Appendix 7: Evidence for systematic effects of antecedent temperature and precipitation on the inter-annual variability of macroinvertebrate taxa in upland streams and lakes.

Abstract

UK upland surface waters are important havens of freshwater biodiversity. Over recent decades, water quality in many upland regions has improved in response to large changes in acid deposition, promoting the gradual re-establishment of some acid sensitive species. Concomittantly, however, the temperature of upland lake and stream water has increased in response to regional warming, while changes in the seasonality of discharge are expected in some regions. Most aquatic macroinvertebrate species are considered to have relatively wide climatic tolerances but some European species are largely confined to cool montane stream habitats. As acid deposition begins to approach background levels in some areas, climate change is likely to become an increasingly important determinant of upland surface water species composition and ecological functioning. Long term records of macroinvertebrate communities, generated primarily by programmes designed to assess recovery from acidification, provide the opportunity to assess the influence of recent variation in temperature and hydrology on these assemblages. However, there is little a priori understanding of which periods in the year effects of climate may be most influential in affecting populations and the degree to which climatic conditions in previous years may have contributed to current status.

Using macroinvertebrate data provided by the UK Upland Waters Monitoring Network (UWMN) and the Llyn Brianne experimental catchments project, we developed a series of site-specific statistical climate models that best explained variation in the absolute abundances of individual taxa in upland surface waters after allowing for responses to changing sulphur deposition and linear change. By exploiting a recently developed statistical technique, effects of variables representing site-specific monthly air temperature and precipitation, as surrogates for water temperature and hydrological variation, were modelled to exert a sinusoidal seasonally varying effect which was allowed to decay over a maximum of 5 years. Inclusion of either a temperature or precipitation variable improved the model fit significantly in almost half of the 512 site-taxon combinations investigated, compared to a model comprised of terms for linear change and sulphur deposition alone. The modelling procedure generates monthly temperature or precipitation coefficients, and we calculated the mean of these to represent the net annual effect of temperature or precipitation on the taxa.

Across all macroinvertebrate taxa investigated there was a tendency for more site-taxon combinations to show negative than positive effects of air temperature (when averaged over the year). In contrast, effects of precipitation were more equivocal. Negative effects of temperature were particularly apparent for: 1) Caddisflies (particularly within the Polycentropidae (and particularly Plectrocnemia species) and Limnephilidae; 2) Stoneflies (particularly Nemouridae (particularly Nemoura species), Chloroperlidae (particularly Siphonoperla torrentium) and Leuctridae (particularly Leuctra inermis); and 3) various Dipterans taxa mostly recorded at family level only.

An examination of the seasonally varying temperature coefficient revealed seasonal differences in sensitivity to temperature between these taxa, and, in the case of Plectrocnemia spp. even within species of the same genus. In several cases, air temperature during previous summers and/or autumns appeared to exert the predominant negative effect. The likely relative importance of temperature effects on adult flying stages, hatching times and larval development, and the
possibility that apparent responses are due more to changing phenology than more chronic effects on these populations, will require more detailed investigation.

In comparison there was considerably less evidence for consistent positive or negative effects of rainfall (although given the less seasonal and more episodic behaviour of rain we are unlikely to be modelling its influence as robustly as for temperature). However, there was a clear tendency for negative effects on: 1) Coleopterans (particularly *Nebrioporus depressus* and *Elmis aenea*) and 2) Perlodid stoneflies (again *Siphonoperla torrentium*), in addition to *Isoperla grammatica*).

Since all of our climate models included terms for linear change and sulphur deposition the model output provides an indication of the importance of temperature or precipitation in influencing inter-annual variability only as opposed to long-term change. However some of the species identified as showing the greatest short-term (i.e. inter-annual) sensitivity to temperature, e.g. *Plectrocnemia conspersa* and *Nemoura* spp. have previously been identified as undergoing long term decline in some of the study sites. Furthermore, the most negative mean temperature coefficients were found for those taxa deemed by their European geographical distributions to be most restricted to cooler waters. Furthermore, mean temperature coefficients for stonefly taxa tended to be more negative at the higher altitude sites, again consistent with heightened temperature sensitivity of the taxa that dominate cooler waters. Further work is now required to explore the likely relative importance of warming and recovery from acidification on these and other taxa.
Introduction

The surface waters of the UK uplands are important havens of freshwater biodiversity and are often considered sensitive sentinels of environmental change[1]. Over recent decades, water quality in many upland regions has improved in response to large changes in acid deposition[2], promoting the gradual re-establishment of some acid sensitive species [3-5]. However, these taxa continue to remain vulnerable to acid episodes in upland streams caused both by high rainfall events and seasalt episodes [6-8]. Furthermore surface waters draining commercially forested catchments, while also recovering chemically, remain more acidic than neighbouring moorland systems [9, 10]. More generally while most upland surface waters continue to experience elevated levels of nitrogen, heavy metals and persistent organic pollutants, legacies of two centuries of atmospheric deposition of industrially-derived pollutants[1].

Aquatic macroinvertebrates, are routinely used as bioindicators of water quality, due to the high sensitivity of various species to water quality and particularly organic pollution and acidity[11]. As the 21st century progresses, and the threats from industrial contaminants begin to diminish, it is expected that changes in climate, e.g. ramping temperatures and changes in the seasonality of flow regimes, will increasingly pose the chief threat to the ecological health of upland surface waters [1, 12]. Several key macroinvertebrate taxa are considered stenothermic, but to date there have been few attempts to assess the sensitivity of populations to recent variations in climate despite the availability of long time series. Such studies are hampered by the paucity of basic ecophysiological understanding of many taxa, including the degree of seasonal variation in sensitivity, and mechanisms behind observed effects remain poorly understood[13].

The most common approach to assessing statistical links between populations and potential drivers, is to apply predictor variables representing effects for discreet periods, i.e. individual antecedent days, months or seasons, often within a forward selection framework. However, such approaches normally assume some a priori understanding of the antecedent period over which an effect operates, and, due to limits of degrees of freedom, most models are tightly restricted with respect to the number of variables that can be incorporated. It is reasonable to expect that antecedent weather throughout the life history of an organism, and possibly over the course of previous generations, will impact on the probability of it being recorded during a given sampling event, and hence on the total abundance of the species. However, due to the restriction on variables, these conventional approaches are unable to incorporate much of the potentially informative climatic information that could be available.

In this paper we investigate evidence for sensitivity of upland macroinvertebrate taxa using a novel approach, described in the statistical appendix of this report, in which the effect of a string of antecedent months of mean monthly air temperature or total monthly precipitation, tracking back as far as five years, are summarised using just three parameters describing the average level of effect, the amplitude of a seasonally varying term and the rate of decay of the effect.

We apply the method to identify which taxa in each of the 31 streams and lakes monitored by two upland water monitoring programmes exhibit statistically significant evidence of effects of either antecedent air temperature or antecedent precipitation on inter-annual variability in abundance. We then go on to consider the distribution of these effects across taxonomic groups, families and species and attempt to pull out patterns that are common across several sites, and therefore most
likely to indicate real sensitivity to changes in climate. The seasonal structure of correlation coefficients for some potential indicators of climate change are then considered in more detail, and possible mechanisms behind some of the more frequently observed species-climate relationships are discussed.

Materials and methods

Upland macroinvertebrate time series data were drawn from two sources, the UK Upland Waters Monitoring Network (UWMN - formerly known as the UK Acid Waters Monitoring Network) and the Llyn Brianne experimental catchment dataset. The UWMN was established in 1988 to assess the impact of acid emission reductions on 11 acid sensitive lakes and 11 acid sensitive streams spanning much of the UK uplands and involves integrated measurements of chemical and biological (aquatic macrophytes, diatoms, macroinvertebrates and fish [14]. Stream macroinvertebrates in the Llyn Brianne experimental catchments in south Wales since were first sampled in 1981–1982 by Stoner et al.[15] and from 1985 to 2005 (except 1991) by Cardiff University using a consistent methodology. The programme enables the assessment of the influence of differences in catchment sensitivity and contrasting land-use (forestry and upland grazing) on responses to acid deposition[16].

In both programmes macroinvertebrates are sampled annually during Spring – normally a time of year when community is most exposed to effects of acid episodes as a consequence of periods of high stream discharge and/or seasalt deposition events ([16, 17]). Llyn Brianne streams are sampled in mid-April, while UWMN sites are sampled during April–May, with the most southerly sites sampled earliest within this period and the most northerly latest to allow for the later onset of spring further north.

The UWMN sampling protocol consists of five, one-minute kick or sweep samples at each site using a 0.33 mm mesh hand-net. Sampling locations are selected with the aim of obtaining consistent replicate samples from the same habitat year over time; stony riffsles at stream sites and stony or sandy littoral habitats, including rooted zones, in lakes[18]. Samples are 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). The Llyn Brianne streams are kick-sampled for a total duration of three minutes, two minutes for riffsles and the remaining time for marginal habitats using a 0.9 mm mesh hand-net. Individual animals are identified where practicable to species (i.e. except for Diptera and Oligochaeta), and absolute abundances for each recorded.

Macroinvertebrate data for each taxon, determined where possible at species level but otherwise at genus or family level, were aggregated for each annual sampling visit for each site. The UWMN and Llyn Brianne data were brought together in common MS Access database at the Environmental Change Research Centre at UCL and taxa were allocated common taxon codes wherever possible to facilitate directly comparable analyses of data drawn from both networks.

Long-term water temperature and water flow data were available for several but not all sites, although few lake discharge data are available. However as many of the macroinvertebrate taxa have terrestrial stages during their life cycles, and as temporal variation in mean monthly water
temperature and mean water discharge tend to correlate strongly with mean monthly air
temperature and total monthly precipitation respectively, particularly for surface water dominated
upland systems, and the polymictic lakes that dominate the UWMN, we used the latter as our
climate surrogates in this analysis. Modelled monthly time series data for both mean air
temperature and total precipitation for the 1 km grid square within which each site lies, were
obtained from the Centre for Ecology & Hydrology's 1 km resolution CHESS model. For each site,
these climate time series were selected to span the period five years prior to the first
macroinvertebrate sampling year to the final year of macroinvertebrate sampling – 2008.

In order to control for the possible influence of chemical recovery from acidification on the
macroinvertebrate assemblages, it was necessary to derive a simple metric to represent annual-
scale change in acidification pressure at all sites. Detailed spot sampled water chemistry data,
including measurements of pH, Gran Alkalinity and inorganic (labile) aluminium, dissolved organic
carbon (DOC) and base cations are available for UWMN streams at monthly and quarterly
frequencies and at quarterly at UWMN lakes. However, unlike the modelled climate data they only
provide instantaneous snapshots of water acidity which may not always provide a representative
reflection of antecedent conditions. Furthermore, the primary limiting chemical factor or factors are
likely to vary between sites depending on geological sensitivity and the degree of buffering provided
by organic acids [9]. For example, while most sites are undergoing increases in water pH and
dissolved organic carbon concentration, reductions in the potentially toxic inorganic aluminium are
likely to dominate chemical recovery in the most acidified sites, whereas the main change in
chemistry in the most geologically buffered sites is likely to be an increase in alkalinity (possibly of
greatest benefit to algal grazing specialists due to the potential influence on benthic primary
productivity). Chemical recovery from acidification has been dominated by reductions in sulphur
deposition, as reflected by aqueous sulphate concentration, and improvements in Acid Neutralising
Capacity (a generic metric of acid-base response) has been shown to be strongly correlated with the
rate of change in sulphate concentration. We therefore chose change in mean annual sulphate
concentration as the acidification metric for the study. Sulphate concentration in upland waters over
recent decades varied with remarkable coherence across the UK reflecting the regional nature of the
pressure derived from long range transboundary air pollution [19].

Data analysis

We confined our analysis to taxa for which data were available for at least 15 years at individual
sites. For each site-taxon combination we generated four models to explain interannual variation in
total abundance. In the most simple of these (the NULL model), sample year (YEAR) was applied as
the single explanatory variable. The second model included YEAR and a variable representing the
mean annual sulphate concentration z-score (SULPH) (i.e. YEAR + SULPH) in order to remove effects
beyond a simple linear trend that might be associated with interannual variability in sulphur
deposition. The final two models (the “full” models) included YEAR, SULPH and further parameters
to model effects of antecedent monthly-scale variation in either temperature (YEAR + SULPH +
TEMP) and precipitation (YEAR + SULPH + PRECIP), and its decay over a period of up to five years,
using the approach described in the statistical appendix. The monthly variation was described by a
sin curve of the form:
\[ s(i) = s_0 + s_1 \sin(2\pi i/12) + s_2 \cos(2\pi i/12) \]

with parameters \( s_1 \) and \( s_2 \) of the sine and cosine terms augmented by a third parameter, \( s_0 \), which represents the median effect of temperature throughout a seasonal cycle.

With only 20 years of biological data available there were insufficient degrees of freedom available to include both temperature and precipitation within the same model and the relative effects of the two for a given site-taxon combination were assessed through comparison of Likelihood Ratio Test (LRT) of each of the two models against the YEAR + SULPH model.

Some of the variance in the abundance of a taxon that may in reality be directly linked to changes in sulphur deposition, temperature or precipitation will be consumed by YEAR if the abundance changes relatively monotonically over time. Nevertheless, use of YEAR in all models reduces the likelihood that other effects, that may also change through time, e.g. changes in land use etc., are mistakenly attributed to the drivers of interest.

Macroinvertebrate data for each annual sample were linked to climate data series extending retrospectively from the month of sampling (if sampling occurred later than the 15th of the month) or from the previous month (for samples taken on or before the 15th of the month). The two climate models generate an annual sinusoidally varying temperature or precipitation coefficient for each month, the sign of which may be either consistently positive or negative or vary either side of zero. In order to summarise the overall influence of temperature or precipitation for each site-taxon combination, we calculated the mean coefficient to summarise the overall direction and size of the climatic effect in question for each taxa at each site.

Results

The CHESS modelled air temperature and precipitation climate time series used in our analysis are summarised in Figure 1. Over the period of macroinvertebrate sample collection (1988-2008) there was relatively little evidence for directional trends in most metrics, with the exception of slight increases in mean autumn and winter temperature. In all cases inter-annual variability in these climate variables dominated over any long term trend.

Figure 2a illustrates the large reduction in sulphate concentration measured in upland waters across the UK since the establishment of the UWMN, a direct response to reductions in the industrial emission of sulphur over the past three decades ([2]. The magnitude of reduction in sulphur deposition has been greatest in central areas of the UK, declining in strength toward the cleaner areas in the north and west [20]. Nevertheless, the pattern of change in concentration at an annual scale at individual sites has been highly coherent across the network, to the extent that a z-score (i.e. the mean of standardised concentrations) (Figure 2b) can be used as an effective surrogate for sulphur deposition nationally [19]. This z-score was averaged at an annual scale to produce the variable SULPH which was applied in the same form in all YEAR + SULPH models. While sulphate concentrations show broad long-term reductions, much of which can be represented by YEAR in this modelling exercise, the major changes occur between 1995 and 2000 [9].
The models were run for a total of 566 site-taxon combinations, an average of 18 taxa per site. The most widespread taxa in our dataset (reduced to exclude non-constant species) were the Oligochaetes and Chironomidae which were represented at all sites, albeit at family level only. The most commonly occurring taxon identified to species level was the stonefly *Siphonoperla torrentium* (27 sites). Four further species, all stonefly taxa, also occurred at over half of all sites: *Leuctra hippopus, L. inermis, Amphinemura sulcicollis* and *Isoperla grammatica*. All other species in the dataset occurred at fewer than half of all sites.

Of the 566 site-taxon combinations, the YEAR + SULPH model explained significantly more variation (LRT = 5; equivalent to p = 0.05) than the NULL model in 23 cases. While this number is no greater than would be expected to occur by chance there was some consistency with respect to the taxa. Twelve were for stonefly taxa, of which *A. sulcicollis* and *Nemoura* spp. were most frequently represented at 3 sites each.

The addition of either antecedent precipitation or air temperature made a significant improvement over the YEAR + SULPH models (LRT > 10; equivalent to p = 0.05 for this more complex model) for 268 and 257 site-taxon combinations respectively. This means that both climate variables were able to explain significant inter-annual variation in the abundance of these taxa beyond any linear change or additional change that can be directly attributable to reductions in acid deposition in just under half of all cases for which sufficient macroinvertebrate data were available. For 135 site-taxon combinations both precipitation and temperature improved the model significantly.

There was a general tendency for those taxa most frequently showing significant temperature effects (i.e. LRT >10 for YEAR + SULPH + TEMP model vs YEAR + SULPH model) to also show the largest number of significant precipitation effects (i.e. LRT >10 for YEAR + SULPH + PRECIP model vs YEAR + SULPH model), partly because they tended to be the most widespread. However, the majority of stonefly taxa, including *Leuctra inermis, L. nigra*, and *A. sulcicollis* showed significant temperature effects at more sites than they showed significant precipitation effects. *Siphoperla torrentium* and *L. hippopus* were the only widespread taxa recorded at species level to show significant precipitation effects at more sites than significant temperature effects although in both cases overall numbers were very similar.

**Group level effects**

Mean coefficients were calculated to represent the overall annual temperature and precipitation effect for each site-taxon combination. The distribution of mean precipitation coefficients, when amalgamated at a taxonomic group level, showed a general tendency for balance in the number of site-taxon combinations showing positive and negative mean coefficients (Figure 3a). In contrast there was a clear bias towards negative mean coefficients with respect to temperature effects. This was particularly apparent with respect to statistically significant (i.e. LRT >10) models for stonefly and caddisfly groups (Figure 3b).

From now on we consider only those models for which the LRT for the full model (either temperature- or precipitation-based) relative to the YEAR + SULPH model exceeded 10 (comparable with a p-value of 0.05) – i.e. those for which the climate variables were deemed to have a significant effect on interannual variation in abundance. Furthermore, in the following boxplots we include only those site-taxon combinations for which mean coefficients fell between -10 and 10.
(approximately 80% of the dataset) to minimise the occasionally distorting effect of extreme outliers. Figures 4a and 4b summarise the distribution of temperature and precipitation coefficients respectively for taxa within the most widely occurring caddisfly families.

Effects on caddisflies

Two caddisfly families, the Limnephilidae and Polycentropidae, showed very clear negatively biased temperature coefficients, implying consistent negative effects of temperature on the constituent species. In contrast, these groups, and the less well represented Rhyacophilidae, showed more equivocal precipitation coefficient distributions. The distribution of statistically significant temperature and precipitation coefficients of the most frequently occurring caddisfly species is illustrated in Figures 5a and 5b respectively. Only two species of Polycentropidae, the predatory, net spinning *Plectrocnemia conspersa* and *P. geniculata*, provided sufficient numbers of significant climate relationships to merit inclusion (i.e. five or more). *P. conspersa* showed a very distinct and discreet negative temperature coefficient distribution, in contrast to the more equivocal *P. geniculata*.

Effects on stoneflies

In common with the dominant caddisfly families, Chloroperlidae, Leuctridae, Chloroperlidae and Taniopterygidae stonefly families, all exhibited marked negative biases in their temperature coefficients, while Perlodidae temperature coefficients were consistently very small and clustered around zero (Figure 6a). It is notable that the Perlodidae were the only widespread stonefly or caddisfly families we studied to show consistently negative precipitation coefficients (Figure 6b), suggesting that the significant but very small temperature effects for this group may represent an artefact of real responses to variation in hydrology. Two stonefly families, the Leuctridae and Nemouridae showed positive biases in their precipitation coefficients. Of the more frequently occurring stonefly species, *Leuctra inermis* (Leuctridae), *Brachyptera risi* (Taniopterygidae), *Protonemura* spp. (Nemouridae) and *Siphonoperla torrentium* (Chloroperlidae) all exhibited negative mean temperature coefficient distributions while *Isoperla grammatica* showed no obvious bias. Perhaps significantly, *I. grammatica* did show a consistently negative mean precipitation coefficient, indicative of deleterious effects of high flows on this species.

Discussion

The upland macroinvertebrate datasets examined in this analysis have been collated over a period (1988 – 2008) of relative stability in climate (Figure 1), when compared with the pronounced warming experienced over the previous two decades. While these datasets therefore provide a highly robust baseline against which the impacts of future climate change may be assessed, they are unlikely to provide definitive evidence for chronic climate change effects per se. Rather, the purpose of this analysis was to examine evidence for sensitivity in the abundance of taxa to changes in temperature and precipitation on these upland ecosystems at an inter-annual level and, by doing so, identify potential indicators of future climate change. Some of the the Llyn Brianne stream macroinvertebrate data stretch back to the early 1980s, although the data used in this study was restricted to post-1987 to match the UWMN monitoring period. There is therefore an opportunity to
use these longer records to test the indicator value of some of the taxa identified as most clearly climate sensitive over a period of more marked temperature change.

Attribution of specific climate-related effects is complicated by the fact that most meteorological variables tend to be correlated at various temporal and spatial scales as a consequence of their common dependence on the state of synoptic-scale weather systems. Hence, wet UK winters that are often associated with a positive NAO tend also to be relatively warm while warm summers tend to result from the establishment of stable high pressure over the UK and are consequently also relatively dry. This might partly explain an apparent association between site-taxon combinations showing significant effects of both temperature and precipitation. Care is therefore required in the interpretation of the results to minimise mis- attribution of effects. Our approach of focusing on evidence for common behaviour across multiple sites, with respect both to the prevalence of significant effects and the sign of mean coefficients, at group, family or species level was intended to mitigate these concerns.

In general our analysis indicated stronger directional (predominantly negative) effects of temperature on macroinvertebrate taxa than for effects of precipitation, and effects seem to be most marked for some of the more commonly occurring taxa. This is consistent with the observations of Durance & Ormerod [21] who noted a negative relationship between temperature and the total abundance of individuals within the non acid-sensitive Brianne streams that are included within this dataset, but the tendency appears to extend to upland surface waters more widely, regardless of acidification status. Aquatic insect responses to temperature are complex, embracing thermal conditions ranging from diel and seasonal variation superimposed upon a range of other factors and stressors[22].

Modelling was restricted to species that occurred at individual sites in all but five years, or more, while our assessment of trend distribution of individual species was necessarily limited to the more widespread taxa. Consequently, this has been a relatively conservative assessment of climate sensitivity within these upland macroinvertebrate populations, with the focus resting ultimately on species showing the most consistent responses across a range of sites. Strongly significant effects of both temperature and precipitation were identified for other species at individual sites, but without replicated evidence for these effects links with climate are more tenuous. Nevertheless, the spring sampled abundance of a clear majority of all caddisfly and stonefly individuals in these systems appear to be negatively affected by temperature, and several species beyond those with the more widespread distributions, and particularly across the Nemourid and Leuctrid stonefly families, and Limnephilid caddis families contributed to this tendency.

The case of *Plectrocnemia spp.*

A few caddisflies and stoneflies stood out as species showing very consistent negative effects of antecedent temperature on their interannual abundances. Of the caddisflies, the most consistent was the net spinning predatory *Plectrocnemia conspersa*. This species showed significant temperature effects at 9 sites as opposed to 5 sites for temperature. At the two sites for which relationships with both climate variables were significant, the LRT value for the temperature model was greater than for precipitation suggesting the former had a greater effect. Times series for the full model fits for this species for eight of the nine sites included in Figure 5a are presented in Figure 8. At most sites, abundances show an overall downward trend. Ormerod and Durance [5] have
previously identified this species as having undergone long term reductions in abundance in some Llyn Brianne streams, and since it tends to be more abundant in more acidic streams, attributed the changes to partial recovery from acidification. However, our analyses show that even when annual scale variation in sulphur deposition and linear long term change were factored in temperature (and/or precipitation) remained a highly significant explanatory variables for this species at several sites. Figure 8 makes it clear that short term (inter-annual) variation can only be accounted for by inclusion of a climate variable (in this case temperature).

The nature of the temperature effect can be explored in more detail by examining the seasonal distribution of monthly temperature coefficients generated by the modelling procedure. Figure 9a demonstrates a consistent tendency among several Llyn Brianne streams for temperature over the previous summer months to have the dominant effect on *P. conspersa* abundance sampled the following spring. This species is not recorded on the UWMN. However, *Plectrocnemia* spp. is recorded at the majority of UWMN sites. This taxon (possibly also *P. conspersa* - check with QMUL) shows similar seasonal variation in the temperature coefficient – again focussed on conditions during the previous summer(s) Figure 9b. *Plectrocnemia conspersa* lays eggs in an egg mass adhered to submerged substrates and the summer incubation of the eggs has been shown to be highly sensitive to temperature, with eggs taking 93 days to hatch at 6 °C compared to 20 days at 18 °C in a laboratory experiment [23]. However, there was no indication in the laboratory study for detrimental impacts on hatching success. Several Caddis species at higher altitudes and towards their northern limits, including *P. conspersa*, adopt a semi-voltine life cycle strategy, i.e. they require more than one year to complete their life cycle. This flexibility is considered to provide an evolutionary advantage in cold water systems where water temperature exerts a major influence on development times[24].

Interestingly, the relationship with temperature would appear to be species specific. Figure 10 provides a comparable plot for *P. geniculata*. This shows rather different seasonal temperature effects, and suggests this species is as much benefitted by a warmer autumn/winter period as it is negatively affected by the temperature of the previous summer. One feasible mechanism for a negative impact of temperature on *P. conspersa* could be through effects of interspecific competition of species (perhaps including *P. geniculata*) that might be more successful in a warmer environment.

**Stonefly species**

Several stonefly species exhibited consistent negative effects of temperature on their abundance. Among these were the three Leuctrid species represented in the dataset: *Leuctra inermis*, *L. nigra* and *L. hippopus*. Water temperature has been shown to exert a very strong influence on the development and hatching success of Leuctrid eggs[25] . Each of six species in that study was shown to have a different temperature optima for egg development and temperature dependent hatching period, characteristics that encourage the different species to hatch at different times of year, thus reducing the potential degree of inter-specific competition. Separately, *L. nigra* was found to show marked reductions in both larval survival and egg production when temperatures rose from 12.1 to 15.8 °C[26]. In this study there was a highly significant positive relationship between the rate of growth and water temperature when this species was cultured in the laboratory, to the extent that it
switched from a semi-voltine to uni-voltine life cycle [26] with consequent deleterious impacts on survival.

Figure 11 illustrates the seasonal pattern in monthly temperature coefficients for the six sites at which *Leuctra inermis* shows significant temperature effects. There is a general tendency for temperature to exert a negative effect throughout the year, but at the majority of sites the dominant temperature effect appeared to be concentrated in the Autumn, when the early stages are larval development are likely to occur. Similar, although slightly less coherent, patterns are seen for *L. nigra* in addition to the Nemourid *Protonemura* spp. (Figure 12).

Figures 14-15 provide an example of where there is little evidence in seasonal coherence in the variation in the temperature coefficient, indicating that the statistically significant “effect” of temperature on this species, the Chloroperlid *Isoperla grammatica*, may be an artefact resulting from covariant relationships with other drivers. Interestingly, there is a far clearer pattern with respect to seasonal variation in the precipitation coefficient (Figure 15) and a suggestion that the abundance of this species may be either primarily determined (either positively or negatively depending on the site) on rainfall, and hence discharge, immediately prior to sampling.

*Other groups*

While effects of temperature, and in some cases precipitation, appear most marked with respect to stonefly and caddis species, some taxa from some other groups also provide evidence of consistent seasonal responses. For example, Figure 16 shows coherent seasonal variation in the temperature coefficient for the ubiquitous, bank dwelling beetle, *Anacaena globulus*, that shows evidence both for negative effects of temperature during winter and positive effects during summer.

*Links between observed temperature sensitivity and regional and local geographical distribution*

Our analysis has drawn out evidence for sensitivity to inter-annual variation in temperature for a range of species, particular stonefly and caddisfly species. One independent test of this evidence is to examine the relationship between the mean temperature coefficients of the species in our dataset and the distributional range of these taxa. A “temperature tolerance index” has been drawn up to classify a large number of commonly occurring European macroinvertebrate species (Green pers. Comm.). We could find only 20 species for which we had multiple records of significant temperature coefficients that were also represented in this database, but these are sufficient to demonstrate a very clear tendency for the species that were most negatively affected in our dataset to be amongst the least “temperature tolerant” on the basis of their European distributions as determined by a meta analysis of macroinvertebrate ecological literature (Figure 17).

We hypothesised that negative effects of warmer years would be felt most by the more stenothermic species that inhabit cooler waters, and thus that we would see more negative mean temperature coefficients at higher altitude sites. It is difficult to perceive an obvious relationship when all species in our dataset were considered together. However, there is a clear tendency among stonefly taxa for the size of negative coefficients to increase in colder, higher altitude sites (Figure 18), with some evidence for an effect threshold of around 300 m. Our work therefore strengthens the argument that the biota of upland waters may be particularly sensitive to effects of regional warming.
Conclusions

This study, involving a novel statistical technique developed specifically for the Bicco-Net2 project by BIOSS, provides clear evidence that inter-annual variation in temperature exerts a consistent negative influence on a range of macroinvertebrate taxa inhabiting upland waters. Effects are most pronounced amongst certain families of stonefly and caddisfly and are consistent with the limited number of laboratory and field studies into their temperature sensitivity, and with respect to their current geographical distribution. Effects appear more marked for sites above 300 m altitude, but show subtle differences between species with respect to periods of the year that temperature has most effect, with predominant effects generally occurring during the Autumn. Our study was only able to demonstrate the influence of temperature on the interannual variation in the taxa caught during fixed spring sampling events. However, it is reasonable to assume that long-term warming will have significant negative effects on their populations and drive significant changes in the ecosystem structure and function of upland waters.

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References


Figure 1. Mean (and standard deviation) annual-scale variation in mean annual and mean seasonal air temperature and total annual and total seasonal precipitation across the upland water study sites. Data are derived from 1 km resolution nationally interpolated CHESS model. Temperature data available to 2008 only. Note there is relatively little evidence for directional trends in most metrics over the period of macroinvertebrate sampling (1988 – 2008) with the possible exception of autumn and winter temperature.
Figure 2. a) Variation in sulphate concentration (µeq L⁻¹), a proxy for sulphur deposition, measured in a range of Upland Waters Monitoring Network (UWMN) lakes and streams between 1988-2008. b) Standardised sulphate concentration for all UWMN sites (red lines) superimposed with the mean standardised score (i.e. the sulphate z-score) used as a national-scale sulphur deposition metric (SULPH) in the following analyses.
Figure 3

a) Sign distribution of temperature coefficients of all site-taxa variables. Temperature effects deemed to be significant when likelihood ratio test (LRT) for the full model vs the sample year + sulphate model >10.

b) Sign distribution of precipitation coefficients of all site-taxa variables. Precipitation effects deemed to be significant when likelihood ratio test (LRT) for the full model vs the sample year + sulphate model >10.
Figure 4

a) Temperature coefficients for the most frequently occurring caddisfly families

b) Precipitation coefficients for the most frequently occurring caddisfly families
Figure 5

a) Temperature coefficients for the most frequently occurring caddisfly species

b) Precipitation coefficients for the most frequently occurring caddisfly species
Figure 6

(a) Temperature coefficients for the most frequently occurring stonefly families

(b) Precipitation coefficients for the most frequently occurring stonefly families
Figure 7a. Temperature coefficients for the most frequently occurring stonefly species. Note: no individuals of *Protonemura* sp. are identified down to species level.

Figure 7b. Precipitation coefficients for the most frequently occurring stonefly species. Note: no individuals of *Protonemura* sp. are identified down to species level.
Figure 8. Time series of model fits for the caddisfly *Plectrocnemia conspersa* at eight sites at which antecedent temperature exerts a significant negative effect. Blue line = Null model; Green Line = YEAR + SULPH model; Black line = full model (including negative temperature effect); observations = red circles.
Figure 9a. Distribution of monthly temperature coefficient for the caddisfly *Plectrocnemia conspersa* in fourteen Llyn Brianne streams.

Figure 9b. Distribution of monthly temperature coefficient for the caddisfly *Plectrocnemia spp* in nine UWMN streams and lakes.
Figure 10. Distribution of monthly temperature coefficient for the caddisfly *Plectrocnemia geniculata* in seven Llyn Brianne streams.
Figure 11  Distribution of monthly temperature coefficient for the stonefly *Leuctra inermis* in three UWMN and three Llyn Brianne streams.

Figure 12  Distribution of monthly temperature coefficient for the stonefly *Leuctra nigra* in four Llyn Brianne streams and one UWMN stream.
Figure 13  Distribution of monthly temperature coefficient for the stonefly Protonemura spp. in two UWMN streams, one UWMN lake and two Llyn Brianne streams.

Figure 14  Distribution of monthly temperature coefficient for the stonefly Isoperla grammatica in two UWMN streams, one UWMN lake and two Llyn Brianne streams.
Figure 15  Distribution of monthly precipitation coefficient for the stonefly Isoperla grammatica in two UWMN streams, one UWMN lake and two Llyn Brianne streams.
Figure 16 Distribution of monthly temperature coefficient for the beetle *Anacaena globulus* in five Llyn Brianne streams
Figure 17 The relationship between the mean of mean temperature coefficients of species or genus level taxa and their temperature tolerances inferred through the collation of a range of European studies focussed primarily on geographic distribution. The higher the temperature tolerance index, the more confined a species to the coolest rivers. Note, only site-taxon combinations where LRT for the full model over the YEAR + SULPH model is greater than 10, and mean temperature coefficients ranged between -10 and 10 were used to compute the averages. In the case of four taxa, only two records were available. While temperature tolerance values are available for species only, data were used to represent three taxa at genus level when there was no difference in the tolerance values between species.
Figure 18

a) Relationship between the mean temperature coefficient and site altitude for all stonefly records, across the full altitude gradient

b) Relationship between the mean temperature coefficient and site altitude for all stonefly records – truncated altitude gradient