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Frame, Jessica L.; Jones, J. Iwan; Ormerod, Steve J.; Sadler, Jonathan P.; Ledger, Mark E.

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Biological barriers to restoration: testing the biotic resistance hypothesis in an upland stream recovering from acidification

Jessica L. Frame · J. Iwan Jones · Steve J. Ormerod ·
Jonathan P. Sadler · Mark E. Ledger

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Abstract The biotic resistance hypothesis provides one of several explanations for the limited biological recovery of streams recovering chemically from acidification. The hypothesis proposes that acidification has changed the presence, abundance and interactions among species in acidified streams to the extent that acid-sensitive colonists cannot re-invade even where acidity has ameliorated. As a first step in testing for biotic resistance in streams, we conducted a field experiment to determine whether the success (growth rate) of acid-sensitive recolonists (mayfly nymphs, *Baetis rhodani*) is reduced by competition with abundant acid-tolerant residents (stonefly nymphs, *Leuctra inermis*) in a chemically recovering Welsh stream (UK). Gut contents analysis revealed a marked overlap in resource use between the two species. However,

when *Baetis* was exposed to several (0, 0.25, 0.5 and 1 times ambient) densities of its putative competitor, *Leuctra*, growth rates of the colonist were not affected by the residents at any of the densities tested. These results do not support the hypothesis that resident species constrain colonist populations by affecting growth rates through competition for limited resources or interference. Further work is required to assess whether independent and/or interactive ecological effects of other common residents might affect colonists in ecosystems recovering from past stressors.

Keywords Acidification · Community · Herbivory · Macroinvertebrates · Stressor

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J. L. Frame · J. P. Sadler · M. E. Ledger (✉)
School of Geography, Earth and Environmental Sciences,
University of Birmingham, Edgbaston,
Birmingham B15 2TT, UK
e-mail: m.e.ledger@bham.ac.uk

J. I. Jones
School of Biological and Chemical Sciences, Queen
Mary, University of London, Mile End Road,
London E1 4NS, UK

S. J. Ormerod
Cardiff School of Biosciences, The Sir Martin Evans
Building, Museum Avenue, Cardiff CF10 3AX, UK

Introduction

Declining acid deposition across the northern hemisphere over recent decades has led to widespread improvements in the water quality of formerly acidified surface waters (Battarbee et al., 2014; Monteith et al., 2014). In some areas, mitigation programmes have attempted to restore water quality by applying calcium carbonate to catchments or river channels through ‘liming’ (McKie et al., 2006), yet despite continuing de-acidification in many river systems, there has been only partial biological recovery (e.g. Ormerod & Durance, 2009; Battarbee et al., 2014). Several hypotheses have been proposed to explain the

failure of acid-sensitive species to recolonise watercourses that are apparently recovering chemically (Yan et al., 2003). These hypotheses, which are not necessarily mutually exclusive, include (1) dispersal limitation, (2) abiotic resistance, (3) resource limitation and (4) biotic resistance (e.g. Ledger & Hildrew, 2005; Murphy et al., 2014).

Dispersal limitation could be an important consideration for less mobile aquatic organisms (e.g. Gastropoda) but recent research indicates it is unlikely to be a major factor constraining recolonisation of insects with highly dispersible winged adults (Masters et al., 2007; Murphy et al., 2014). *Abiotic resistance* may also limit the persistence of recolonists where continued acid episodicity arises after heavy rainfall or snowmelt (Lepori et al., 2003; Kowalik & Ormerod, 2006), but episodes may not be a straightforward explanation for limited biological recovery where acidity is ameliorating (Monteith et al., 2014). *Resource limitation* may occur where the low quantity and/or quality of available food constrains recovery (Ledger & Hildrew, 2001a, b; Thomsen & Friberg, 2002). For example, the absence of herbivorous invertebrates in acid streams has been attributed to the impoverished nature of the biofilms at these sites (e.g. Townsend et al., 1983; Dangles & Guérol, 2000). Whilst much evidence points to marked changes at acidified sites in the taxonomic composition of algal assemblages (Hirst et al., 2004; Bray et al., 2008), consequent effects on food quality/quantity remain uncertain (Ledger & Hildrew, 2001a, b; Murphy et al., 2014). Evidence for *biotic resistance* to recovery has been found in lakes (e.g. Vinebrooke et al., 2003), but the hypothesis has received little consideration in rivers and streams (but see Ledger & Hildrew, 2005; Layer et al., 2013). For instance, we do not know whether changes in the presence and abundance of species, and biotic interactions, which occur under acid conditions in turn influence the invasibility of riverine communities as acidity ameliorates (Ledger & Hildrew, 2005; Murphy et al., 2014).

Acidification leads to major shifts in the presence and abundance of species across the food web. Changes in assemblage structure and resulting shifts in the strength and number of species interactions may impart a degree of *biotic resistance* to the re-establishment of acid-sensitive populations once water quality is restored. ‘Community closure’ (sensu

Lundberg et al., 2000) may arise where species loss occurs unevenly across functional groups, altering trophic structure (Hildrew et al., 1984; Planas, 1996). Evidence of ‘competitive resistance’ has been found in acidified lake communities (e.g. Keller & Yan, 1998; Holt & Yan, 2003; Vinebrooke et al., 2003; Frost et al., 2006), where delayed recovery of algal and zooplankton communities following chemical restoration has been attributed to competition with persistent acid-tolerant species, but support in streams remains circumstantial.

In this paper, we examine the potential for biotic resistance to recovery within the benthic macroinvertebrate community of a chemically recovering stream in mid-Wales, UK. Acidified streams in the catchments of the River Wye are improving chemically, both through declining acid deposition and restoration projects (liming), but to date evidence for biological recovery is scarce, with limited persistence of many acid-sensitive invertebrates, especially mayflies (e.g. Bradley & Ormerod, 2002; Ormerod & Durance, 2009). One of the untested mechanisms of community resistance to reinvasion in streams is competition with residents, and it could be that changes in species abundances and interactions occurring as streams acidified place tight constraints upon the subsequent return of sensitive species when acidity ameliorates (Ledger & Hildrew, 2005; Layer et al., 2013).

Stoneflies occur commonly in upland streams and abundances can increase dramatically under high acidity, reflecting compensatory dynamics arising as resources are released by species deletions (Ledger & Hildrew, 2005; Frost et al., 2006; Tixier et al., 2012). We have shown elsewhere that species within the order feed (Ledger & Hildrew, 2000a) and grow (Ledger & Hildrew, 2001a, b) on the sparse algal layers of impoverished acid streams, and it could be that as acidity declines, recolonists such as baetid mayflies face competition with these highly abundant, herbivorous residents. Stoneflies can depress the abundance of algal resources available on stones (Ledger & Hildrew, 2000b) and where they are abundant could limit the availability of algal food for new colonists. As a first step in testing for biotic resistance in streams, we examined the effect of a numerically dominant acid-tolerant resident (Plecoptera: *Leuctra inermis* Kempny, 1899) on the success (growth) of a widespread, acid-sensitive and early recolonist (Ephemeroptera: *Baetis rhodani*

Pictet, 1845) that has so far shown limited persistence in de-acidifying streams (e.g. Bradley & Ormerod, 2002). Several field experiments have demonstrated the potential for competition within the stoneflies (e.g. Ledger & Hildrew, 2000a, b) and mayflies (e.g. Kohler, 1992) characteristic of upland streams, but the extent to which competition occurs between these groups has not been tested within the context of acidification. Specifically, we assessed the degree of resource overlap between *Leuctra inermis* and *Baetis rhodani* and, using a field experiment, tested the hypothesis that the growth rate of a recolonist, *Baetis*, could be affected by *Leuctra*, its putative competitor. Consistent with the biotic resistance hypothesis, we predicted that the growth of *Baetis* would be constrained by *Leuctra* and that mayfly growth rates would decrease with the increasing abundance of the stonefly resident.

Methods

Site description

Research was conducted in a reach of the upper River Wye (site WY34, 52.414772N, -3.659245W) in August 2007. Catchment land use was a combination of pasture, with acid grassland and forests of exotic species such as *Picea sitchensis* (Bong.) Carrière (Forestry Commission, 2002). The site has been monitored routinely as part of long-term research in the Wye and Usk catchments (see Lewis et al., 2007; Frame, 2010). In July 2003, hydrological source areas of the Wye were treated with limestone as part of a large-scale mitigation programme designed to improve water quality and ecological status of sites impacted by acidification (Lewis et al., 2007). Although the main channel of the river was not limed directly, it was subject to the cumulative influence of lime-treated tributaries and was characterised by stable pH during the summer months. River water at the study site was moderately acidic (minimum pH 5.7, mean pH 6.0, maximum pH 6.3 for 2003–2006; study pH 6.1 August 2007) and nutrient poor (mean dissolved calcium 2 mg l⁻¹, nitrate 0.4 mg l⁻¹, phosphate 0.012 mg l⁻¹ for 2003–2006), with relatively low mean dissolved aluminium concentrations (maximum 0.080 mg l⁻¹, minimum 0.009 mg l⁻¹, mean 0.032 mg l⁻¹ 2003–2006, Frame, 2010). Research

was conducted in a physically unstable (Pfankuch index = 73; Pfankuch, 1975) stony riffle dominated by cobbles and gravel where the river was 10 m wide and 0.5 m deep (Frame, 2010).

Life histories of study invertebrates

In the UK *Baetis rhodani* is typically bivoltine, with a summer generation emerging between August and February and an overwintering generation on the wing between March and July (Elliott et al., 1988). The acid-tolerant stonefly *Leuctra inermis* is a univoltine primary consumer with an overwintering generation in which adults are on the wing from April to September and at the study site, nymphs were present all year (Frame, 2010). *Baetis* colonists potentially co-occur with resident *Leuctra* across their aquatic life cycle, from neonate to late instar nymphs, and competition could occur across these life stages. For *B. rhodani*, repopulation of chemically recovering streams is likely to be by dispersal of winged adults from neighbouring catchments (Masters et al., 2007), although drift from less acid upstream reaches is also possible. As neonate colonists hatching from eggs grow and redistribute, the success of this initially sparse population of nymphs is potentially influenced by interactions with acid-tolerant residents, including stoneflies and other macroinvertebrates, over several months. Here, we report the results of an experiment to test the interspecific competitive effect of *Leuctra* residents on *Baetis* in mid-summer when nymphs are maturing, overwintering generations of *Baetis* and *Leuctra* are the dominant life stage in the stream, and the likelihood of confounding acid episodes is at its lowest. Our experiment therefore represents a snapshot of the interactions occurring between the two species later in their life cycles as the nymphs develop.

Competition experiment

An additive experimental design (Inouye, 2001) tested the effect of interspecific competition from resident *Leuctra inermis* on the performance (growth rate) of a focal colonist species, *Baetis rhodani*, using *in-stream* enclosures (plastic containers with lids, 15 cm length × 10 cm width × 5 cm height). Seven treatments were established, representing various density combinations of *Baetis* and *Leuctra* (Table 1). In four treatments, the number of *B. rhodani* individuals was

Table 1 Experimental design of four treatments exposing *Baetis rhodani* (at 10% reference density) to different densities of its competitor, *Leuctra inermis*

Treatment		Number of nymphs in enclosure	
Code	Label	<i>Baetis rhodani</i>	<i>Leuctra inermis</i>
1	10% B, 0% L	5 (10%)	0 (0%)
2	10% B, 25% L	5 (10%)	5 (25%)
3	10% B, 50% L	5 (10%)	10 (50%)
4	10% B, 100% L	5 (10%)	20 (100%)
5	<i>Leuctra</i> only	0	20
6	<i>Baetis</i> only	20	0
7	Ungrazed control	0	0

held constant (5 individuals per enclosure, equivalent to 10% of densities in reference streams, consistent with sparse populations recolonising restored habitats) and exposed to varying densities (0, 25, 50 and 100% ambient density) of its putative competitor, *L. inermis*; treatments 1–4, Table 1). The maximum ambient density of the resident *Leuctra inermis* (1333 m⁻²) was determined from Surber samples (0.0625 m², 250 µm mesh net) collected from 30 sites in the Wye catchment (Frame, 2010). In two further treatments, each species was held in the absence of the other (at equivalent densities, 20 individuals per enclosure) to compare growth rates and assess the potential impact of each species on algal resources (treatment 5–6, Table 1). Finally in an ungrazed control, algal resources were enclosed without animals (treatment 7, Table 1).

Enclosures were fully submerged and placed longitudinally to the flow in blocks of seven in well-lit stony riffles for a period of 23 days. Blocks were replicated seven times (i.e. a total of 49 enclosures), resulting in a 7 × 7 randomised block design. The side walls of the enclosures contained six circular apertures (each 4 cm diameter) of 300 µm mesh. The mesh size allowed water flow through enclosures but prevented departure or arrival of macroinvertebrates. The mesh walls were cleaned weekly during the experiment to reduce clogging. Enclosures were placed in areas of moderately high water velocity, thereby offsetting flow reductions caused by the mesh (cf. Ledger & Hildrew, 2000a, b), and secured to the stream bed using metal stakes. Two rinsed biofilm-coated cobbles from the same stretch of river were placed into each cage, together with clean sand. Biofilms were

characterised by diatoms *Achnantheidium minutissimum* (Kützing) Czarnecki, *Fragilaria* cf. *capucina* var. *gracilis* and *Eunotia exigua* (Bréb. ex Kütz.) (I. Juttner, pers. comm.). Nymphs of *Baetis rhodani* and *Leuctra inermis* were collected from riffles downstream of the experimental area using a hand net (250 µm mesh) and were placed in the enclosures. For each species, nymphs of approximately equal size (mean body length ± SE: *Baetis* 4.8 ± 0.1 mm, *Leuctra* 7.4 ± 0.1 mm) were selected for use in the experiment. Nymphs from the pool of individuals collected from the river were subsampled initially (day 0) for growth determination (100 *Baetis*, 100 *Leuctra*) and gut content analysis (12 *Baetis*, 12 *Leuctra*). At the end of the experiment, all nymphs recovered from enclosures were collected and preserved in 70% industrial methylated spirit and subsequently processed in the laboratory. Water temperature (weekly spot samples) during the experiment ranged from 13 to 17°C.

Algal biomass determination

At the end of the experiment, algal biofilm was scraped from the upper surfaces of two cobbles in each enclosure using a toothbrush, washed into separate scintillation vials with filtered stream water, and frozen immediately in the dark. The scrubbed surface of each cobble was carefully traced onto an acetate sheet and the area (cm²) subsequently determined from the mass of the tracings (Ledger & Hildrew, 1998). The biofilm samples were later defrosted in the laboratory and made up to 24 ml with water. Estimates of ash-free dry mass (AFDM) were made by placing aliquots of biofilm slurry (5–10 ml) in crucibles for ashing at 500°C for 4 h. AFDM was estimated as the difference between oven-dried (80°C) and ashed crucibles (Ledger & Hildrew, 1998).

Gut content analysis

The diet of *B. rhodani* and *L. inermis* was determined to establish the extent of resource overlap between the two species collected from the river ($n = 12$ individuals for each taxon) and experimental enclosures ($n = 12$). Nymphs were rinsed in deionised water to remove preservative, and the contents of the fore-gut teased onto a glass slide, enclosed by a coverslip, and examined under a stereoscopic microscope (Zenith

STZ-4500) (Ledger & Hildrew, 2000a, b; Ledger et al., 2002). The contents were then dispersed into a drop of deionised water using forceps. Prepared slides were examined under a compound microscope (Nikon Alphaphot YS2-T), and particles present in the gut contents were assigned to one of five categories, namely diatoms, filamentous green algae, coccoid green algae, amorphous detritus and inorganic debris (after Ledger & Hildrew, 2000a, b; Ledger et al., 2002). Particle areas were calculated for each nymph by multiplying the number of particles of each type in the field of view by the mean area of each particle type, calculated from 50 randomly selected particles (Ledger & Hildrew, 2000a, b).

Measuring macroinvertebrate growth

In the laboratory, the species identity of all macroinvertebrates collected at the beginning and end of the experiment was confirmed, and the length of individuals was measured using a dissecting microscope ($\times 100$ magnification) and eye-piece graticule (to the nearest 0.1 mm). The length of individuals (BL, mm) was converted to dry mass (DM, mg) using length-dry mass regression equations previously determined for *B. rhodani* and *L. inermis* (Burgherr & Meyer, 1997). The mean instantaneous growth rates (G_w) for each species in each enclosure was then calculated from the initial (W_0) and final dry mass (W_t) of individuals by $G_w \% d^{-1} = (LnW_t - LnW_0/t) \times 100\%$,

where t is the duration of the experiment in days (Ledger & Winterbourn, 2000; Burrell & Ledger, 2003).

Data analysis

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

Dietary overlap of *Baetis rhodani* and *Leuctra inermis* was assessed using detrended correspondence

analysis (DCA) in CANOCO 4.5 (ter Braak & Šmilauer, 2002). Data comprised the percentage contribution of food particle types (diatoms, filamentous greens, coccoid greens, detritus and inorganic debris) in the diet of individual nymphs of each species collected from the benthos (at the start of the experiment) and from the experimental enclosures (at the end of the experimental period).

Results

Gut content analysis revealed substantial overlap in the diet of *Baetis rhodani* and *Leuctra inermis* (Fig. 1). Individuals of both species, collected from the benthos and experimental enclosures, consumed diatoms, filamentous green algae, coccoid green algae, detritus and inorganic debris of varying proportions. Benthic *B. rhodani* consumed proportionally more diatoms and coccoid green algae, and less detritus, than *L. inermis* (Fig. 1). Detrended correspondence analysis (DCA) of gut contents revealed inter-individual variation in the proportions of different food types consumed was greater for *B. rhodani* than *L. inermis*, and greater for caged than benthic nymphs of both species (Fig. 2).

During the 23-day enclosure experiment, individuals of bivoltine *B. rhodani* grew an average of 2.29% dry mass day^{-1} , compared to a rate of 0.85% dry mass day^{-1} for univoltine *L. inermis*. One-way ANOVA revealed no significant effect of *L. inermis* density on *B. rhodani* growth rate ($df = 18$, $F = 0.378$, $P = 0.770$; Fig. 3), whilst a t test revealed that *B. rhodani* growth rates were similar ($df = 9$, $t = 0.319$, $P = 0.757$) at low and high density. There was no statistically significant difference in growth of *L. inermis* among the treatments (One-way ANOVA, $F = 0.977$, $P = 0.423$). One-way ANOVA revealed no significant difference in biofilm biomass (AFDM per cm^2) among treatments ($df = 48$, $F = 0.253$, $P = 0.955$; Fig. 4). Thus, neither *B. rhodani* nor *L. inermis* significantly reduced algal abundance in enclosures.

Discussion

We tested the hypothesis that abundant acid-tolerant resident *Leuctra inermis* could influence the success (growth) of an acid-sensitive recolonist *Baetis*

Fig. 1 Mean percentage composition (food category area as percentage total particle area) of gut contents collected from nymphs of *Baetis rhodani* and *Leuctra inermis* collected from the benthos prior to the start of the experiment, and from the experimental enclosures at the end of the experiment

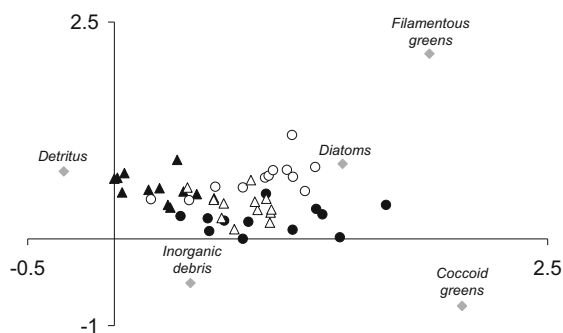
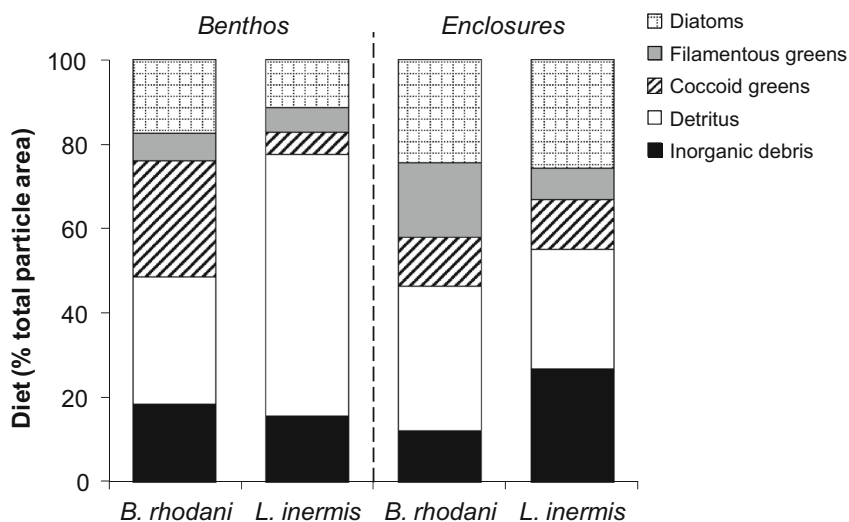


Fig. 2 DCA ordination diagram of diet for *Baetis rhodani* (circles) and *Leuctra inermis* (triangles) from benthic samples (open symbols) and enclosures (closed symbols). Centroids for each food particle type (grey diamonds) are also shown. Axis one has a length of 1.568 SD and an eigenvalue of 0.154, whereas axis two has a length of 1.197 SD and an eigenvalue of 0.062

rhodani, thereby potentially slowing the rate of recovery of mayfly populations via interspecific competition. The results of our enclosure experiment revealed that whilst the two species feed and grow on similar resources, they are unlikely to be in competition for those resources as mature nymphs at ambient densities in these upland streams. Although our data suggest residents are unlikely to influence the rate of invertebrate recolonisation at this stage in their life cycle, more research is required to test for competitive effects acting on early life stages of recolonists (e.g. neonates).

In theory, resident species should compete more strongly with, and limit the establishment of,

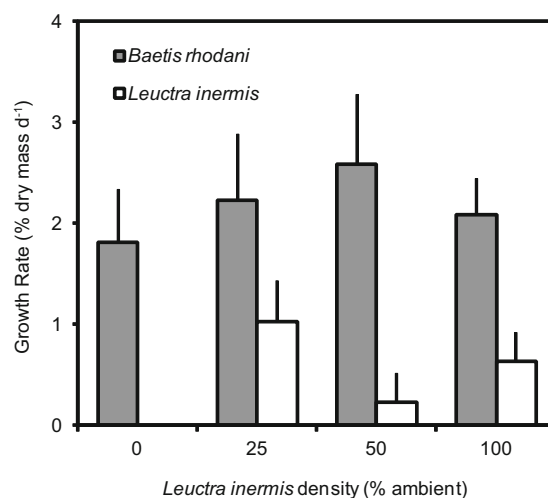


Fig. 3 Mean (+1 SE) instantaneous growth rate (% increase in dry mass per day) of *Baetis rhodani* and *Leuctra inermis* in experimental enclosures. Each of the four treatments exposed the putative colonist *B. rhodani* to different densities of the putative resident competitor, *L. inermis*

recolonising species that have similar resource requirements (Fargione et al., 2003). Our analysis of diet confirmed a significant degree of resource overlap between *Baetis* and *Leuctra*, with both species feeding on biofilm in the benthos and enclosures. Nymphs consumed a range of algae including diatoms and both coccooid and filamentous green algae. Filamentous greens were found in the diet of both species, and although their nutritional value is uncertain (Reynolds, 1992; Junger & Planas, 1993), they could be an

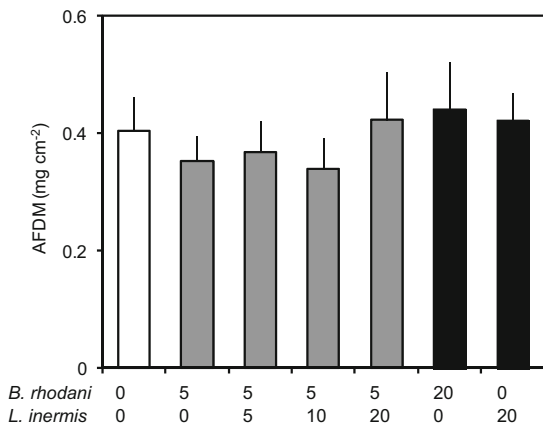


Fig. 4 Final (mean + 1 SE) ash-free dry mass (AFDM) of epilithic biofilm in ungrazed controls (open bar), under grazing by low densities of *Baetis* exposed to a gradient of *Leuctra* (grey bars; Fig. 3), and where *Baetis* and *Leuctra* are enclosed at equivalent high densities (black bars)

important part of the diet, especially in summer months when abundant (Brown, 1961). The occurrence of algae in the diet of *L. inermis* is somewhat unusual for a genus that is assumed to be dominated by obligate detritivores (see Moog, 1995). Nevertheless, the composition of the diets of the species reported here is consistent with data collected from other streams in the UK (e.g. Ledger & Hildrew, 2005; Lauridsen et al., 2012). We also observed broad overlap in the diet of nymphs in benthos and enclosures, suggesting that any interaction between *B. rhodani* and *L. inermis* in the experiment was predicated on realistic resource exploitation, although the lower proportion of detritus in the diet of *Leuctra* in the enclosures compared with the benthos may suggest either increased consumption of algae or a dearth of particulate detritus in the enclosures possibly caused by the washing of cobbles to remove animals during the setup of the experiment, or limited ingress. Nevertheless, detritus was an important component of the diet of both species, although it was proportionally much more abundant in the diet of *Leuctra* collected from the benthos. This, together with lower inter-individual variation in diet among *Leuctra* nymphs, is consistent with contrasting foraging strategies of the two herbivore–detritivores. *Baetis* can swim actively in search of food-rich algal patches, whereas *Leuctra* is a slim-bodied crawler likely to have higher patch fidelity. These contrasting strategies

of food acquisition could lessen any interspecific competition for shared resources, even at small spatial scales.

Resource availability is a key determinant of invasion success in ecosystems recovering from perturbation (Fargione et al., 2003). In streams, herbivorous residents could affect the success of colonists if they exert strong control on algal resource abundance. Many studies have shown that stream invertebrates, including mayflies and stoneflies, reduce algal standing crop and compete for these resources in small streams (e.g. Kohler, 1992; Ledger & Hildrew, 2000b), but we found no evidence for resource depression by either species in this experiment. Weak grazing pressure can be explained by low numerical abundance of consumers, poor match between consumer mouthparts and resource structure, and rates of algal primary production that counteract consumptive losses (e.g. Ledger & Hildrew, 2000a, b). *L. inermis* are relatively small, generalist, nymphs that may not feed as intensively on biofilms as larger, more specialised consumers (e.g. some caddis, snails). Despite small per capita effects, stonefly nymphs can reach high densities in small stony streams, and they may compensate functionally for the loss of acid-sensitive feeders (Layer et al., 2013). Here we used realistic densities of *L. inermis* to define the experimental conditions and failed to detect an effect of competition on *B. rhodani*. Compensatory dynamics have been observed in acid lakes, such that decreases in abundances of acid-sensitive species are coupled to increases in abundance of acid-tolerant functionally similar species (Klug et al., 2000), but the data in streams are equivocal. Our experiment measured the impact of one of the most abundant acid-tolerant herbivores in acid streams, but more research is needed to determine the community-wide consumption of algae by consumers across a range of natural densities.

Food quality may also influence invertebrate growth rates in recovering streams and could present a more immediate threat to the success of recolonists than competition (e.g. Townsend et al., 1983; Winterbourn et al., 1992, but see Hogsden et al., 2013). Indeed, in the present investigation, the growth rate of *B. rhodani* was 2.29% dry mass day⁻¹, lower than values reported elsewhere (Frame, 2010). Low growth rates for *Baetis* in other Welsh streams have been attributed to the chemically impoverished nature of

biofilms in limed streams (Frame, 2010). By contrast, growth of *L. inermis* was greater than in an acid stream in the Peak District (0.37% DM d⁻¹, Pretty et al., 2005), suggesting a more suitable resource environment for this species.

Acidification has had a profound impact on the biota of freshwaters and over recent decades research has documented reduced biodiversity (e.g. Hildrew & Ormerod, 1995; Layer et al., 2013) and marked changes in the relative abundances of species with the decreasing pH (e.g. Townsend et al., 1983). However, far less is known about how these pronounced impacts influence community assembly processes as acidity ameliorates (Ledger & Hildrew, 2005; Layer et al., 2013). We do not yet understand recovery processes in previously acidified streams to know whether biological recovery is limited entirely by only limited abiotic improvement (Kowalik et al., 2007; Ormerod & Durance, 2009; Murphy et al., 2014) or whether altered biota, and feedbacks between abiotic and biotic factors, might delay or divert recovery (Ledger & Hildrew, 2005; Pye et al., 2012), especially in streams. Where abiotic factors constrain biological recovery and biotic factors such as resource availability, competition or predation are trivial, we may expect the empty niches of lost species to be refilled as conditions improve sufficiently to enable re-establishment, and for communities to return to their former state (Sudling et al., 2004). However, where biotic factors intervene, as revealed in recovering lakes (e.g. Keller & Yan, 1998; Holt & Yan, 2003; Vinebrooke et al., 2003; Frost et al., 2006), niches may be filled or modified by residents (Sudling et al., 2004), shifting successional endpoints as acidity declines. Although the research reported here found no evidence for competition as a mechanism limiting biological recovery, we have addressed just one competitive interaction, within the benthic food web, at one point in time. Our experiment was conducted in summer using relatively mature nymphs of both species (medium-sized *Baetis* and larger *Leuctra*), and important questions remain regarding the effect of *Leuctra* (and other taxa) on *Baetis* earlier in the life cycle. In many cases, the dominant route for *Baetis* recolonisation is via egg oviposition as winged adults disperse from unpolluted source streams, and thus, any effect of residents on egg hatchlings could have profound implications for the re-establishment of mayfly populations in chemically recovering streams.

Although practically difficult to manipulate, neonates of *B. rhodani* may be more vulnerable to the competitive effect leuctrid (and other) nymphs than the larger nymphs of the summer generation investigated here. Clearly, much remains to be done to explore interactions among the resources, primary consumers and predators that characterise running waters. Since a recent meta-analysis suggests biotic resistance in freshwaters may be more frequently driven by consumption rather than competition (Alofs & Jackson, 2014), a key priority for future research is to investigate the role of invertebrate predators in the survival and persistence of recolonising species. It could also be that periods of episodic acidity during high flow represents a more immediate threat to the survival and fitness of many acid-sensitive recolonists (Lepori et al., 2003), but more research is needed to determine whether ecological processes and biotic resistance also influence the rate of recovery following restoration in streams.

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References

- Alofs, K. M. & D. A. Jackson, 2014. Meta-analysis suggests biotic resistance in freshwater environments is driven by consumption rather than competition. *Ecology* 95: 3259–3270.
- Battarbee, R. W., E. M. Shilland, M. Kernan, D. T. Monteith & C. J. Curtis, 2014. Recovery of acidified surface waters from acidification in the United Kingdom after 20 years of chemical and biological monitoring (1988–2008). *Ecological Indicators* 37: 267–273.
- Bradley, D. C. & S. J. Ormerod, 2002. Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater Biology* 47: 161–171.
- Bray, J. P., P. A. Broady, D. K. Niyogi & J. S. Harding, 2008. Periphyton communities in New Zealand streams impacted

- by acid mine drainage. *Marine and Freshwater Research* 59: 1084–1091.
- Brown, D. S., 1961. The food of the larvae of *Cloeon dipterum* L. and *Baetis rhodani* (Pictet) (Insecta, Ephemeroptera). *Journal of Animal Ecology* 30: 55–75.
- Burgherr, P. & E. I. Meyer, 1997. Regression analysis of linear body dimensions versus dry mass in stream macroinvertebrates. *Archiv für Hydrobiologie* 139: 101–112.
- Burrell, G. P. & M. E. Ledger, 2003. Growth of a stream-dwelling caddisfly (*Olinga feredayi*: Conosucidae) on surface and hyporheic food resources. *Journal of the North American Benthological Society* 22: 92–104.
- Dangles, O. J. & F. A. Guérol, 2000. Structural and functional responses of benthic macroinvertebrates to acid precipitation in two forested headwater streams (Vosges Mountains, northeastern France). *Hydrobiologia* 418: 25–31.
- Elliott, J. M., U. H. Humpesch & T. T. Macan, 1988. Larvae of the British Ephemeroptera: A Key with Ecological Notes. Freshwater Biological Association, Ambleside.
- Fargione, J., C. S. Brown & D. Tilman, 2003. Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences of the United States of America* 100: 8916–8920.
- Forestry Commission, 2002. National Inventory of Woodland and Trees: Wales. Forestry Commission, Edinburgh.
- Frame, J. L., 2010. Ecology of acidification and recovery in Welsh upland streams. PhD thesis, University of Birmingham.
- Frost, T. M., J. M. Fischer, J. L. Klug, S. E. Arnott & P. K. Montz, 2006. Trajectories of zooplankton recovery in the Little Rock Lake whole-lake acidification experiment. *Ecological Applications* 16: 353–367.
- Hildrew, A. G., C. R. Townsend & J. Francis, 1984. Community structure in some southern English streams: the influence of species interactions. *Freshwater Biology* 14: 297–310.
- Hildrew, A. G. & S. J. Ormerod, 1995. Acidification: causes, consequences and solutions. In Harper, D. M. & A. J. D. Ferguson (eds), *The ecological basis for river management*. Wiley, London: 147–160.
- Hirst, H., F. Chaud, C. Delabie, I. Jüttner & S. J. Ormerod, 2004. Assessing the short-term response of stream diatoms to acidity using inter-basin transplantations and chemical diffusing substrates. *Freshwater Biology* 49: 1072–1088.
- Hogsdon, K. L., M. J. Winterbourn & J. S. Harding, 2013. Do food quantity and quality affect food webs in streams polluted by acid mine drainage? *Marine and Freshwater Research* 64: 1112–1122.
- Holt, C. & N. D. Yan, 2003. Recovery of crustacean zooplankton communities from acidification in Killarney Park, Ontario, 1971–2000: pH 6 as a recovery goal. *Ambio* 32: 203–207.
- Inouye, B. D., 2001. Response surface experimental designs for investigating interspecific competition. *Ecology* 82: 2696–2706.
- Junger, M. & D. Planas, 1993. Alteration of trophic interactions between periphyton and invertebrates in an acidified stream: a stable carbon isotope study. *Hydrobiologia* 262: 97–107.
- Keller, W. & N. D. Yan, 1998. Biological recovery from lake acidification: zooplankton communities as a model of patterns and processes. *Restoration Ecology* 6: 364–375.
- Klug, J. L., J. M. Fischer, A. R. Ives & B. Dennis, 2000. Compensatory dynamics in planktonic community responses to pH perturbations. *Ecology* 81: 387–398.
- Kohler, S. L., 1992. Competition and the structure of a benthic stream community. *Ecological Monographs* 62: 165–188.
- Kowalik, R. A., D. M. Cooper, C. D. Evans & S. J. Ormerod, 2007. Acidic episodes retard the biological recovery of upland British streams from chronic acidification. *Global Change Biology* 13: 2439–2452.
- Kowalik, R. A. & S. J. Ormerod, 2006. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology* 51: 180–191.
- Lauridsen, R. L., F. K. Edwards, M. J. Bowes, G. Woodward, A. G. Hildrew, A. T. Ibbotson & J. I. Jones, 2012. Consumer-resource elemental imbalances in a nutrient rich stream. *Freshwater Science* 31: 408–422.
- Layer, K., A. G. Hildrew & G. Woodward, 2013. Grazing and detritivory in 20 stream food webs across a broad pH gradient. *Oecologia* 171: 459–471.
- Ledger, M. E. & A. G. Hildrew, 1998. Temporal and spatial variation in the epilithic biofilm of an acid stream. *Freshwater Biology* 40: 655–670.
- Ledger, M. E. & A. G. Hildrew, 2000a. Herbivory in an acid stream. *Freshwater Biology* 43: 545–556.
- Ledger, M. E. & A. G. Hildrew, 2000b. Resource depression by a trophic generalist in an acid stream. *Oikos* 90: 271–278.
- Ledger, M. E. & M. J. Winterbourn, 2000. Growth of New Zealand stream insect larvae in relation to food type. *Archiv für Hydrobiologie* 149: 353–364.
- Ledger, M. E. & A. G. Hildrew, 2001a. Growth of an acid-tolerant stonefly on epilithic biofilms from streams of contrasting pH. *Freshwater Biology* 46: 1457–1470.
- Ledger, M. E. & A. G. Hildrew, 2001b. Recolonization by the benthos of an acid stream following drought. *Archiv für Hydrobiologie* 152: 1–17.
- Ledger, M. E. & A. G. Hildrew, 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution* 137: 103–118.
- Ledger, M. E., A. L. M. Crowe, G. Woodward & M. J. Winterbourn, 2002. Is the mobility of stream insects related to their diet? *Archiv für Hydrobiologie* 154: 41–59.
- Lepori, F., A. Barbieri & S. J. Ormerod, 2003. Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams. *Freshwater Biology* 48: 1873–1885.
- Lewis, B. R., I. Jüttner, B. Reynolds & S. J. Ormerod, 2007. Comparative assessment of stream acidity using diatoms and macroinvertebrates: implications for river management and conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 17: 502–519.
- Lundberg, P., E. Ranta & V. Kaitala, 2000. Species loss leads to community closure. *Ecology Letters* 3: 465–468.
- Masters, Z., I. Peteresen, A. G. Hildrew & S. J. Ormerod, 2007. Insect dispersal does not limit the biological recovery of streams from acidification. *Aquatic Conservation: Marine and Freshwater Ecosystems* 17: 375–383.
- McKie, B. G., Z. Petrin & B. Malmqvist, 2006. Mitigation or disturbance? Effects of liming on macroinvertebrate assemblage structure and leaf-litter decomposition in the

- humic streams of northern Sweden. *Journal of Applied Ecology* 43: 780–791.
- Monteith, D. T., C. D. Evans, P. A. Henrys, G. L. Simpson & I. A. Malcolm, 2014. Trends in the hydrochemistry of acid-sensitive surface waters in the UK 1988–2008. *Ecological Indicators* 37: 287–303.
- Moog, O. (ed.), 1995. *Fauna Aquatic Austriaca*, Version 1995. Wasserwirtschaftskataster, Bundesministerium für Land- und Forstwirtschaft, Wien.
- Murphy, J. F., J. H. Winterbottom, S. Orton, G. L. Simpson, E. M. Shilland & A. G. Hildrew, 2014. Evidence of recovery from acidification in the macroinvertebrate assemblages of UK fresh waters: a 20-year time series. *Ecological Indicators* 37: 330–340.
- Ormerod, S. J. & I. Durance, 2009. Restoration and recovery from acidification in upland Welsh streams over 25 years. *Journal of Applied Ecology* 46: 164–174.
- Pfankuch, D. J., 1975. *Stream Reach Inventory and Channel Stability Evaluation*. United States Department of Agriculture Forest Service, Region 1, Missoula.
- Planas, D., 1996. Acidification effects. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds), *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, San Diego: 497–522.
- Pretty, J. L., D. J. Giberson & M. Dobson, 2005. Resource dynamics and detritivore production in an acid stream. *Freshwater Biology* 50: 578–591.
- Pye, M. C., I. P. Vaughan & S. J. Ormerod, 2012. Episodic acidification affects the breakdown and invertebrate colonisation of oak litter. *Freshwater Biology* 57: 2318–2329.
- Reynolds, C. S., 1992. Algae. In Calow, P. & G. E. Petts (eds), *The Rivers Handbook*, Vol. 1. Blackwell Scientific Publications, Oxford: 195–215.
- Sudling, K. N., K. L. Gross & G. R. Houseman, 2004. Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology and Evolution* 19: 46–53.
- ter Braak, C. J. F. & P. Šmilauer, 2002. *CANOCO Reference Manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5)*. Microcomputer Power, New York.
- Thomsen, A. G. & N. Friberg, 2002. Growth and emergence of the stonefly *Leuctra nigra* in coniferous forest streams with contrasting pH. *Freshwater Biology* 47: 1159–1172.
- Tixier, G., V. Felten & F. Guérol, 2012. Population characteristics and feeding habits of *Siphonoperla torrentium* subject to varying prey, predator, and competitor abundance across an acidification gradient. *Freshwater Science* 31: 191–204.
- Townsend, C. R., A. G. Hildrew & J. Francis, 1983. Community structure in some southern English streams: the influence of physiological factors. *Freshwater Biology* 13: 521–544.
- Vinebrooke, R. D., M. D. Graham, D. L. Findlay & M. A. Turner, 2003. Resilience of epilithic algal assemblages in atmospherically and experimentally acidified boreal lakes. *Ambio* 32: 196–202.
- Winterbourn, M. J., A. G. Hildrew & S. Orton, 1992. Nutrients, algae and grazers in some British streams of contrasting pH. *Freshwater Biology* 28: 173–182.
- Yan, N. B., B. Leung, W. Keller, S. E. Arnott, J. M. Gunn & G. G. Raddum, 2003. Developing conceptual frameworks for the recovery of aquatic biota from acidification. *Ambio* 32: 165–169.