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Article

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Changes in macroinvertebrate community structure provide evidence of neutral mine drainage impacts

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Abstract

Contamination of aquatic environments as a consequence of metal mining is an international issue. Most historic studies have considered the impact of acid mine drainage (pH < 6) on instream communities and comparatively little attention has been given to sites where drainage is typically circum-neutral (6 > pH < 8). Here, the impacts of historic mining activities on the benthic macroinvertebrate community of a circum-neutral river in central Wales are assessed. Biotic and diversity indices, widely used for biomonitoring purposes, indicate aquatic macroinvertebrate assemblages within the Afon Twymyn to be in a good condition, despite severe metal contamination of bed sediments and river water. However, Canonical Correspondence Analysis identifies differences in community structure between mining impacted and unimpacted reaches of the river associated with chalcophile (Zn, Pb, Cu, Cd) and common (Fe and Mn) metals. Stream pH was not a significant factor structuring the macroinvertebrate community. Widely utilised macroinvertebrate indices failed to identify impacts at the community level because they either seek to identify impacts of a specific contaminant or are dependent on a model community response to a given stress. The nature of metal mine discharges is temporally complex, having highly variable chemical signatures and as a result, care is advised when interpreting and modelling community impacts. The use of standard macroinvertebrate biotic and diversity indices in the context of the EU Water Framework Directive could lead to erroneous classifications of aquatic ecosystem health when used for bio-monitoring rivers affected by neutral mine drainage where other indicators are unavailable.

Keywords: metal mine, neutral mine drainage, toxic metals, macroinvertebrate, bioindices, canonical correspondence analysis, water quality

1. Introduction

Contaminated drainage from abandoned metal mines is an environmental problem of international significance that can impact stream hydrochemistry, sediment geochemistry and ecological communities. [1, 2, 3, 4] Documented impacts associated with abandoned mines have been primarily associated with the weathering of metal sulphides (in particular the iron disulphide, pyrite) and the generation and release of acid mine drainage (AMD), a leachate typically high in dissolved toxic metals, sulphates and acidity. [5] Contaminants can be released from mine sites into river systems through surface runoff from mine spoil / tailings, drainage from adits / shafts, and dry aerial deposits that arise from smelting. [2] Contamination from mine drainage can persist for centuries after the closure of a mine as long as there is a supply of reactive sulphidic material. [6] In addition, toxic metals will persist in riverbed and floodplain sediments long after mining has ceased. [7] Metal mine drainage can severely impact aquatic ecosystems by affecting primary and secondary production, nutrient cycling, energy flow and decomposition processes. [4, 8, 9] As a consequence, riverine ecosystems in metal mining districts are generally impoverished and suffer chronic long-term contamination.

A large number of experimental studies have documented the impact of contaminated mine drainages on instream macroinvertebrate communities and inferred the general health of aquatic ecosystems in mining-impacted river systems. [2, 10] Such impacts include changes in community structure and composition, [3, 11] changes in organism physiology and behaviour, [12, 13] genetic and morphological mutations, [14, 15] and direct mortality. [16] However, in other studies, effects have not been detected or only limited changes to the community have been observed. [17]

The majority of experimental studies associated with metal mining activity have considered the impacts of AMD (pH < 6) on macroinvertebrate communities, since this is generally considered to be the most toxic form of mine drainage and typically leads to an impoverished aquatic ecosystem. [18] Many taxa appear to display a pH-dependent response to AMD. [19] Acidic conditions may result in toxicity, although acidity in mine discharges is also usually associated with elevated concentrations of toxic aqueous metal species. Most commonly, a decrease in pH will lead to an increase in the availability of toxic free metal ions due to changes in metal speciation, mobility and bioavailability. [18] It is often the combined effects of dissolved metals and acidity which result in the greatest impacts upon aquatic communities. [20] However, not all mine discharges are acidic and water chemistry can vary considerable between regions as a consequence of lithological and mineralogical setting. For example, in regions where carbonate lithology predominates (e.g., Carboniferous Limestone), neutral to basic mine discharges are common and these can have significantly lower concentrations of dissolved toxic metals than is typical of AMD. [21] Relatively little research has been undertaken on the impact of neutral mine drainage (NMD) (6 > pH < 8) on instream communities. However, rivers receiving NMD may still have highly elevated concentrations of dissolved metals and the impacts of these discharges on aquatic ecosystems need to be quantified in order to meet the strict water quality and ecological guidelines specified by the EU Water Framework Directive (EU/2000/60/E).

In this study, we investigate the impact of NMD on the benthic macroinvertebrate community of a mining-impacted river in central Wales. We examine whether traditional macroinvertebrate bio-monitoring metrics and indices, based on the effect of specific contaminants or model community responses to stress, can detect changes in community structure arising from NMD when compared to multivariate techniques. Our objectives are to: a) quantify macroinvertebrate community health using a range of commonly utilised biotic and diversity indices used in routine bio-monitoring programmes in the UK; b) compare the effectiveness of macroinvertebrate indices and multivariate statistical techniques for elucidating impacts of NMD on a stream ecosystem; and c) identify the chemical variables associated with NMD that potentially influence the structure of the macroinvertebrate community.

2. Methods

2.1 Study area

Dylife mine is located in the Central Wales Mining District, approximately 20 km south-west of Machynlleth at an elevation of 375 m AOD **(Figure 1)**. The mine site is drained by the Afon Twymyn, which at its junction with the Afon Laen, has a catchment area of 35 km^2 and considerable range of elevation (98 – 530 m AOD). Mining ceased at Dylife in the 1920s. Although it was worked primarily for Pb and smaller quantities of Zn, Cu and Ag from Roman times; the principle ore extraction occurred in the mid to late 19th century. The catchment lies on Upper Silurian argillaceous sediments, mainly comprised of mudstone and siltstone turbidites and hemipelagites with interbedded sandstones. [22] Diagenesis associated with igneous activity during the Caledonian (444 – 416 Ma) and Hercynian (390 – 310 Ma) orogenies gave rise to mineralisation related to hydrothermal and meteoric groundwater solutions. [22] The ores are primarily sphalerite and galena. Calcite and dolomite are rare, as are secondary minerals. [23]

The impact of mine drainage on the water quality of the Afon Twymyn has been evaluated previously. [24, 25] Dissolved metal concentrations are high downstream of the mine under base flow conditions (mean values: 269 $\mu g\ l^{-1}$ Pb and 1044 $\mu g\ l^{-1}$ Zn) [26] with significant flushing of dissolved metals (up to 6150 $\mu g\ l^{-1}$ Pb and 1790 $\mu g\ l^{-1}$ Zn) occurring during storm flow events. [25] The pH of the river water is circum-neutral and ranges from 6.0 to 7.5. Mine spoil leachate and water obtained from mine portals have a pH range of 5.9 to 6.9. The absence of significantly acidic drainage at Dylife is primarily related to the sedimentary lithology and the lack of pyrite in the country rock. There has been no comprehensive geochemical characterisation of the mineralisation or the mine wastes at Dylife, although the geochemistry of the river surface sediments has been characterised. [27, 28] Lead and Zn concentrations in the stream sediments are highly elevated and above levels reported to have deleterious impacts on aquatic ecology. [29] Most toxic metals exist in the most mobile, easily exchangeable and carbonate-bound geochemical phases, and as such pose a threat to the ecosystem.

2.2 Biological data

Three baseline surveys of the macroinvertebrate community were undertaken – in June 2007, October 2007, and March 2008, representing high, medium and low flow conditions in the Afon Twymyn. Samples were collected at 29 sites along the channel (**Figure 2**). Ten sites were located upstream of Dylife mine and represent a ‘reference condition’ biological community, unimpacted by contamination from the mine and were classed as ‘control’ sites. Thirteen sites were located at Dylife mine and were classified as ‘impacted’ due to their proximity to the mine and former workings. The remaining six sites were located downstream of Dylife mine and are classified as ‘recovery’ sites, because a gradual improvement in water and sediment quality occurs downstream of the mine. [4, 24, 27] Samples were collected using a Surber sampler (250 µm mesh and 0.1 m^2 frame) over a 1-minute period. Large rocks and woody debris were removed from the sample frame and any attached invertebrates washed into the net. Three replicate samples were collected at each sample site and combined to form one composite sample. In the laboratory, macroinvertebrates were identified to species level where possible, although some groups, such as Oligochaeta were recorded at order level and others such as Diptera were identified to family level and some families of mayfly and stonefly larvae were recorded at genus level (e.g., Heptegenidae – *Heptegenia* sp. and Chloropelidae – *Chloroperla* sp.).

2.3 Univariate biological indices

Due to the complex nature of metal mine discharges, a range of diversity and biotic indices were used to characterise the health of the macroinvertebrate community. The diversity indices used were the Shannon-Weiner diversity index (H’) [30] and Berger-Parker dominance index (d) [31] which consider the number of species and distribution of density among samples. The following biotic indices, widely used in the UK for bio-monitoring purposes, were derived: Biological Monitoring Working Party (BMWP) index, [32] Average Score Per Taxon (ASPT), [33] Acid Waters Indicator Community Index (AWIC) [34] and the number of Ephemeroptera (mayfly), Plecoptera (stonefly) and Trichoptera (caddis fly) (EPT) taxa. [35] These indices consider the responses and sensitivity / tolerance of organisms at a variable taxonomic resolution along known stressor gradients. The number of taxa present and the abundance of individuals were also used to characterise the macroinvertebrate community. All biotic and diversity indices, except AWIC, were derived using the Species Diversity and Richness software (Version 2). [36] The AWIC index was calculated using the methodology and equation outlined in the original publication. [34] One-way Analysis of Variance (ANOVA) was used to test for significant differences in indices between sample locations (control, mining-impacted and recovery) and time periods (March, June, October). Where the data failed to comply with the normality of distribution assumption, the non-parametric equivalent (Kolmogorov-Smirnov test – Non-parametric ANOVA) was used. Where significant differences occurred, Mann Whitney U tests were used to determine where significant differences between groups occurred.

2.4 Environmental data

Physico-chemical (pH, temperature and conductivity) and water quality (dissolved metals – Pb, Zn, Cu, Cd, Fe, Mn) data were available for the 29 macroinvertebrate sample locations on the Afon Twymyn and for the three time periods under consideration. [37] Bioavailable metal concentrations (Pb, Zn, Cu, Cd, Fe, Mn) in the river bed sediment were also available for June 2007. [28] Bioavailable metals represent the acid-soluble metal phase (easily exchangeable and bound to carbonates) and were determined according to a three-step sequential extraction procedure. [38] Bioavailable metals are used in preference to total metal concentrations in this contaminant-ecosystem assessment as they present a more realistic picture of the risk of metal toxicity.

2.5 Multi-variate analysis of biological and environmental data

Structural difference in the macroinvertebrate community data from all 29 sites and from the three sample time periods were examined using Canonical Correspondence Analysis (CCA) within the program CANOCO 4.5. [39] Species abundances were transformed (+1, log) to reduce the clustering of common and abundant taxa at the centre of the ordination plot. Rare taxa were down-weighted in preliminary analyses, although this had little effect on the output. As a result, all taxa with an abundance less than or equal to 4 were removed from CCA analyses due to the overriding influence these taxa had on the output. Macroinvertebrate community and environmental data were examined in a series of analyses to explore the associations between mine-derived contaminants and the macroinvertebrate community. A series of six analyses were undertaken to determine if the community response to the environmental variables considered were similar between control, impact and recovery sites and also between surveys (**Table 1**). If the different surveys produced markedly different community responses when considering the same environmental variables, it might suggest that there may be other factors (not sampled) influencing the community. The statistical significance of each of the individual environmental parameters and the axes were examined using the forward selection procedure via Monte Carlo random permutation test (999 random permutations).

3. Results

3.1 Spatial variation in macroinvertebrate community

A total of 87 macroinvertebrate samples (29 samples times 3 sample periods) were collected from the channel bed and 39 taxa were recorded in these samples. Overall, Ephemeroptera (mayfly larvae) comprised 42% of the macroinvertebrate community, with Plecoptera (stonefly larvae) (25%) and Diptera (true fly larvae) (23%) being the next most abundant orders. However, this masks significant differences in community structure that appear to be strongly associated with metal-related environmental impacts (**Table 2**). At control sites, the major macroinvertebrate taxa recorded were Diptera – 46% (true fly larvae – primarily Chironomidae and Simuliidae), Plecoptera – 23% (stonefly larvae from four families: Leuctridae (primarily *Leuctra hippopus*), Chloroperlidae, Nemouridae (*Amphinemura sulcicollis* and *Protonemura praecox*) and Perlodidae (primarily *Isoperla grammatica*) and Ephemeroptera – 21% (mayfly larvae from two families: Baetidae (primarily *Baetis rhodani*) and Heptegeniidae). In contrast, there was a different community structure at impacted sites, where the assemblage was dominated by Ephemeroptera (55%) and the numbers of both Plecoptera (21%) and Diptera (16%) were reduced. Downstream from the mine, at recovery sites, macroinvertebrate communities were less dominated by a single order. Here, the relative abundance of Ephemeroptera decreased (38%) and Plecoptera increased (34%), while the percentage of Diptera (16%) remained similar. The proportion contribution to the benthic community of other major macroinvertebrate orders remained stable throughout the Afon Twymyn, with the exception of Trichoptera (caddisfly larvae – Hydropsychidae, primarily

Hydropsyche siltalai; Polycentropidae, primarily P*olycentropus flavomaculatus;* Rhyacophilidae, primarily *Rhyacophila dorsalis*; and cased caddis from the family Limnephilidae), which increased from 2.9% at control and impacted sites to 5.7% at recovery sites. Macroinvertebrate taxa recorded under 'Other' were encountered infrequently and included Oligochaeta, Gammaridae, Sphaeriidae, Zygoptera, Ansioptera, Lymnaeidae, Planariidae and Ostracoda.

3.2 Spatial and temporal variation in biological indices scores

Macroinvertebrate community diversity (Shannon-Weiner) and dominance (Berger-Parker) indices were generally not significantly different between either location or sample period groups (**Table 3**; **Figure 3a and 3b**). However, mean scores for the entire study period did increase (dominance) and decrease (diversity), respectively, at impacted sites. No significant differences were recorded for BMWP or ASPT scores between locations or sample periods (**Table 3; Figure 3c and 3d**). Using the Environment Agency of England and Wales biological quality classification scheme (BMWP scores), the biological quality of the Afon Twymyn during the study period is classified as fair to good. The Number of EPT taxa at impacted and recovery sites were significantly higher (p = < 0.01) when compared with control site values (**Table 3; Figure 4a**). From a total of 51 possible AWIC scoring taxa, 28 occurred at the study sites. The majority of the missing taxa were from the acid sensitive AWIC groups. AWIC scores increased with distance downstream (**Figure 4b**) and impacted and recovery sites had significantly higher scores (p = < 0.01) than the upstream control sites (**Table 3**). Spatially, macroinvertebrate abundances were relatively in variable between sample groups in March. However, there was a significant increase recorded in values at impacted and recovery sites in June (p = < 0.01) and October

(p = < 0.05) (**Table 3; Figure 4c**). An increase in abundance was observed with distance downstream in the catchment. However, the number of macroinvertebrate taxa present in the Afon Twymyn was comparable throughout the year (**Figure 4d**) and no significant differences were observed between sample location or time period (**Table 3**).

3.3 Multi-variate analysis of species-environment relations

Each of the six CCA's incorporating macroinvertebrate abundance, physico-chemical, water and sediment quality data identified significant patterns in species composition associated with environmental conditions. All of the CCA's clearly identified three groups of environmental variables that distinguished the control, (metal mine) impacted and recovery sites: i) chalcophile metals - Zn, Pb, Cu and Cd), ii) common metals - Fe and Mn and iii) physico-chemical measurements - pH, temperature and conductivity (**Figure 5a and 6a**). In all six CCAs, the first canonical axis confirmed the presence of a significant environmental gradient within the data (p < 0.05). Examination of the individual variables using the forward selection procedure indicated that one or more of the following parameters were significant in accounting for macroinvertebrate community variability in all analyses: Fe, Pb, Zn or sediment-bound bioavailable Zn (**Table 4**). The remaining variables were significant factors in three (conductivity), two (water temperature) and one CCA (Cu and Cd), respectively. pH and Mn were not significant factors in any of the analyses. The fraction of variance in the species-environment relation explained by the first canonical axis ranged from 27.5% (Run 6) to 46.9% (Run 3) and the cumulative variance explained by the first four axes ranged from 68.7 (Run 4) to 91.2% (Run 3)

(**Table 5**).

Examination of the distribution of taxa on the first two canonical axes of all analyses identified similar association between taxa and environmental parameters, even though the pattern and direction of the axes varied (**Figures 5 and 6**). Three taxa were consistently associated with bioavailable sediment-bound metals – *Oulimnius* sp., *Limnephilidae* sp. and *Polycentropus flavomaculatus* (**Figure 5b**). The majority of taxa were not associated with any metal gradient and none were strongly associated with dissolved chalcophile metals (Cd, Cu, Pb and Zn; **Figure 6b**). A number of taxa displayed no discernible sensitivity or association with any of the environmental variables, being located consistently at the origin of the bi-plots. These included *Chloroperla* sp., *Baetis rhodani*, Heptageniidae, Simuliidae, *Rhyacophila dorsalis*, *Hydropsyche siltalai*, and *Leuctra hippopus*.

4. Discussion

4.1 The performance of biotic and diversity indices

A number of studies have reported a reduction in diversity and abundance in metal mining-impacted rivers and, in some cases, an associated increase in the numbers of tolerant taxa. [40, 41, 42] However, none of the diversity and biotic indices used in this study detected any significant adverse impacts on macroinvertebrate assemblages that could be associated with the metal contamination of the river water and bed sediment. The Shannon-Weiner diversity and Berger-Parker dominance indices were generally not significantly different between locations or sampling periods. This is in marked contrast to the results of some other studies associated with metal mine pollution. [41, 43, 44, 45, 11] Diversity indices might be favoured over traditional biotic indices in mine drainage pollution studies because they measure total environmental stress and not the impact of a specific contaminant. [46, 47] They are based on a theoretical response of a community to a contaminant, where as stress increases, sensitive taxa are progressively excluded, so that taxa richness falls and tolerant taxa are found in high abundances. [48] In the present study, some taxa (e.g., *Baetis rhodani*, *Leuctra hippopus*) appeared to dominate the contaminated environment but not to the extent of exclusion of other taxa.

The BMWP score and ASPT were not significantly different between locations or sample periods. Despite some success in detecting the effects of AMD in previous studies, [49, 50, 47] these biotic indices are based on saprobity and are generally not suitable for detecting the effects of toxic contaminants. [51] However, they have been used to assess ecological impacts in metal mining-impacted rivers. Measurements of EPT taxa have been reported to elucidate the effects of AMD, [51, 52] although seasonal variation in EPT taxa abundance in response to temperature variation could confound this pattern. [53] In this study, slightly higher (but not significantly different) EPT scores were recorded at impacted sites, influenced by the presence of large numbers of Plecoptera taxa, which are generally considered to be tolerant of metal pollution. [54, 55] The AWIC index scores generally improved with distance downstream corresponding to the gradual increase in stream pH with increasing distance from the source of the river. Therefore, this pressure-specific index successfully identified changes in acidity and its impact on macroinvertebrate communities. However, the index could not be expected to detect the impact of NMD at Dylife because mine drainage did not reduce the pH of impacted sites below that of control sites. Little is also known of how appropriate the AWIC index is for discharges with high concentrations of metals [2] and it has been suggested that a modification may be required to incorporate metal toxicity. [47]

4.2 Multivariate analysis of species-environment relationships

The results presented in this paper illustrate that traditional bioassessment indices may not be able to detect impacts on benthic communities in rivers receiving NMD even when the river water and bed sediment are highly contaminated by metals. However, Canonical Correspondence Analysis (CCA), which contained both biological and environmental diagnostics, identified significant environmental (metal) gradients within the data. The most significant environmental parameters influencing community composition across all six CCAs were dissolved Fe, Pb, Zn, sediment-bound Zn and conductivity. This supports findings reported in previous research where dissolved Pb, [55] and particularly dissolved Zn [49, 52, 55] have been observed to influence macroinvertebrate community composition in metal mining-impacted rivers. However, river pH was not a significant parameter influencing macroinvertebrate community composition in this study, suggesting that the principal environmental stress on the community is associated with dissolved and sediment-bound bioavailable metals.

Metal contaminants (dissolved and sediment-bound) accounted for between 75 and 91% of the variation in macroinvertebrate community composition. The greatest variance in community structure was explained by the bioavailable metal fractions in the river bed sediments, possibly indicating the importance of biological uptake of metals from the sediment and surface biofilms. Given the high concentrations of bioavailable metals in the sediment of the Afon Twymyn, [28] metals would be expected to accumulate to a high degree in primary producers and, as a result might have an impact on the abundance of those taxa feeding on them. [56, 57, 58] In this study, Ephemeroptera were found to be the most abundant macroinvertebrate at mining-impacted sites. This appears to contradict the results of many other studies. [59, 40, 60, 61, 52, 41, 62, 55] Stonefly larvae were also widespread at impacted sites, although this order of insects has been noted for its metal and acid tolerance. [55] In both instances, the abundance was dominated by a single species of each order, Ephemeroptera by *Baetis rhodani* and Plecoptera by *Leuctra hippopus*. These taxa are both algal scrapers feeding on algae and biofilms on the surface of sediments, and both have an acknowledged tolerance of metal contamination. [54, 60, 63] Other herbivorous mayfly taxa (e.g., *Siphlonurus lacustris* and *Ephemerella ignita*) were rare at impacted sites when compared with control and recovery sites. A study of metal bioaccumulation in macroinvertebrates at Dylife mine found *Baetis* sp. and another Ephemeroptera herbivore, *Rhithrogena* sp. (Heptageniidae), to contain far higher Zn concentrations than other taxa. [24] It was reported that Plecoptera (*Protonemura praecox* and *Amphinemura sulcicollis*) sampled at Dylife mine contained far lower Zn concentrations in their bodies than *Baetis* sp. and *Rhithrogena* sp and that Ephemeroptera appeared to reach equilibrium with Zn concentrations in the local environment. [24] It appears that Plecoptera may be able to resist uptake of Zn and other metals through absorption on to external cuticle surfaces and / or accumulation and concentration in the gut prior to excretion. [64] However, Zn is not the only contaminant in the river system and, in terms of overall concentration in the sediment, bioavailable Pb is by far the greatest concern. In the absence of other measured metal tissue concentrations, it may be hypothesised that over time, these macroinvertebrate taxa have acquired tolerances to Pb through exposure over evolutionary timescales. [65, 66, 67, 68]

4.3 Moving forward with bioassessment tools for metal mining-impacted rivers

The scientific literature reports highly variable performances of macroinvertebrate bioindices as indicators of contamination in metal mining-impacted rivers. [4] This is undoubtedly related to the varying character of metal mine pollution, the individual response of species to contamination, and the length of time the ecological community has been exposed to the contamination. Currently, a universal index for determining ecological health in metal mining-impacted rivers is not available, due to the difficulties posed by the many biotic and abiotic factors influencing community structure. Such an index would need to incorporate the effects on a community of multiple environmental stressors, each of which varies in importance over time and distance from source. Whether this is achievable is unclear given the complex multi-parameter interactions that need to be considered. However, this research suggests caution is required when interpreting ecological status at abandoned metal mines for the EU Water Framework Directive based on benthic invertebrates alone. The results presented highlight that widely used macroinvertebrate indices were largely insensitive and some failed to identify the effects of high metal concentrations. Therefore, wherever possible, a multi-proxy approach including both biotic (diatoms, macrophytes, benthic invertebrate and fish) and chemical parameters should be used to avoid errors in the classification of rivers receiving NMD.

In the absence of a reliable biological macroinvertebrate index for metal mining-impacted rivers, assessments of mine water impacts on ecological communities should incorporate several biodiagnostic and multivariate statistical analyses in order to detect impacts from this multi-factor contaminant which varies widely in chemical signature. Assessments should make use of a weight-of-evidence approach, utilizing a combination of water quality, sediment quality, physico-chemical, habitat and toxicity characterisations rather than a single tool approach. [69] Such assessments would be time- and labour-intensive and so would be best suited to the most severely contaminated river systems where traditional monitoring and management techniques have not been able to identify or adequately address the problem.

5. Conclusions

The investigation of ecological impacts of mine water contaminants in the Afon Twymyn using traditional univariate biological indices did not reveal any adverse effects. These results were surprising given the known severe level of metal contamination of the water and sediment in the river and it might be reasonable to assume that the lack of significantly acidic drainage from the mine is an important factor confounding the analyses using biological indices. However, Canonical Correspondence Analysis was able to reveal differences in community structure between control and mining-impacted survey sites in the river which are likely to be related to significant metal contamination of the river water and bed sediments in the vicinity of the abandoned mine. Up to 90% of the variation in taxa composition in the river could be explained by dissolved and sediment-bound bioavailable metals, whereas stream pH was not a significant factor in any of the analyses undertaken. Sediment-bound bioavailable metal contaminants (Pb, Zn, and Fe) accounted for most of the variation recorded in macroinvertebrate community structure and impacted sites were dominated by metal-tolerant, benthic taxa which feed on biofilm and algae.

The univariate biological indices utilised in this study did not identify impacts associated with the mine drainage probably because they either seek to identify impacts of a specific contaminant (when mine drainage is typically multi-factorial) or are dependent on a model community response to stress (which might only occur with the most severe acidic metal mine drainages). In this regard, it is recommended that several biological assessment methods are used together to identify possible ecological impacts in rivers receiving neutral mine drainage. Where biotic and diversity indices are unresponsive to metal mine drainage, multivariate analysis methods may provide a powerful alternative capable of identifying changes in community structure associated with the complexity of metal mine drainage.

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Figure captions

Figure 1. Central Wales mining district with location of Afon Twymyn catchment and Dylife mine.

Figure 2. Location of instream macroinvertebrate, water and sediment sample sites in the Afon Twymyn catchment.

Figure 3. Mean (±) of (a) Shannon-Weiner Diversity, (b) Berger-Parker Dominance, (c) BMWP and (d) ASPT scores for three sample time periods at control, impacted and recovery sites in the Afon Twymyn.

Figure 4. Mean (±) of (a) number of EPT taxa, (b) AWIC index, (c) abundance and (d) number of taxa for three sample time periods at control, impacted and recovery sites in the Afon Twymyn.

Figure 5. CCA bi-plots for Run 3 (June, sediment data only): (a) site-environment and (b) species-environment. Isope = *Isoperla grammatica*, Rhyac = *Rhyacophila dorsalis*, Empid = Empididae, Perlo = Perlodidae, Oligo = Oligochaeta, Dicr = *Dicranota* sp., Hydra = *Hydraena* sp., Ephem = *Ephemerella ignita*, Chiro = Chironomidae, Simul = Simuliidae, Siphl = *Siphlonurus lacustris*, Helop = *Helophorus* sp., Scirt = Scirtidae, Chlor = *Chloroperla* sp., Hydro = Hydrophilidae, Hepta =

Heptageniid sp., Elmis = *Elmis aenea*, Limni = *Limnephilidae* sp., Baeti = *Baetis rhodani*, Leuct = *Leuctra hippopus*, Oulim = *Oulimnius* sp., Polyc = *Polycentropus flavomaculatus* sp., Tipul = Tipulidae.

Figure 6. CCA bi-plots for Run 6 (combined water quality dataset): (a) site-environment and (b) species-environment. Isope = *Isoperla grammatica*, Rhyac = *Rhyacophila dorsalis*, Empid = Empididae, Perlo = Perlodidae, Oligo = Oligochaeta, Dicr = *Dicranota* sp., Hydra = *Hydraena* sp., Ephem = *Ephemerella ignita*, Chiro = Chironomidae, Simul = Simuliidae, Siphl = *Siphlonurus lacustris*, Helop = *Helophorus* sp., Scirt = Scirtidae, Chlor = *Chloroperla* sp., Hydro = Hydrophildae, Hepta = *Heptageniid* sp., Elmis = *Elmis aenea*, Limni = *Limniphilidae* sp., Baeti = *Baetis rhodani*, Leuct = *Leutra hippopus*, Oulim = *Oulimnius* sp., Polyc = *Polcentropus flavomaculatus* sp., Tipul = Tipulidae, Amphi = *Amphinemura sulicollis*, Proto = *Protonemura praecox*, Taban = Tabanidae.

Table 1. Details of the six CCA model runs including biological and environmental data.

CCA Model Run	**Data included in each run**
Run 1	Macroinvertebrate abundance and water quality data (March)
Run 2	Macroinvertebrate abundance and water quality data (June)
Run 3	Macroinvertebrate abundance and sediment quality data (June)
Run 4	Macroinvertebrate abundance, water and sediment quality data (June)
Run 5	Macroinvertebrate abundance and water quality data (October)
Run 6	Macroinvertebrate abundances and water quality data (March, June, October)

Table 2. Percentage of the most common macroinvertebrate families at control, impacted and recovery sites in the Afon Twymyn. Values in the 'All sites' column represent the percentage of each family making up the macroinvertebrate order at all sample sites.

Macroinvertebrate order and *family*	**Control (%)**	**Impacted (%)**	**Recovery (%)**	**All sample sites (%)**
Diptera				
Chironomidae	49	23	28	35
Simuliidae	43	42	15	57
Coleoptera				
Elmidae	8	88	4	46
Scirtidae	1	1	98	39
Oulimnius	8	67	25	2
Trichoptera				
Hydropsychidae	20	64	16	50
Rhyacophilidae	13	67	20	25
Limnephilidae	59	30	11	10
Polycentropidae	57	43	0	10
Ephemeroptera				
Baetidae	10	63	27	84
Heptageniidae	15	65	20	11
Plecoptera				

Chloroperlidae	39	28	32	22
Leuctridae	13	37	50	65
Nemouridae	6	88	6	5
Perlodidae	49	46	5	8
Other				
Oligochaeta	46	20	34	90

Table 3. Kruskal-Wallis and Mann Whitney U tests of significance for univariate biological indices between sample locations and time periods.

Main effects	**Diversity**	**Dominance**	**No.Taxa**	**Abundance**	**No. EPT taxa**	**ASPT**	**BMWP**	**AWIC**
Location	.134	.042*	.793	.018*	.002**	.708	.192	.006**
Season	.057	.087	.754	.002**	.013**	.669	.061	.000**
Post Hoc tests	**Diversity**	**Dominance**	**No.Taxa**	**Abundance**	**No. EPT taxa**	**ASPT**	**BMWP**	**AWIC**
Time								
Jun07*Oct07	.741	.616	.621	.386	.152	.595	.741	.226
Jun07*Mar08	.098	.211	.473	.001**	.004**	.694	.057	.000**
Oct07*Mar08	.014*	.015*	.792	.012*	.120	.375	.062	.007**
Location								
Control*Impacted	.191	.095	.453	.451	.001**	.379	.100	.003**
Control*Recovery	.442	.367	.695	.019*	.021*	.705	.616	.006**
Impacted*Recovery	.064	.020*	.832	.020*	.306	.680	.167	.998

**significant at 0.01

*significant at 0.05

Table 4. Significant (p < 0.05) variables in each of the CCA runs identified using unrestricted Monte Carlo significance tests.

CCA Model Run	**Variable**	**Lambda-A**	**p-value**	**F-ratio**
Run1	Temp	0.161	0.001	4.19
	Fe	0.124	0.034	2.06
	Pb	0.111	0.022	2.29
Run2	Fe	0.183	0.001	4.49
	Cond	0.166	0.005	2.66
	Pb	0.118	0.006	2.44
Run3	Zn*	0.132	0.004	3.06
Run4	Cond	0.150	0.001	4.07
	Zn*	1.118	0.001	3.56
	Fe	0.074	0.007	2.35
	Cu*	0.057	0.039	1.87
Run5	Pb	0.171	0.001	4.22
	Zn	0.156	0.002	2.74
Run6	Fe	0.130	0.001	5.76
	Temp	0.094	0.001	4.55
	Pb	0.092	0.001	3.66
	Zn	0.087	0.004	2.61
	Cond	0.069	0.017	1.92
	Cd	0.064	0.001	2.38

* sediment-bound bioavailable metals; Temp = water column temperature; cond = water column conductivity.

Table 5. Cumulative percent variance of species-environment data explained by the four canonical axes in the six CCA runs.

CCA Model Run	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
Run 1	36.1	58.6	76.7	84.7
Run 2	34.8	58.3	70.7	79.2
Run 3	46.9	68.9	82.1	91.2
Run 4	28.4	50.7	60.0	68.7
Run 5	34.3	54	68.0	78.9
Run 6	27.5	51.1	70.5	80.4