1				
	Crunoecia irrorata	1									
	Lepidostoma hirtum	1									
	Sericostoma personatum	1		1			1				1
Diptera	TIPULIDAE	1	1	1	1	1	1	1	1	1	1
	PEDICIIDAE				1	1					
	LIMONIIDAE										
	PSYCHODIDAE									1	

Major Group	TAXON	NART	LAG	MYN	HAFR	GWY	BEAH	BENC	BLU	CONY	VNG9402
	Dixa sp.	1									
	CHAOBORIDAE										
	CULICIDAE		1	1		1				1	
	CERATOPOGONIDAE		1	1			1			1	1
	CHIRONOMIDAE	1	1	1	1	1	1	1	1	1	1
	SIMULIIDAE	1			1	1	1	1		1	
	EMPIDIDAE	1	1		1	1	1	1	1	1	1