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Habitat recovery from diverted acid mine drainage pollution determined by increased biodiversity of river and estuarine benthic species

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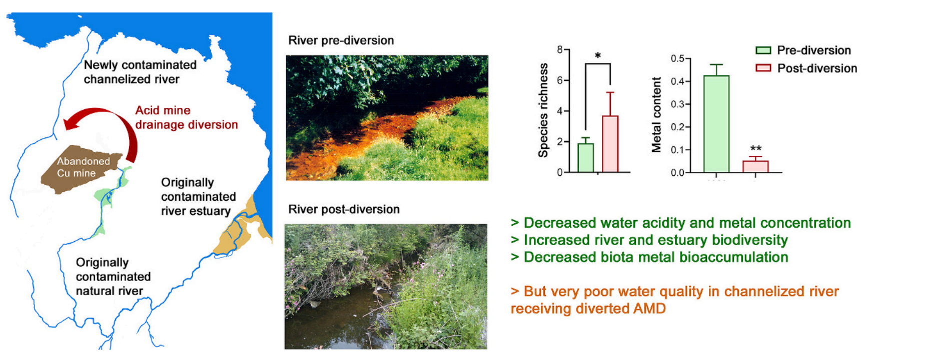
HIGHLIGHTS

- An AMD polluted river catchment was assessed for long term ecological recovery.
- Invertebrate biodiversity improved at most river sites following AMD diversion.
- Biodiversity improved for rocky shore and infaunal species at the river estuary.
- Redirection of AMD into another stream channel caused significant loss of species.
- Within a decade a river catchment can show partial ecological recovery to AMD.

GRAPHICAL ABSTRACT



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ABSTRACT

Acid mine drainage (AMD) is a frequent cause of ecological damage to many river and estuarine habitats. Once AMD pollution is halted our understanding of subsequent habitat recovery requires long-term ecological assessment. This study examines the consequences of diverting AMD away from a highly contaminated river and estuary using water quality and ecological data from pre- and post-diversion sample periods. 10–12 years following diversion, water quality and benthic macroinvertebrate biodiversity significantly improved at all sample sites of the river, indicative of ecological recovery but upstream sites that were closer to the pollution source were less improved. However, redirection of the AMD into a nearby stream channel caused an almost complete loss of benthic macroinvertebrates. Habitat recovery at the river estuary was demonstrated by increased richness of infaunal invertebrates and rocky shore species, including crustaceans, barnacles and mollusc species. Measurements of copper bioaccumulation in the barnacle *Austrominius modestus* showed a significant reduction in present day samples compared to those collected before AMD diversion. This study shows that within a decade, an estuarine and river system can demonstrate ecological recovery from AMD pollution, yet within this time period, recovery did not fully match uncontaminated sites.

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1. Introduction

The mining, extraction and processing of metals are critical to global industrial activity and economy, yet the contamination that results from these activities is extremely damaging to the environment and severely threatens the health of ecosystems (Luckeneder et al., 2021). Much of this pollution results from abandoned mines and mine tailings, such that it has been estimated that metal mining has affected just over 480,000 km of river channels worldwide with over 365,000 km of rivers contaminated from inactive mines (Macklin et al., 2023). A large proportion of mining waste waters are not only metal-rich but also highly acidic, referred to as acid mine drainage (AMD), derived mainly from the oxidation of sulphide-rich ores (Akcil and Koldas, 2006). Due to the toxic nature of AMD, such pollution is highly degrading to both aquatic and terrestrial environments.

The ecological consequences of AMD pollution in freshwaters can be substantial, due to the very low pH (often below pH 3.0), high concentrations of dissolved metals, and metal precipitate accumulation on riverbed sediments. These factors lead to reductions in abundance and biodiversity of pollution-sensitive microorganisms, benthic macroinvertebrates, and fish, while also causing dominance of pollution-tolerant organisms (Hogsden and Harding, 2012). As such, changes in the community structures of organisms like macroinvertebrates or diatoms, as well as metal bioaccumulation into organisms are good bioindicators of AMD damage (Byrne et al., 2012; Cadmus et al., 2016). Physical consequences of mining including the channelisation of water courses can also reduce biodiversity and inhibit biotic recovery in a stream, even after water quality has improved by remediation (Nelson and Roline, 1996). River estuary habitats, composed of mud- and sand-flats, salt marshes and rocky shores, are also prone to AMD pollution and ecosystem damage, particularly due to the hydrodynamics of estuarine systems that facilitate the build-up of metal-rich sediments (Parkman et al., 1996; Simons et al., 2011). Estuaries are important ecosystems composed of diverse habitats that include many pollution-sensitive species, as well as species that could act as bioindicators of metal pollution. While studies have examined the ecological consequences of metal pollution from various sources in estuaries (Bryan and Langston, 1992; Truchet et al., 2021), the analysis of ecological impacts in estuaries due to AMD are less extensive than studies of AMD-polluted river catchments.

Approaches to treat and prevent AMD contamination in aquatic environments can include a variety of options that have been extensively investigated and reviewed. These include chemical and physical removal and prevention methods, such as alkalinisation, metal chelation, and capping and draining of mine adits (Johnson and Hallberg, 2005; Park et al., 2019; Thisani et al., 2021), passive biological-based approaches, such as wetlands (Dean et al., 2013; Nyquist and Greger, 2009; Pat-Espadas et al., 2018) and microbial-based treatment methods (Neculita et al., 2007; RoyChowdhury et al., 2015), as well as hybrid treatment approaches (Wibowo et al., 2024). A major challenge for many AMD-contaminated environments is that pollution typically derives from older abandoned mine sites with no responsible ownership except public bodies and that treatment is often needed in perpetuity. As such, costly remediation solutions are often unfeasible, requiring the need for low-cost and sustainable solutions (Rezaie and Anderson, 2020). Therefore, it may be expedient to simply rapidly divert AMD entirely away from a vulnerable ecosystem to a location where the effluent will be less damaging like an underground storage location, or to divert the AMD for treatment (Gunn et al., 2010; Kotalik et al., 2023). Regardless of the method of AMD remediation or removal employed, it is important to monitor ecological recovery over long time scales, although this is often not done.

A few long-term studies have shown that AMD removal can lead to partial or complete ecological recovery, such as determined by benthic macroinvertebrate community data (Dean et al., 2013; Herbst et al., 2018; Kruse et al., 2013; Nelson and Roline, 1996; Steyn et al., 2019). However, this success is sometimes limited due to other residual factors such as habitat degradation (Kotalik et al., 2023), or only occurring after many years (Clements et al., 2021; Gunn et al., 2010). Indeed, in some cases assessment over very long time scales such as over 40 years may be needed to demonstrate full recovery (Williams and Turner, 2015). Water quality improvements are rarely immediate, and often positive changes to the water chemistry occur much faster than improvements to a biological community such as fish populations (Cravotta et al., 2010). There is also a need to better understand the spatial patterns of recovery, which requires an ecological assessment over a full catchment (Johnson et al., 2014).

The Afon Goch catchment in Anglesey, Wales is an example of a small river system that has been severely impacted by AMD due to drainage waters from the abandoned Parys Mountain Cu mine (Fig. 1). Mining at Parys Mountain extends back over 4000 years although the peak activity was from the mid-18th century until mining ceased in 1911 (Wilson and Pyatt, 2007). This has left a landscape of exposed pyrite-rich mine workings that generate significant AMD. For many years AMD from Parys Mountain drained into the southern Afon Goch (also known as Afon Goch Dulas) via the Mona adit making it one of the most metal- and acid-contaminated streams in the UK (Boult et al., 1994; Dean et al., 2013; Todd et al., 2024). In addition, the flux of metal precipitates from the river has caused substantial contamination of the shallow Dulas estuary, leading to metal transfer into the estuarine biosphere and the wider marine environment (Chalkley et al., 2019; Parkman et al., 1996). This river and estuary pollution continued until 2003 when the removal of an underground dam (Younger and Potter, 2012) resulted in the mine water instead directly entering the nearby northern Afon Goch (also known as Afon Goch Amlwch) via the Dyffryn Adda adit (Fig. 1), with future contamination of the southern Afon Goch only occurring from spoil leachate runoff. Following the drainage diversion, contamination in the southern Afon Goch fell to a level at which a natural wetland could further bioremediate the AMD pollution, leading to a marked improvement in water quality (Aguinaga et al., 2018; Dean et al., 2013). However, this diversion led to the northern Afon Goch becoming the main route by which AMD from the Parys Mountain mine enters the Irish Sea (Aguinaga et al., 2018). With no remediation in place at the northern Afon Goch, the discharges from Parys Mountain are the single biggest contributors of Cu and Zn to the Irish Sea (Hudson-Edwards et al., 2008), and account for the largest proportion of total Fe release nationally (Mayes et al., 2010).

Although the diversion of AMD in the Afon Goch catchment was not originally implemented for the purpose of remediation, it nevertheless provides an ideal case study to understand how rivers and estuaries may recover from severe AMD pollution that occur over many years, and what may be expected in terms of river and estuary ecological recovery. This is particularly important considering the global scale of mine drainage contaminated rivers (Macklin et al., 2023). Very few studies have examined ecosystem recovery in response to AMD reduction over a whole river catchment including in river estuaries. Successful demonstration of habitat improvement at a watershed scale from such case studies could be instrumental at incentivising remediation and recovery approaches elsewhere. The aim of this study was to make use of previously unpublished historic data collected between 1989 and 1995 prior to the diversion of AMD outflow away from the southern Afon Goch and into the northern Afon Goch, alongside data collected between 2013 and 2016 after AMD diversion in 2003 to examine water quality and biodiversity changes before and after the diversion of AMD. This aim was addressed by four objectives (Fig. 2), in which we determined 1) southern Afon Goch river chemistry and biodiversity, 2) northern Afon Goch river chemistry and biodiversity, 3) biodiversity and sediment metals at Dulas estuary, and 4) metal bioaccumulation in selected bioindicator species at Dulas estuary. Comparison of these data pre- and post-diversion will facilitate an understanding of the implications of the AMD diversion on ecosystem recovery and degradation across the catchment.

2. Materials and methods

2.1. Study site

The southern Afon Goch is 11 km in length and runs south of Parys Mountain (Fig. 1). Historically (before 2003) AMD flowed into the river via the Mona adit near site S1. At ~500 m downstream of the AMD source, the river flows through a natural wetland of ~0.1 km^2, after which the river flows south before turning north-east towards Dulas bay where it enters the Irish Sea. Ten sites (S1–S10) were sampled for water chemistry and biodiversity analysis along the course of the southern Afon Goch (S5 and S8 are on tributaries), with S10 located just above the tidal limit at Dulas estuary (Fig. 1). In addition to being subject to AMD pollution, causing acidification and metal contamination in the water column and riverbed sediment, long stretches of the river at the upper sites (S1–S3) are channelised.

The northern Afon Goch rises to the west side of Parys Mountain and runs ~5.5 km in length to the town of Amlwch where it enters the Irish Sea through an industrial site next to the port. The river is mostly channelised along its entire length, with the Dyffryn Adda adit (at site NA) providing AMD input at ~2.5 km along the river. Four sites (N1–N4) in addition to site NA were used as sampling locations for water chemistry and biodiversity analysis along this river (Fig. 1).

The Dulas estuary largely consists of soft substrate (sand and soft sediment), though there is a rocky shore along the northern edge of the estuary and along the spit of land that divides the estuary from Dulas bay. At low tide seawater retreats and only the narrow channel of the southern Afon Goch is visible. Dulas estuary was sampled for biodiversity analysis, sediment metal concentration, and organismal metal bioaccumulation. Soft substrate communities were sampled at sites DS1–DS9, and rocky shore communities at sites DR1–DR9 (Fig. 1). As a control comparison for Dulas estuary, the Afon Alaw estuary on the west coast of Anglesey that is not affected by AMD and is physically similar, was also sampled (Fig. S1). The same parameters were sampled at Alaw estuary at soft substrate sites AS1–AS8 and rocky shore sites AR1–AR3 (Fig. S1). The approximate locations of all the sampling sites are given in Table S1.

2.2. Data collection and analysis

Data was collected across the catchment at the sample sites described in Section 2.1 at time points between 1989 and 1995 (pre-diversion of AMD) and between 2013 and 2016 (post-diversion of AMD) in order to meet the four objectives of the study (Fig. 2). While most of the

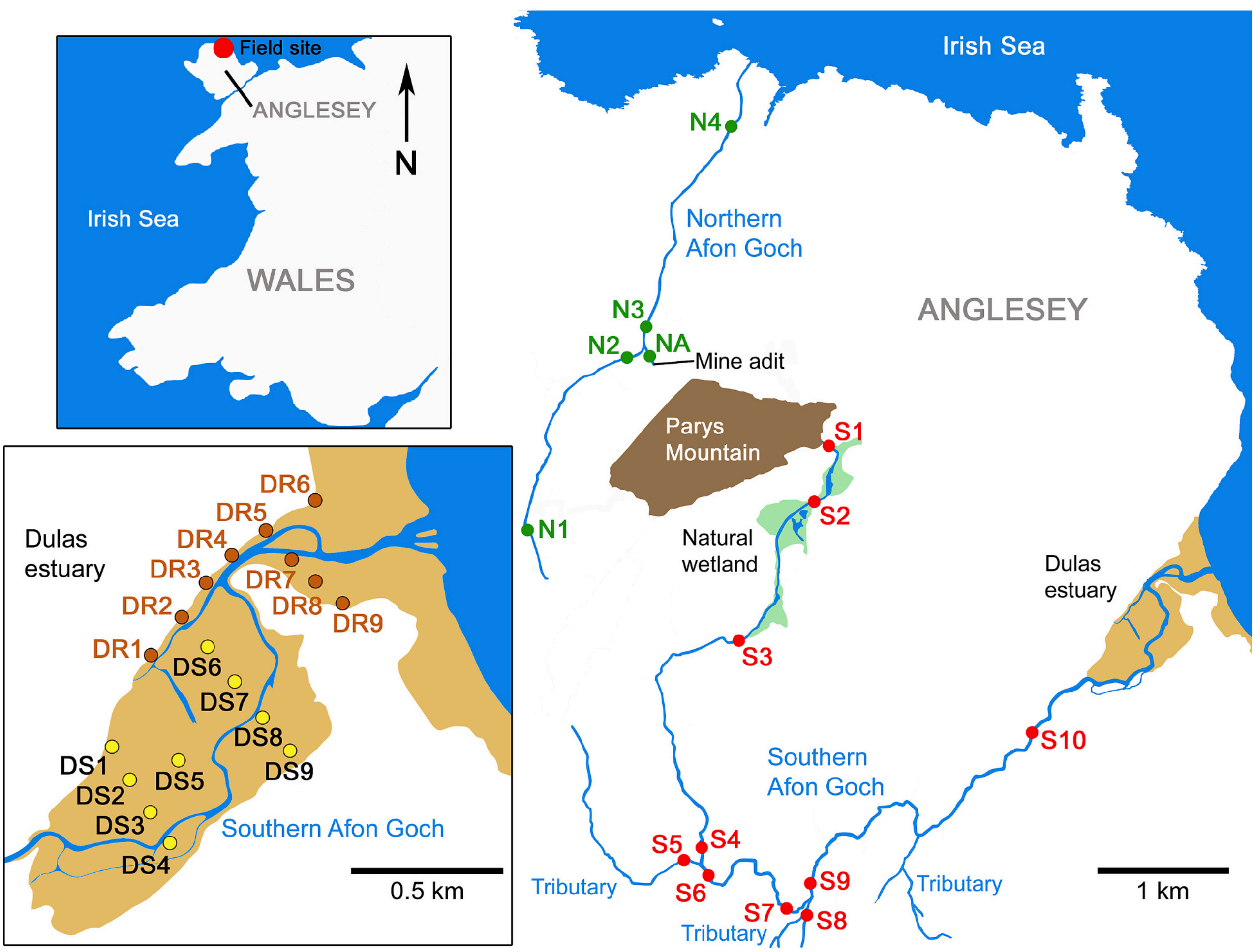

Fig. 1. Location of sampling sites along the southern and northern Afon Goch rivers in Anglesey, Wales that receive acid mine drainage from the abandoned Parys Mountain mine site. Bottom-left inset map shows the location of sampling sites within Dulas estuary where the southern Afon Goch flows into the Irish Sea. Sites S1–S10 are on or nearby the southern Afon Goch and were sampled in 1990/1995 and 2013/2015. Sites S1 and S3 are above and below a natural wetland, and sites S5 and S8 are on tributaries that flow into the main river. Sites N1–N4 are on the northern Afon Goch with site NA located at the mouth of a mine drainage adit, and were sampled in 2013/2014. Sites DR1–DR9 are at rocky shore locations within Dulas estuary. Sites DR1–DR6 form a transect along the northern side of the estuary and were sampled in 1993. Sites DR5–DR9 form a transect across the estuary where the river enters the beach and were sampled in 2016. Site DR6 was also sampled in 1989 and 2016. Sites DS1–DS9 are at soft substrate/sand locations within Dulas estuary. Sites DS1–DS4 and DS6–DS9 form two transects across the estuary and were sampled in 2016. Sites DS3, DS5 and DS7 form a transect along the estuary and was sampled in 1993.

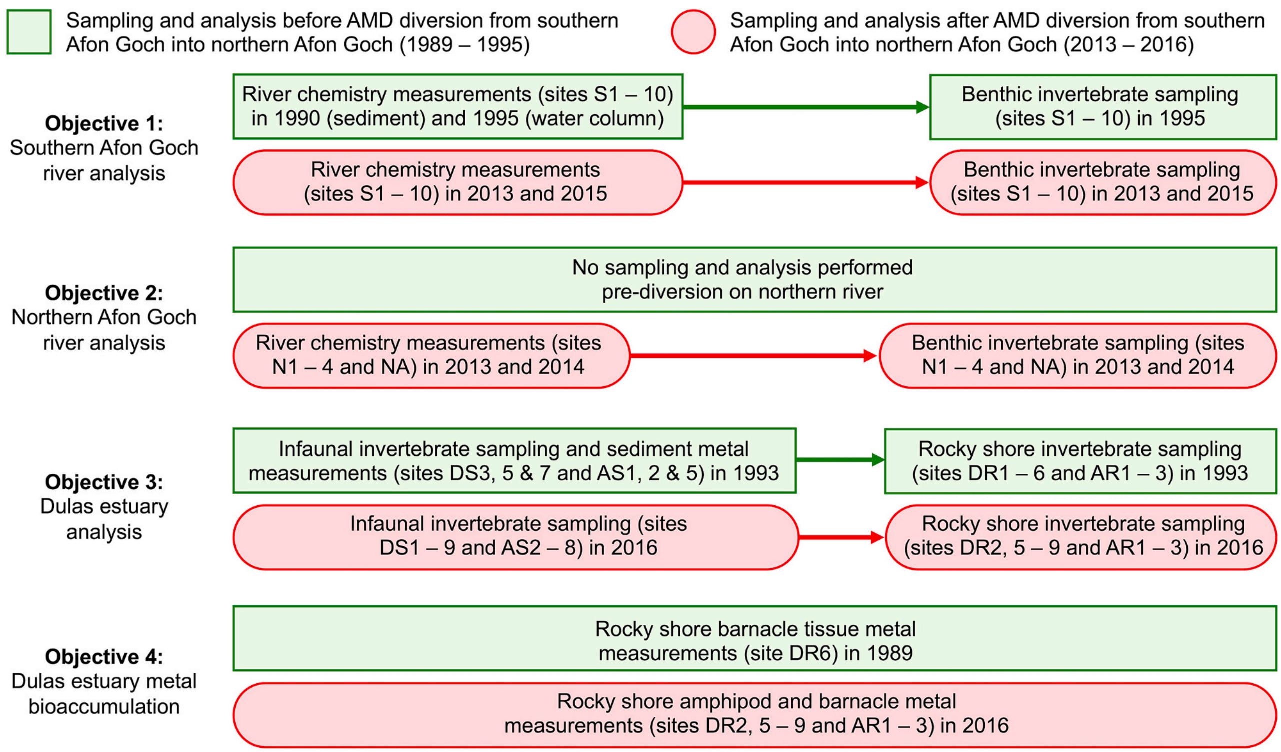

Fig. 2. Summary of the sampling dates, sampling points, and the analyses conducted during the time periods before and after AMD diversion from the southern Afon Goch, required to address each research objective.

parameters measured and methods of collection were consistent between the two time periods (pre- and post-diversion data collection periods), there was some variation with regard to the time of year that sampling occurred and the amount of replication performed. As such, the heterogeneity of the dataset meant that some of the comparisons are descriptive, while other comparisons are statistically analysed, where stated. All statistical analyses (principal components analysis (PCA), linear regression, unpaired *t*-tests and one- and two-way ANOVA) were performed using GraphPad Prism v.9 except for non–metric multidimensional scaling (NMDS) analysis that was performed using RStudio v.2024.09.1+394 and the Vegan package. Data was tested for parametric determination by using the D'Agostino-Pearson normality test. The threshold for statistical significance was at least $p < 0.05$, as indicated. In all cases, data was untransformed unless when stated.

2.2.1. *Southern Afon Goch data*

Pre-diversion water chemistry data were collected from sites S1–S10 on two different sampling occasions in June 1995. On each occasion a single sample was taken for water chemistry. Benthic invertebrate data were collected from the ten sites on the same two sampling occasions in June 1995, and in May 1995, with a single sample of invertebrates taken on each date, providing three replicate pre-diversion samples for each site in total. Pre-diversion sediments samples were taken from six sites (but not S1, S5, S6 or S8) in June and July 1990, with a single sample taken on each date. Post-diversion data (water and sediment metals) was collected from the southern Afon Goch sites on one sampling occasion in August 2013 and on one occasion in February 2015 with three replicate samples taken on each occasion from each site.

Invertebrates were collected in August 2013 (two replicate samples) and February 2015 (one sample) providing three replicate post-diversion samples in total. The benthic invertebrate family level data was used to calculate river water quality using both the Whalley Hawkes Paisley and Trigg (WHPT) and the Biological Monitoring Working Party (BMWP) indices, which are derived from the sensitivity of specific invertebrate taxa to pollution, with the most sensitive taxa scoring highest, to indicate the ecological health of the river (Guareschi et al., 2017; Paisley et al., 2014). Although primarily designed to respond to organic pollution, these indices are also suitable for monitoring other types of impact including AMD (Gray and Delaney, 2008). WHPT is a revision of BMWP that includes more families and also scores on the basis of individual taxon abundance variation and so is arguably more accurate (Paisley et al., 2014; Walley and Hawkes, 1997). Both WHPT and BMWP are expressed as a score (the sum of values for each taxon), and as average score per taxon (ASPT), which is the mean WHPT or BMWP score of all scoring taxa present in the sample. All river chemistry and biodiversity data are presented as mean values of replicate samples with data ranges (minimum/maximum values). In addition, invertebrate data are also provided as pooled counts (individuals for each taxa identified) (Table S2).

Variations among southern Afon Goch sample site water chemistry were analysed by PCA. Ordination of the southern Afon Goch benthic invertebrate community data was performed by NMDS in three dimensions using a Bray-Curtis dissimilarity matrix set to 100,000 permutations after square-root transformation. Water chemistry vectors were fitted onto the ordinations using the ENVFIT function. An Adonis test was performed to determine significant difference (with threshold $p < 0.05$) between the 1995 and 2013/2015 sample groups. The NMDS stress value was 0.093. Linear regression analyses were performed to evaluate the influence of water quality parameters on WHPT values with all data $\log_{10}$ transformed except for pH values. The R^2 values for the linear regression models and *p* values to determine the significance of the slope deviation from zero were calculated.

2.2.2. *Northern Afon Goch data*

Data for the northern Afon Goch is only available for post-diversion, with water chemistry data collected from sites N1–N4 and NA from three replicate samples taken on a sampling occasion in August 2013, and from three replicate samples taken on a sampling occasion in March 2014. Data are presented as mean values of replicate samples with data ranges. Two replicate samples of benthic invertebrate data were collected on each of the August 2013 and March 2014 sampling dates, and are presented as number of scoring taxa, WHPT and ASPT scores (mean values and data range), and pooled counts of individuals for each identified taxa from the two sampling occasions (Table S3).

2.2.3. Dulas and Alaw estuary biodiversity and sediment metal data

A survey of Dulas estuary community biodiversity that was performed in 1993 (Jones, 1995) and a further survey was carried out in 2016. The control estuary where the 'clean' Afon Alaw flows into the Irish Sea (Fig. S1) was also sampled in 1993 and 2016. Fauna from hard substrate (rocky shore) and soft substrate (sandy/muddy shore) habitats were identified to species level. Pre-diversion estuary sandy shore/mudflat communities were sampled in January, April, July and October 1993 with samples taken from the soft shore community at three locations of Dulas estuary (DS3, DS5 and DS7) and Alaw estuary (AS1, AS2 and AS5). These soft substrate communities were sampled by collecting eight replicate areas of sediment. Analysis of pre-diversion estuary rocky shore communities was carried out in May 1993, with the rocky shore at Dulas estuary and Alaw estuary sampled from eight replicate quadrats placed along transects between sites DR1–DR6 (at Dulas estuary) and AR1–AR3 (at Alaw estuary). Post-diversion biodiversity analysis from rocky shore and soft substrate communities at Dulas and Alaw estuaries was carried out in April 2016. Sediment samples were taken at sites DS1–DS4 and DS6–DS9 on the Dulas estuary, and at sites AS2–AS4 and AS5–AS8 on the Alaw estuary by sampling three replicate areas at each site. Rocky shore fauna was sampled using three replicate quadrats at sites DR5–DR9 (at Dulas estuary) and AR1–AR3 (at Alaw estuary). Invertebrate species presence and absence at the soft and rocky substrate locations for both estuaries was determined by whether the species was recorded at least once during any one of the sampling occasions at each time period (1993 or 2016). For the soft substrate infaunal species, mean taxa counts (species richness) were quantified and compared between Dulas and Alaw estuary and between 1993 and 2016 by two-way ANOVA with Tukey's post-hoc multiple comparison test (with $p < 0.05$ minimum threshold).

Seasonal differences in infaunal invertebrate species number and in sediment exchangeable metal concentration between Dulas and Alaw estuary samples collected in January, April, July and October 1993 (6 replicate samples on each occasion, from sites DS3, DS5 and DS7 in Dulas estuary and sites AS1, AS2, and AS5 in Alaw estuary) were compared by two-way ANOVA with Tukey's post-hoc multiple comparison test (with $p < 0.05$ minimum threshold). In addition, the relationship between infaunal species richness and sediment metal concentration was examined by Pearson correlation, with all data log($x + 1$) transformed prior to analysis.

2.2.4. Dulas and Alaw estuary metal bioaccumulation data

Bioaccumulation of metals into selected bioindicator species was determined from samples collected from rocky shore sites DR6 at Dulas estuary in July 1989, and DR2, DR5–DR9 (Dulas estuary), AR2 and AR3 (Alaw estuary) in April 2016. This was to determine the degree of metal contamination and bioaccumulation into estuarine biota at the site. The chosen species were the barnacle *Austrominius* (previously classified as *Elminius*) *modestus* and the amphipod *Orchestia gammarellus*, in part due to their high abundance across the rocky sites of Dulas and Alaw estuary in 2016, and their presence in the estuary before the AMD diversion. *O. gammarellus* is a littoral scavenger that feeds on seaweed and detritus, and has been used as a bioindicator of metals for many years (Moore et al., 1991; Mouneyrac et al., 2002). *A. modestus* is invasive although long widespread in European waters, and a suspension feeder that can accumulate very high concentrations of trace metals making it ideal as a coastal bioindicator of metal pollution (Rainbow and Phillips, 1993; Rainbow and Wang, 2001).

Three replicate samples of five pooled individuals of *O. gammarellus*, and three replicate samples, each with 10 pooled individuals of *A. modestus*, were taken from each of the rocky sites. The concentrations of metals in *A. modestus* collected from site DR6 in 2016 were compared with samples collected in 1989. Differences in metal accumulation between pre- and post-diversion data from site DR6 were analysed using unpaired *t*-test (with $p < 0.05$ minimum threshold), and differences in metals accumulation between Dulas and Alaw estuary samples in 2016 were tested using one way ANOVA with Tukey's post-hoc multiple comparison test (with $p < 0.05$ minimum threshold).

2.3. River water quality and metal concentration measurements

At each sampling site on the southern and northern Afon Goch water pH was measured using a pHOX42E meter and conductivity with a pHOX52E meter. Samples from the river water column were collected from 2 or 3 locations at each sample site. For dissolved metals, a known volume of water from each collection point was filtered through 0.45 μm cellulose acetate filter paper with the filtrate acidified to 2 % (*v/v*) nitric acid. The filter papers were retained for the analysis of particulate metals. Sediment samples were collected from a ~1 cm depth of the riverbed, oven dried at 60 °C for 48 h, and passed through a 250 μm sieve. A known weight of sediment (~0.1 g) was digested for the analysis of sediment metals. Both the filter papers and sediment samples were digested in 67 % (v/v) ultra-pure nitric acid at 100 °C for 24 h, then diluted to 2 % (v/v) nitric acid in deionised water. All southern Afon Goch water and particulate samples were analysed for Cd, Cu, Fe, Mn, Pb, and Zn, while the southern Afon Goch sediment samples were analysed for Cu, Fe, Mn and Zn. All northern Afon Goch water, particulate and sediment samples were analysed for Al, As, Cd, Cu, Fe, Mn, Pb, and Zn. Metal concentrations for the 1990 and 1995 water samples and digests were determined by flame atomic absorption spectroscopy (AAS), and for the 2013–2015 samples inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Perkin-Elmer Optima 5300 was used. Both instruments were calibrated using external standards.

2.4. River invertebrate sampling

At each of the southern and northern Afon Goch sites, a 3 min kick net sample was performed using a hand-held net with a mesh size of 1 mm to collect benthic invertebrates followed by a 1 min examination of large stones to dislodge any invertebrates that were attached to them, following Environment Agency, UK guidelines. A volume of 70 % (*v/v*) ethanol was added to each sample to preserve the biota for subsequent identification to family level using standard keys (Elliott et al., 1988; Greenhaigh and Ovenden, 2007; Hynes, 1977; Quigley, 1977). Pooled counts of the identified organisms collected from three sampling occasions for each time period are shown in Table S2 for the southern Afon Goch samples and in Table S3 for the northern Afon Goch samples.

2.5. Estuarine invertebrate sampling

Samples were collected from the sites at Dulas and Alaw estuary as described in Section 2.2.3. For the 1993 samples, the soft substrate communities were identified from eight replicate areas of sediment (0.2 m × 0.2 m) collected to a depth of 0.2 m at each site, which were sifted through a 1 mm mesh sieve, while the rocky shore community was identified from the eight replicate quadrats (0.5 m × 0.5 m) placed at 2 m intervals along the transects at each estuary. For the 2016 samples, the soft substrate communities were identified from three replicate areas of sediment (0.5 m × 0.5 m) to a depth of 0.3 m collected at each site, which were sifted through a 1 mm mesh sieve, while the rocky shore community was identified from the three replicate quadrats (1 m × 1 m) at each site. In all cases, organisms were identified to species level where possible using a marine fauna guide (Haywood and Ryland, 1990).

2.6. Pre-diversion estuarine sediment metal measurements

Sediment samples collected in triplicate at low water from the surface (0–2 cm depth) of each of the sites (all mid-tide level sites) at Dulas and Alaw estuary (as described in Section 2.2.3) in January, April, July and October 1993 were oven dried at 70 °C for 12 h. To determine total metal concentrations, 1 g of dried sediment was digested with 10 ml of 67 % (*v*/v) ultra-pure nitric acid at 70 °C for 4 h, then diluted to 2 % (v/v) nitric acid in deionised water prior to analysis. To determine exchangeable (bioavailable) metal concentrations as described by Luoma and Bryan (1981), 1 g of dried sediment was extracted with 10 ml of 1 N HCl for 2 h before being filtered through Whatman GFC filters. The digests were analysed for Cu, Fe, Mn and Zn by flame AAS and calibrated using external standards.

2.7. Estuarine invertebrate metal analysis

The replicate samples of *O. gammarellus* and *A. modestus*, taken from each of the rocky shore sites (described in Section 2.2.4) were used for metal concentration measurement in their tissues. *A. modestus* individuals with diameters of ~8 mm were taken, by carefully scraping off the rocks. *A. modestus* and *O. gammarellus* samples were kept in aerated seawater for 28 h to ensure that their guts were cleared of any metal particulates. For metal analysis of *A. modestus* the main 'body' (cirri, thorax and head) and a portion of egg mass was removed from each carapace using fine tweezers. For *O. gammarellus* whole individuals of similar sizes were analysed. Both sets of samples were oven dried at 60 °C until a constant weight was reached, then a known weight of each sample was digested in 20 ml of 67 % (v/v) ultra-pure nitric acid at room temperature for 24 h, following which 2 ml of 30 % (v/v) hydrogen peroxide was added and the solution left for another 24 h. A 2 ml volume of each digest was diluted to 10 ml with deionised water prior to metal measurement. The 1989 *A. modestus* samples were analysed for Cu, Fe and Zn concentrations by graphite furnace AAS. The 2016 *A. modestus* and *O. gammarellus* samples were analysed for Cd, Cu, Fe, Mn, Pb, and Zn concentrations using inductively coupled plasma mass spectroscopy (ICP-MS), and calibrated using external standards.

3. Results

3.1. Water quality improvements along the southern Afon Goch

The southern Afon Goch was a highly contaminated river in the 1990s due to AMD pollution, even at the downstream locations, as seen from visual observations of the extensive Fe precipitation (Fig. 3) and confirmed by water chemistry measurements (Figs. 4; S2). As far downstream as site S7, there were high conductivity values (>1 mS), low pH (<pH 3), and high concentrations of dissolved metals including Fe, Cu and Zn. In contrast, the water quality was much better at the two tributary sites S5 and S8, which show consistent water chemistry characteristics across both time periods (Fig. 4D). In post-diversion years (2013/2015) water quality was substantially improved in most sites on the southern Afon Goch, notably downstream of the natural wetland (from site S3). This is seen by reduced conductivity, acidity and dissolved metal concentrations (Figs. 4; S2). However, at S1 and S2 in 2013/2015 there was still poor water quality, particularly due to low mean pH values of 2.65 and 2.95, respectively (Fig. 4D).

PCA of the water chemistry dataset provides a visual comparison between the sites and over time (Fig. 4A). Sites S1–S4, S6 and S7 in 1995 cluster away from all sites in 2013/2015 on the basis of PC1 with this divergence due to differences in conductivity and dissolved metal concentrations (Fig. 4B). All of the 2013/2015 sites are closely grouped but with S1 and S2 slightly separated, and the 1995 S5 and S8 tributary sites are also in this cluster. Sites S9 and S10 in 1995 diverged from the other sites on the basis of PC2 (Fig. 4A), due to differences in concentrations of some of the particulate metals (Fig. 4B). Indeed, there was much higher concentrations of particulate Fe and Cu (Fig. 4F) and particulate Al (Fig. S2) at these sites in the lower reaches of the river in 1995. The concentrations of some metals (Mn and Zn) in the riverbed sediments were markedly lower in 1990 than in 2015/2015 for all sites sampled below the natural wetland (Fig. 5). However, there was very little difference in concentrations of sediment Fe and Cu between the two time periods.

In summary, water quality in the southern Afon Goch was substantially improved following AMD diversion but dissolved metal concentrations and acidity were still high at sites S1 and S2 that are closest to the mine site.

3.2. Improvements in southern river biodiversity alongside water chemistry changes

In 1995 the mean WHPT scores (Fig. 6A) were between 4.1 and 13.8 at each of the southern Afon Goch sites (excluding the tributaries), due to a low number of taxa at each site, with less than five scoring taxa at each of these sites (Fig. 6B). In contrast, the S8 tributary site, has 23 scoring taxa and a mean WHPT score of 103.3, and as such S8 can be considered as a control site of good water quality. Interestingly, the

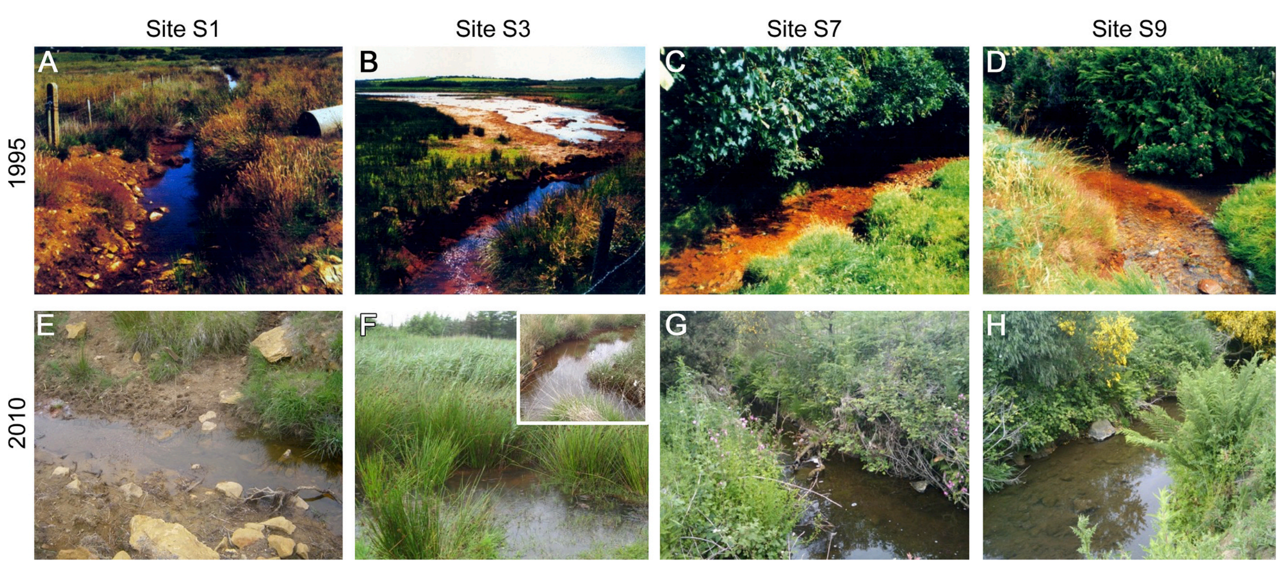

Fig. 3. Photographs of selected sample sites along the southern Afon Goch in 1995 (A–D) in comparison to 2010 (E–H). Ferrihydrite precipitation giving orange colouration to the river bed can be seen at all sites in 1995.

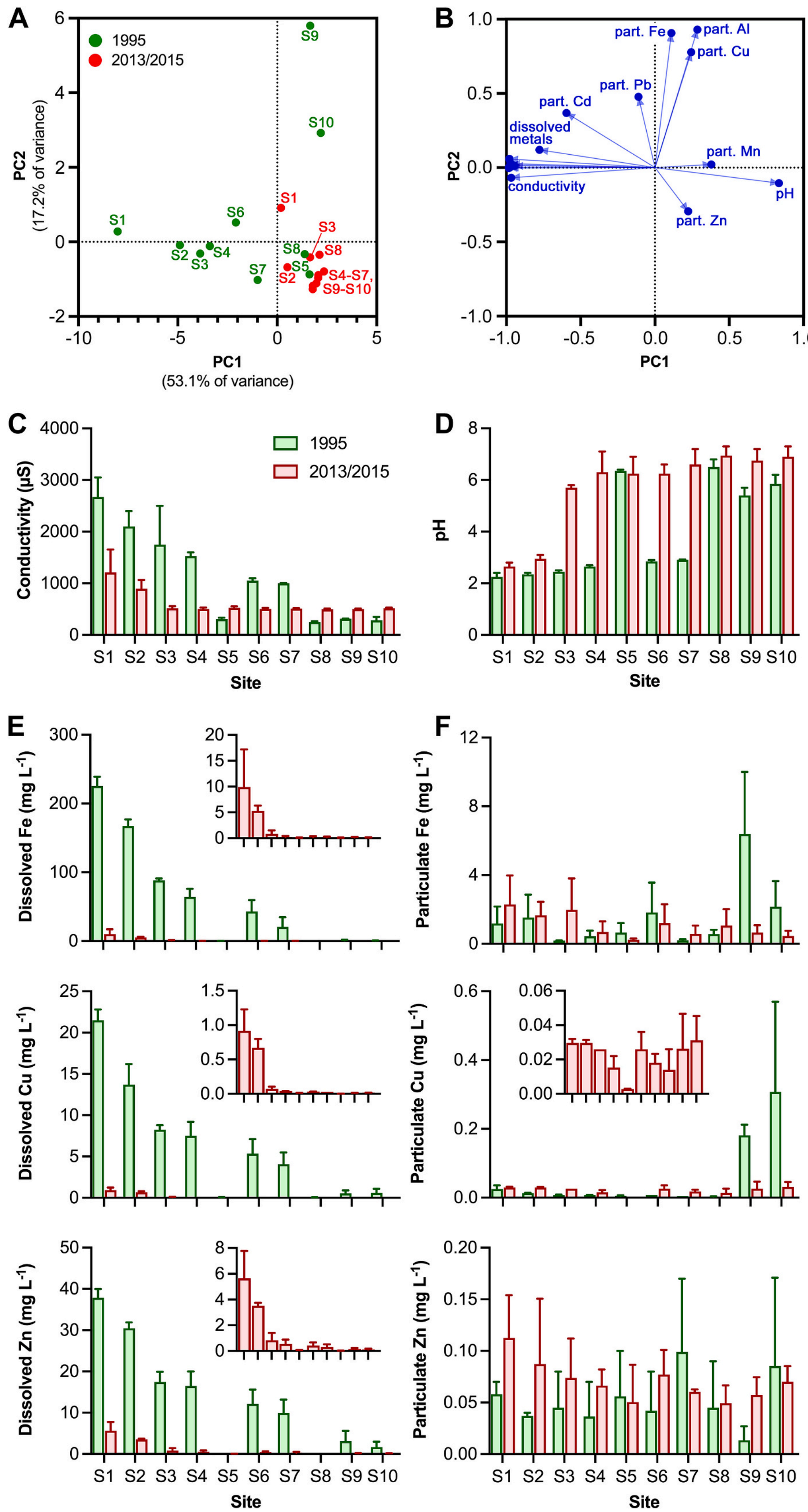

Fig. 4. Water chemistry of the southern Afon Goch in 1995 compared to 2013/2015. PCA (A) and loading plot (B) for conductivity (C), pH (D), and particulate and dissolved metals at 10 sample sites along the river. Concentrations of dissolved Fe, Cu and Zn (E) and particulate Fe, Cu and Zn (F) are shown. Inset graphs show low concentration values of selected metals. Data for other metals are presented in Supplementary Fig. S2. All sites are on the main river apart from S5 and S8 on tributaries. Upstream site S1 is closest to the mine. For 1995, each data point represents the mean of two samples taken in June 1995. For 2013/2015, each data point represents a mean of two triplicate samples taken in August 2013 and February 2015. Error bars show data range.

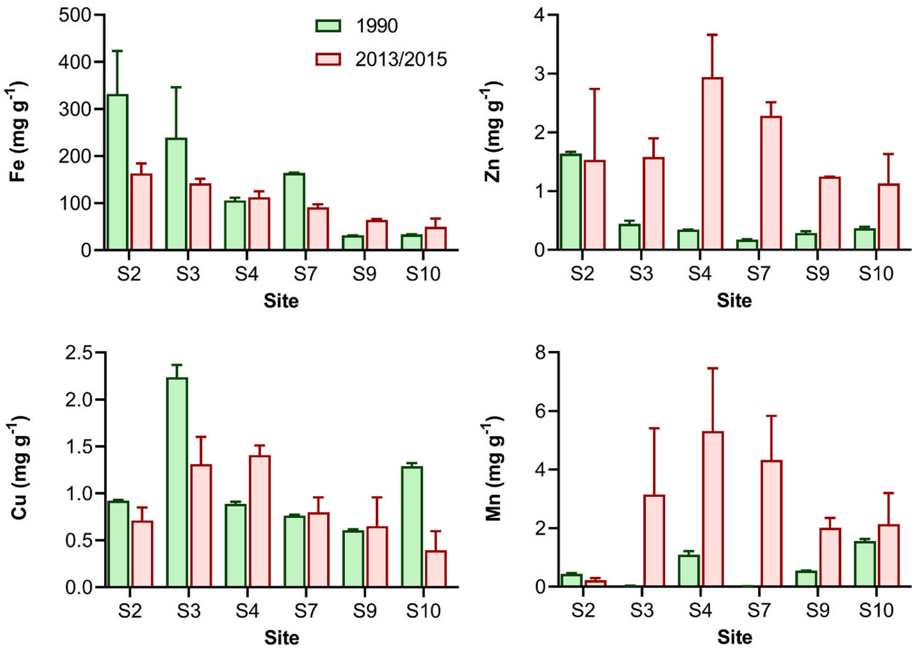

Fig. 5. Sediment metals along the southern Afon Goch in 1990 and 2013/15. For 1990 each bar represents the mean of two samples taken in June and July, and for 2013/15 each bar represents the mean of two triplicate samples taken in August 2013 and February 2015. Error bars show data range.

other tributary at S5 showed a poor invertebrate community composition in 1995 and a mean WHPT score of only 16.5, for unknown reasons. There was a clear dominance of Chironomidae at most of the upstream southern Afon Goch sites, which is a well-known bioindicator of poor water quality (Winner et al., 1980), although it is important to be aware that Chironomidae can include both pollution-sensitive and pollution-tolerant species (Molineri et al., 2020). These high numbers of Chironomidae in the upstream sites (S1, S2 and S3) explain the relatively high numbers of total individual organisms at these sites when compared to the downstream sites S7, S9 and S10 with just 6, 21 and 5 individual organisms observed (Table S2). A substantial improvement in biodiversity was seen at all sites in 2013/2015 as indicated by WHPT scores (Fig. 6A) and number of scoring taxa (Fig. 6B). Mean WHPT scores ranged from 68.9 to 117.9 for sites S4–S10, while mean numbers of taxa were > 12.0. Although the upstream sites (S1–S3) were less improved, the ecological status of these sites was also improved. For example, at site S1 the mean WHPT score increased from 10.0 in 1995 to 23.5 in 2013/2015.

Standard WHPT scores (calculated by taking into account abundance values) were compared with WHPT scores calculated without considering abundance, giving rise to a largely identical profile (Fig. S3B). This confirms that any differences in sampling effort over different time periods that may have resulted in different abundances did not contribute to the temporal differences in scores. ASPT values are also typically less sensitive to sampling effort and seasonal variations. The ASPT values calculated here, whether determined for WHPT (Fig. 6C) or BMWP (Fig. S3A), were low (<5.0) for all sites in 1995 except for tributary site S8 with a mean score of 6.3. In 2013/2015, ASPT scores remained low for the upstream sites S1, S2 and S3 but were >5.0 for all other sites, again indicative of improved water quality.

The improvement in water quality over time as determined by the invertebrate abundance data can be visualised by NMDS ordination (Fig. 6D). Based on the NMDS analysis, the sites were separated on the basis of pre- and post-AMD diversion, with a significant difference between the two sample site groups ($p < 0.002$, $n = 3$ for each site). All of the 2013/2015 downstream sites (S4–S10) were clustered together, while the wetland site S3 (a channelised site just below the wetland) was a slight outlier and the two upstream sites S1 and S2 grouped separately. A second cluster included all of the 1995 sites apart from the S8 (tributary) site. Incorporating water chemistry variables into the NMDS show that dissolved metal concentration changes mainly explain the distinction between the 1995 and 2013/2015 invertebrate community assemblage (Fig. 6D). The pH changes (reversal of acidic conditions) correlate significantly ($p < 0.05$) with the 1995 S8 tributary site and all 2013/2015 sites except S1 and S2.

To further demonstrate that changes in water chemistry correlate with improvements to water quality indices such as WHPT, linear regression analysis was performed between WHPT scores and water chemistry parameters. For example, there was a significant positive correlation between increasing WHPT score and pH ($R^2 = 0.581$, $p < 0.0001$) (Fig. 6E), a significant negative correlation between increasing WHPT score and decreasing conductivity ($R^2 = 0.234$, $p = 0.031$) (Fig. 6F), and a significant negative correlation between increasing WHPT score and decreasing dissolved Cu concentration ($R^2 = 0.749$, $p < 0.0001$) (Fig. 6G) and Fe concentration ($R^2 = 0.603$, $p < 0.0001$) (Fig. 6H). Other dissolved metals such as Cd and Al also show a strong negative correlation with WHPT score, although particulate metals do not, except for concentration of particulate Mn that shows a significant positive correlation with increasing WHPT score ($R^2 = 0.381, p = 0.004$) (Fig. S4). This correlation between particulate Mn and the improved 2013/2015 benthic invertebrate assemblage was also observed on the NMDS plot (Fig. 6D).

In summary, the benthic invertebrate examination indicates improved biodiversity and therefore habitat recovery at all southern Afon Goch site during the post-AMD diversion period, but most clearly at all sites downstream of the natural wetland.

3.3. Degradation in northern river biodiversity alongside water chemistry changes

Since the 2003 diversion of AMD, the water chemistry of the northern Afon Goch is currently highly acidic and metal-rich downstream from the adit (site NA), as indicated by low pH values ($<$ pH 3), high conductivity values (> 1 mS), and high concentrations of dissolved metals at sites N3 and N4 (Fig. 7A; Table S4). As such, the invertebrate community is highly degraded. Across the three sampling occasions, no more than ten individual organisms were found in the river downstream of the adit and all were Chironomidae (Table S3), giving a mean WHPT

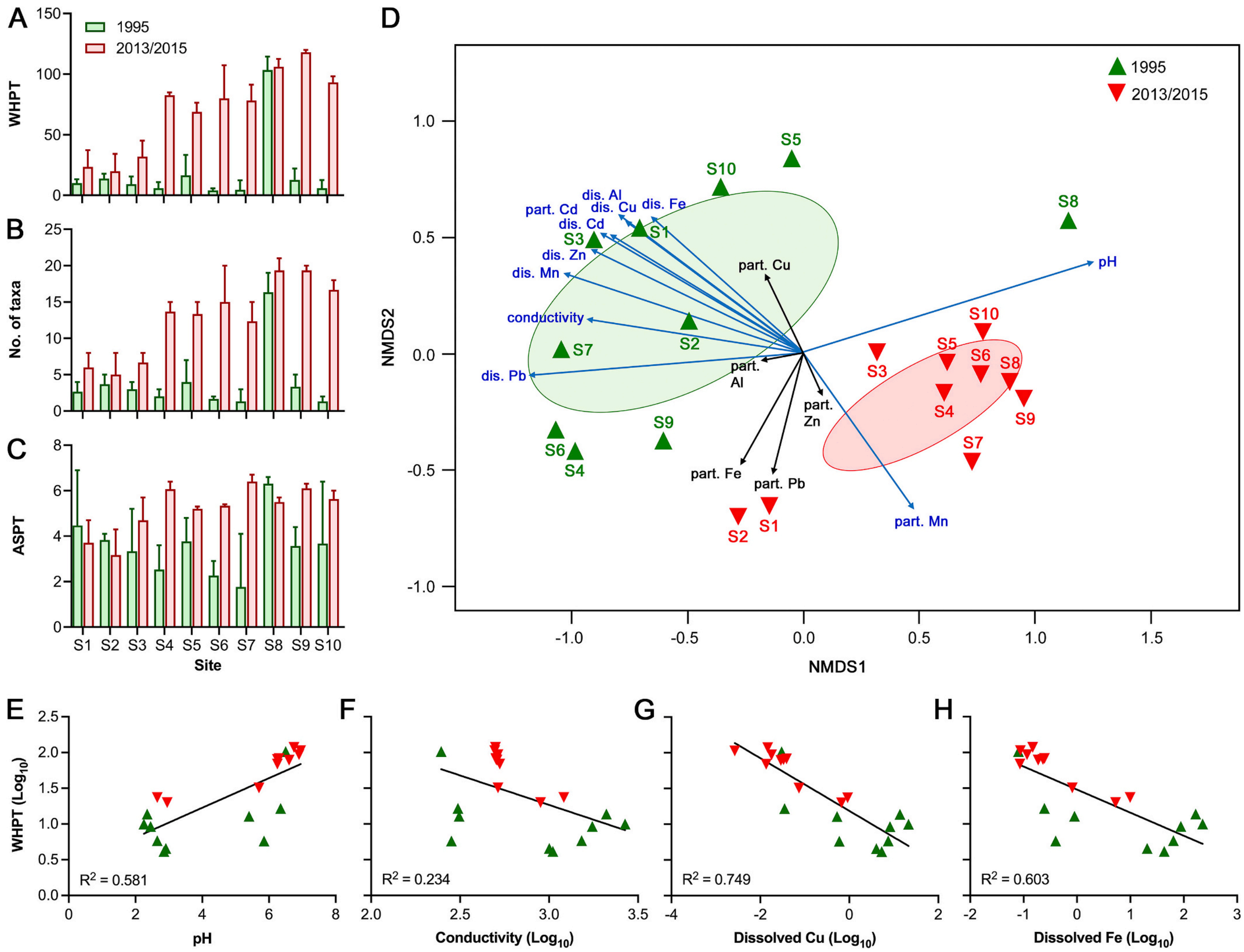

Fig. 6. Benthic invertebrate water quality assessment along the southern Afon Goch in 1995 compared to 2013/2015. (A–C) WHPT (A), number of scoring taxa (taxa richness) (B) and average score per scoring taxa (ASPT) for WHPT (C) values. Each bar represents the mean of three sampling occasions in June 1995, and from three sampling occasions in 2013/2015 (August 2013 and February 2015). Error bars show data range. (D) NMDS of the benthic invertebrates sampled at sites along the southern Afon Goch in 1995 compared to 2013/2015 with water chemistry variables indicated by loading vector arrows. Blue and black vectors indicate significant ($p < 0.05$) and non-significant ($p > 0.05$) correlations, respectively. The shaded ellipses indicate 95 % confidence interval ordinations. Analysis was performed using square root transformed mean data. (E–F) Linear regression of WHPT mean data for each site in 1995 and 2013/2015 in relation to pH (E), conductivity (F), dissolved Cu concentration (G) and dissolved Fe concentration (H). All values apart from pH were log_{10} transformed to perform linear regressions. All sites are on the main river apart from S5 and S8 on tributaries. Upstream site S1 is closest to the mine.

score of 0.6 for sites NA, N3 and N4 (Fig. 7B). In contrast, the upstream sites (N1 and N2) are much less contaminated by excess metals and are near-neutral, and the biodiversity of sites N1 and N2 was much better, with mean WHPT scores of 27.0 (N1) and 15.3 (N2), but the ASPT scores were <5.0, with these sites dominated by Chironomidae. As such, the invertebrate community profiles at N1 and N2 are poor, which may be indicative of poor habitat quality due to the channelisation of the river.

In summary, the high degree of AMD pollution in the northern Afon Goch coupled with river channelisation has given rise to extremely poor benthic invertebrate abundance and biodiversity.

3.4. Biodiversity changes at Dulas estuary

At Dulas estuary in 1993, a total of 13 marine invertebrate species were identified; seven from the rocky substrates that were predominantly crustacea, and six species from the soft substrates (Table 1). The barnacles *A. modestus* and *Semibalanus balanoides* were the most abundant species on the rocky shore at this time (Jones, 1995). In comparison, the 2016 survey identified 24 different species; 13 species identified from the rocky shore and 11 species identified from the sediment/sand substrate, with one of the most abundant species being *O. gammarellus*. Collectively, this data indicates that prior to the AMD diversion, Dulas estuary supported a low number of species. At the control Alaw estuary, while there were some minor differences in the taxonomic profile of invertebrates between the two time periods, at both times >20 different species were identified (Table S5). Infaunal species from the soft substrate habitats were quantified by species richness at both estuaries in April 1993 and April 2016, allowing direct comparison. In 1993 there was significantly reduced ($p = 0.0004$, df = 26, $n = 7–8$) species richness at Dulas estuary compared to Alaw estuary but in 2016 there was no significant difference in mean species number between the two sites (Fig. 8). Moreover, the mean species number was significantly higher ($p = 0.0323$, df = 26, n = 7–8) at Dulas estuary in 2016 compared to in 1993.

Nearly all of the species observed at Dulas estuary in 1993 were also seen in 2016 (Table 1). Additionally, a number of species were found in the 2016 survey species but were not recorded in 1993, including a beadlet anemone *Actinia equina*, four marine annelid worm species, the

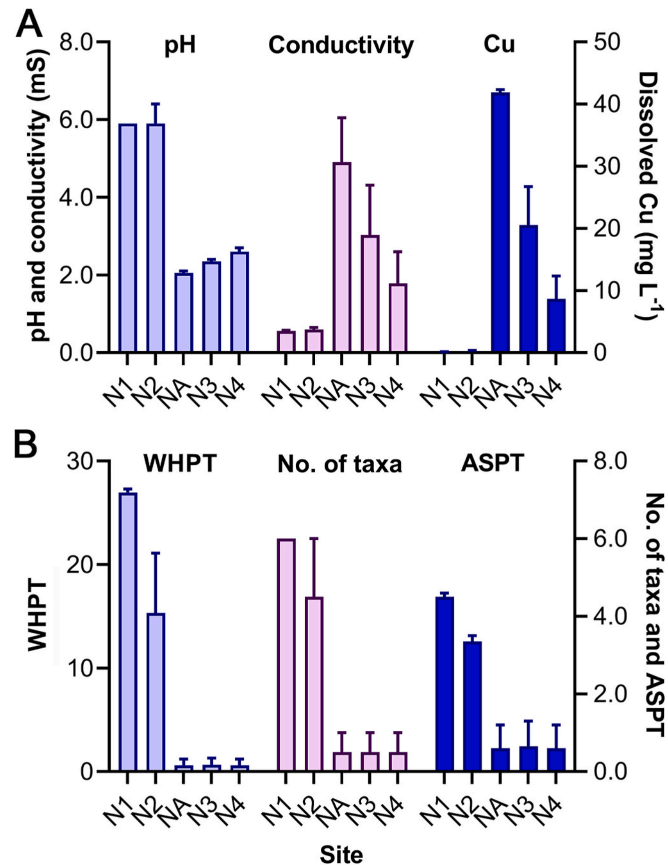

Fig. 7. Water quality assessment along the northern Afon Goch in 2013/2014 upstream and downstream of the mine adit. (A) pH, conductivity and dissolved Cu concentration at 5 sample sites along the river. Data for other metals are presented in supplementary information. (B) WHPT, number of taxa (taxa richness), and average score per taxa (ASPT) for WHPT values at each site. Sites N1 and N2 are upstream of the mine adit (site NA) and sites N3 and N4 are downstream of the adit. Each bar represents the mean of 4 or 6 replicate samples collected in August 2013 and March 2014. Error bars show data range.

Table 1
Intertidal and infaunal invertebrate species observed on hard and soft substrate at Dulas estuary in 1993 and 2016. The rocky shore (hard substrate) sites used for sampling were DR1–DR6 (1993), and DR2, DR5–DR9 (2016). The sand/sediment (soft substrate) sites used for sampling were DS3, DS5 and DS7 (1993), and DS1–DS4 and DS6–DS9 (2016). The presence of an 'X' symbol indicates the presence of specific taxa.

	Hard substrate locations		Soft substrate locations	
Taxa	1993	2016	1993	2016
PHYLUM: ANNELIDA				
Arenicola marina				X
Hediste diversicolor[a]		X	X	X
Lanice conchilega		X		X
Nephtys hombergii				X
Pygospio elegans				X
Tubificoides sp.			X	
SUBPHYLUM: ANTHOZOA				
Actinia equina		X		
SUBPHYLUM: CRUSTACEA				
Austrominius modestus[b]	X	X		
Bathyporeia pilosa			X	X
Carcinus maenas	X	X	X	X
Corophium volutator		X	X	X
Crangon crangon			X	
Haustorius arenarius				X
Ligia oceanica	X			
Orchestia gammarellus	X	X		
Semibalanus balanoides	X	X		
Spirorbis spirorbis	X	X		
PHYLUM: MOLLUSCA				
Cerastoderma edule				X
Littorina littorea		X		
Littorina obtusata		X		
Littorina saxatilis	X			
Patella vulgata		X		
PHYLUM: NEMERTEA				
Unknown Nemertea species		X		X
Total number of species	**7**	**13**	**6**	**11**

[a] Previously *Nereis diversicolor*.
[b] Previously *Elminius modestus*.

amphipod *Haustorius arenarius*, the cockle *Cerastoderma edule*, two periwinkles *Littorina littorea* and *Littorina obtusata*, and the limpet *Patella vulgate*, indicating that these may be pollution-sensitive species. In contrast, potential pollution-tolerant species that were abundant in 1993 were the ragworm *Hediste diversicolor* (previously known as *Nereis diversicolor*) and the crustacean *Corophium volutator*.

The low diversity of invertebrates at Dulas estuary in 1993 was analysed to determine if this correlates with exchangeable sediment metal concentration. Infaunal species richness was determined across the four seasons in 1993 (January, April, July and October) at both Dulas estuary and Alaw estuary, with species richness significantly reduced at Dulas at all four times of the year (Fig. S5A). In 1993, Dulas estuary sediment was heavily contaminated with metals, with mean total sediment concentrations of 50.8 mg g^{-1} Fe, 2.01 mg g^{-1} Cu, 1.2 mg g^{-1} Zn and 0.43 mg g^{-1} Mn. However, because exchangeable fractions of metals from sediments are considered more relevant since this correlates with bioavailability (Jones et al., 2008), exchangeable (HCl extractable fraction) sediment metal concentrations (for Cu, Zn, Fe and Mn) were determined. For Cu and Zn there were significantly higher concentrations in Dulas estuary sediments than Alaw estuary throughout the year (Fig. S5B). Sediment Fe was only significantly higher in the January sample, and sediment concentrations of Mn were higher at all time points except for January. By comparing the infaunal species richness with the estuary sediment metal concentrations, it was found that there were negative correlations between mean species number and mean sediment metal concentrations for each month studied (Table S6), with significant ($p < 0.01$) negative correlation between species richness and sediment exchangeable Cu and Zn in July 1993 ($r = -0.899$ for Cu and -0.887 for Zn).

In summary, substantial changes in biodiversity at Dulas estuary following the AMD diversion, with the current presence of a number of rocky shore and soft sediment species that could not previously be supported at the site, presumably due to sensitivity to high metal availability.

3.5. Metal bioaccumulation at Dulas estuary

Measurements of six metals (Cd, Cu, Fe, Mn, Pb and Zn) in *O. gammarellus* samples collected from the six Dulas sites in 2016 found tissue metal concentrations that were fairly consistent (Fig. S6). For example, Cu concentrations ranged from ~50 to 60 μg g^{-1}, Zn from ~60 to 100 μg g^{-1}, and Cd from ~0.12 to 0.24 μg g^{-1}. Barnacles are also considered as good bioindicator species (Rainbow, 1995) and so metal bioaccumulation into *A. modestus* samples from Dulas estuary was performed. In contrast to *O. gammarellus*, *A. modestus* metal content was typically much higher and there was more variation in samples from the different sites (Fig. 9A). Metal concentrations in *A. modestus* were higher in samples from sites DR7 and DR8, with concentrations of Cu reaching ~200 μg g^{-1}, Cd at ~5 μg g^{-1}, and Zn at ~3500 μg g^{-1}. In addition,

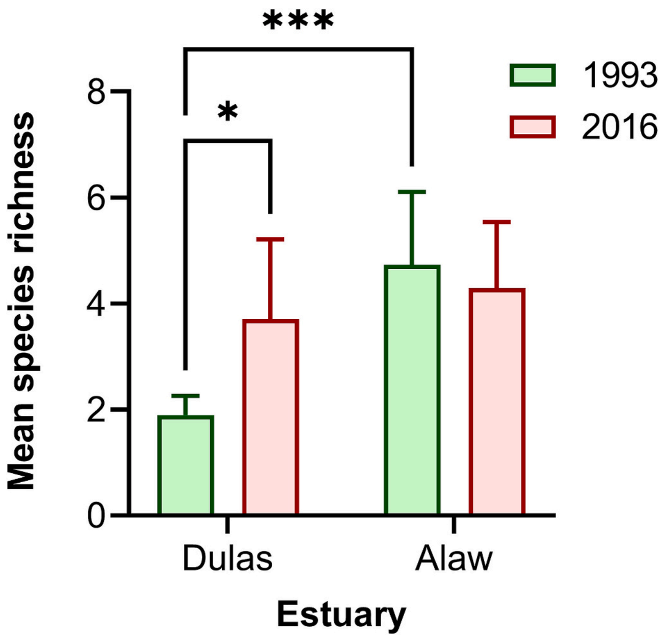

Fig. 8. Mean species number (species richness) of infaunal invertebrate species observed on soft substrate at Dulas and Alaw estuaries in 1993 and 2016. The sand/sediment (soft substrate) sites used for sampling were DS3, DS5 and DS7 in Dulas estuary, and AS1, AS2 and AS5 in Alaw estuary (in April 1993), and DS1–DS4 and DS6–DS9 in Dulas estuary, and AS2–AS8 in Alaw estuary (in April 2016). Values are a mean of the species counts determined at each site and error bars show standard deviation. The asterisks show where there is significant differences (*, $p < 0.05$ and ***, $p < 0.001$) between the indicated samples, as determined by two-way ANOVA.

metal concentrations were determined from *A. modestus* samples collected from two sample sites at the Alaw estuary (AR2 and AR3) in 2016. By comparing with a pooled mean value of *A. modestus* metal concentration from all the Dulas estuary sites, for most metals (such as Fe, Zn and Cd), there was no significant difference between the two estuaries (Fig. S7A). However, barnacle Cu concentrations were significantly higher in the samples from Dulas estuary compared to those from Alaw estuary. Likewise, Cu concentrations in *O. gammarellus* were significantly higher in the samples from Dulas estuary than from both Alaw estuary sites, while there were also differences for *O. gammarellus* Mn and Cd between both estuaries (Fig. S7B).

Measurements of metal accumulation into *A. modestus* samples from site DR6 at the mouth of the Dulas estuary were taken in 1989, and therefore could be directly compared with *A. modestus* metal content at the same site in 2016. It was previously shown that concentrations of Cu, Fe and Zn in the bodies of *A. modestus* examined from Dulas estuary were very similar between spring and summer (Al-Thaqafi and White, 1991), therefore the comparison made here between July 1989 and April 2016 samples was appropriate. There was no difference in Fe content in the *A. modestus* samples between 1989 and 2016, but a ~7-fold decrease in Cu content ($p = 0.0002$, df $= 4$, $n = 3$ for each sample) and ~5-fold decrease in Zn content ($p < 0.0001$, df $= 4$, n $= 3$ for each sample) in the 2016 samples compared to the 1989 samples (Fig. 9B).

In summary, these findings show that bioindicator species metal bioaccumulation can be used as a measure of past and present metal contamination of a location. While Cu and Zn bioaccumulation at Dulas estuary is markedly reduced since the diversion of AMD, there is evidence of some higher metal contamination at Dulas estuary compared to the control Alaw estuary site.

4. Discussion

4.1. Ecological recovery of the southern Afon Goch

In this study the ecological recovery of the southern Afon Goch, 10–12 years after AMD diversion, has been demonstrated using analysis of benthic invertebrate communities, which are well established as bioindicators of AMD (Clapcott et al., 2016; Gray and Delaney, 2008). A previous study from a former mining site in Canada used invertebrate community analysis as a means to demonstrate partial habitat recovery and water quality improvement following AMD diversion (Gunn et al., 2010). Although these authors demonstrated a rapid recolonisation of many families of benthic invertebrates, their site remained significantly impaired 8 years after the AMD diversion, with a low abundance of AMD-sensitive organisms. The efficiency of river recolonization will depend on available colonist pools. For example, Nelson and Roline (1996) demonstrated that macroinvertebrate communities can recover quickly if the concentrations of metals remain low but also if there are nearby colonist pools. It has also been shown that species with more efficient downstream drift propensity (as determined by river drift rate), such as baetis mayflies, are more likely to recover due to their ability to recolonize the river (Cadmus et al., 2016). Here, we showed that two tributaries of the southern Afon Goch (sites S5 and S8) were always uncontaminated with AMD (Fig. 4) and so may have acted as invertebrate colonist pools for recovery of the main river ecosystem. However, it was noticeable that the biodiversity of tributary S5 was low in 1995 (Fig. 6A–C) for unknown reasons, meaning that this site may not have been as important for seeding recolonization.

In 1995 the riverbed of the southern Afon Goch was coated with iron oxide precipitate along the whole course of the river (Fig. 3). In particular, there were very high concentrations of particulate Fe and Cu in the lower reaches (S9 and S10) of the river water pre-diversion as these metals were washed downstream (Fig. 4F), which would have had a deleterious effect on the invertebrate community. This deposition of metal hydroxides including iron hydroxides on the riverbed causes loss of habitat and a consequent decline in the invertebrates. For example, McKnight and Feder (1984) found that precipitation of hydrous metal oxides greatly decreased the abundance of benthic invertebrates and suggested that metals covering the riverbed may have a more deleterious effect on communities than high dissolved metal ion activities. This may occur through fine metal sediments causing direct toxicity and coating of food sources including benthic algae and detritus, thereby reducing food availability. However, studies have also shown that water column chemistry is more important as an indicator of invertebrate toxicity than sediment chemistry (Soucek et al., 2000). By 2010 following the AMD diversion, although iron hydroxide precipitation on the riverbed was reduced (Fig. 3), some metals had presumably dispersed into the sediment, explaining higher concentrations of Mn and Zn in the southern Afon Goch riverbed sediment post-diversion, possibly due to an antagonistic relationship between metal hydroxide availability and Mn and Zn sedimentation (Fig. 5), yet invertebrate abundance remained high during this period (Fig. 6). This may indicate that parameters such as water column pH and dissolved metal concentration are more important than sediment metals with regard to benthic invertebrate recovery.

The invertebrate community data indicates that there was a substantial recovery at two of the downstream sites (S9 and S10), with species richness, and WHPT and ASPT index values from 2013/2015 equivalent to the 1995 site S8 tributary values (considered as a control). There was also increased diversity at sites S4 to S7, but there was still relatively poor invertebrate fauna recovery at upstream sites S1 to S3. While dissolved metal concentrations at these three sites are substantially lower than in 1995, they remain higher than the other southern Afon Goch sites in 2013/2015, and pH remained low at site S1 and S2. These 2013/2015 site S1, S2 and S3 concentrations for dissolved Cd, Cu, Fe, Pb and Zn are still too high and are contravening the UK

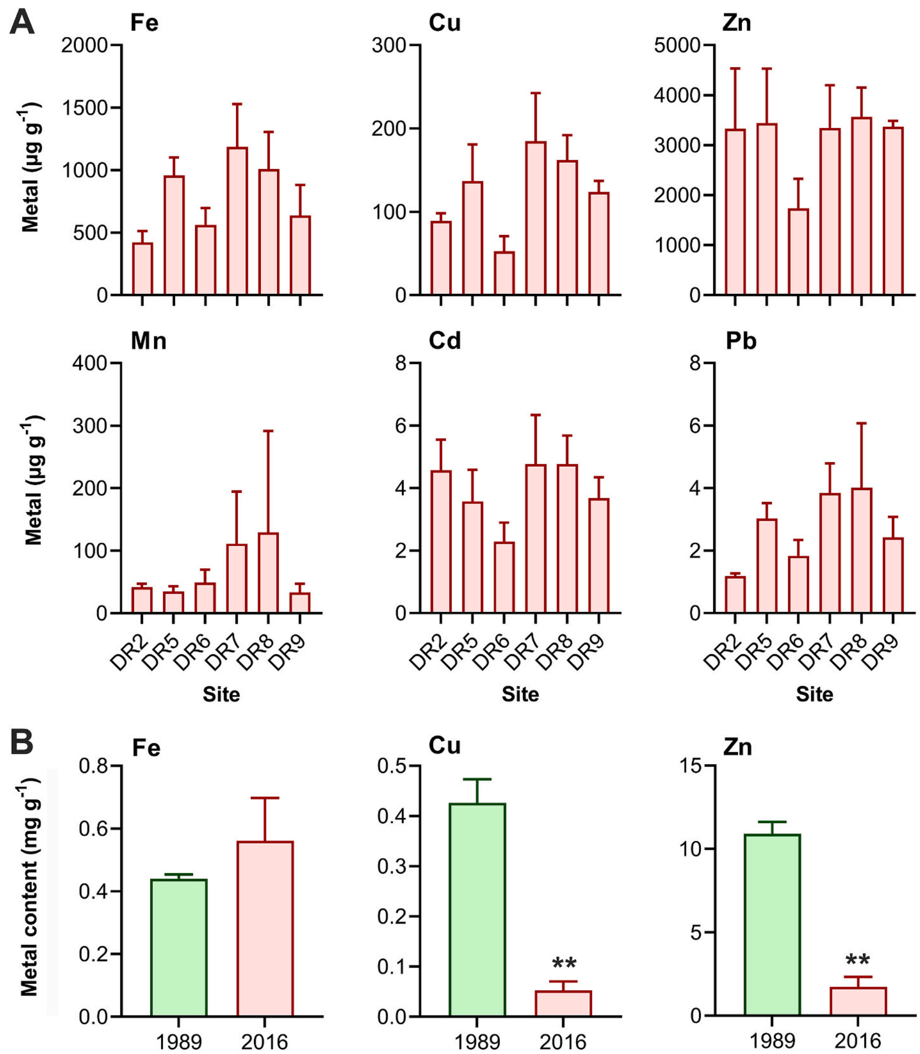

Fig. 9. Metal accumulation into *Austrominius modestus* samples collected at Dulas estuary. (A) Metal content in *A. modestus* samples collected from six rocky shore sampling sites at Dulas estuary in April 2016. (B) Historical comparison between metal content in *A. modestus* samples collected at rocky shore site DR6 at Dulas estuary in July 1989 and April 2016. All data are mean values of three replicate samples (each of ten pooled individuals) and error bars show standard deviation. Statistical analysis was performed on data in (B), and asterisks (**) indicate significant difference ($p < 0.01$) of the 2016 samples compared to the 1989 samples, as determined by unpaired t-tests.

Environment Agency annual average environmental quality standards concentrations for these metals in freshwaters. Site S1 is likely directly impacted by runoff from exposed spoil on Parys Mountain. Sites S2 and S3 are above and below a natural wetland that has been shown to have a significant remedial effect, giving rise to reduced dissolved metals and acidity at site S3 (Aguinaga et al., 2018; Dean et al., 2013). Many variables within wetlands, not only water chemistry, but other factors such as vegetation structure, geomorphology and nutrient loading can affect invertebrate taxa diversity and richness (Meyer et al., 2015). This means that invertebrate community data are not necessarily good bioindicators of water quality within some wetlands due to factors including channelisation and sediment deposition. Furthermore, the three upstream sites, in contrast to those downstream, are largely channelised and embanked with a depositional substrate of silt and sand, creating a poor-quality invertebrate habitat. Likewise, Hoiland et al. (1994) showed that improved water quality alone due to reduced metal concentrations did not lead to increased invertebrate biodiversity where habitat quality was degraded, such as by channelisation, suggesting that the invertebrate community may remain poor at these upstream sites.

Although benthic invertebrates are good bioindicators of ecological recovery to AMD, quantification of fish community recovery following AMD remediation or diversion has been regarded as particularly useful because of their importance in aquatic ecosystems (Greig et al., 2010; Schorr and Backer, 2006). Recovery of fish populations may take longer than for macroinvertebrates; for example, at one AMD-restored site in Pennsylvania it took 40 years to see a doubling of fish species richness (Williams and Turner, 2015). This is in part due to faster recolonisation ability by many invertebrate taxa, including those with a flight stage to their life-cycle (Stoertz et al., 2002). There is currently little available data on the current and historic presence of fish in the Afon Goch catchment (Mullinger, 2004), although individual brown trout were identified in the southern Afon Goch in 2021 that showed patterns of genetic isolation and reduced diversity arising from legacy pollution (Osmond et al., 2024). Although the benthic invertebrate data in this study does provide strong evidence of recovery of the southern Afon Goch, future work should attempt to demonstrate that other aspects of this river ecosystem, including fish populations are also recovered.

4.2. Ecosystem recovery at Dulas estuary

The value of our study is that the river ecosystem improvement was examined over a long time period (>10 years), but that it also encompasses an analysis of the full catchment, including the estuary. Dulas estuary was a sink for metal deposition from Parys Mountain via the southern Afon Goch for many years (Foster et al., 1978), and the level of contamination observed here in 1993 is comparable with other metal-polluted estuaries in the past such as the Fal estuary in Cornwall, and much higher than at non-polluted sites (Williams et al., 1998). The pollution of Dulas estuary in 1993 was also put into context by comparison with the uncontaminated Alaw estuary (Fig. S5), where there was 7.2 mg g^{-1} of exchangeable Fe compared to 23.2 mg g^{-1} at Dulas estuary, and 3.3 μg g^{-1} of exchangeable Cu at Alaw compared to 730.3 μg g^{-1} at Dulas. Since the AMD diversion, the amount of dissolved and particulate metals entering the estuary from the southern Afon Goch has reduced substantially (Chalkley et al., 2019). Although recent estuary sediment metals concentrations are not available to compare with 1993, the reduction in concentrations of Cu and Zn found in recent (2016) samples of the barnacle *A. modestus* compared to historic (1989) measurements (Fig. 9B), are strongly indicative of reduced metal pollution in Dulas estuary over time. Nevertheless, the higher accumulation of Cu in *A. modestus* and *O. gammarellus* at Dulas estuary compared to Alaw estuary in 2016 samples (Fig. S7), indicates that there remains some pollution at Dulas.

Filter feeders, such as barnacles can uptake both dissolved and particulate metals, which can lead to significant bioaccumulation of metals, particularly from particulate metals. As such, barnacles have often been used as bioindicators of metal contamination in coastal waters and estuaries, particularly because they poorly regulate body metal levels and so these tissue concentrations correlate well with amounts in the environment (Powell and White, 1990; Rainbow, 1985, 1995; Reis et al., 2011). For example, samples of the barnacle *S. balanoides* collected from Dulas estuary in the 1970s was found to contain very high concentrations of Zn (up to 50.3 mg g^{-1}) and to a lesser extent Cu (up to 3.8 mg g^{-1}), both in the form of discrete granules in the parenchyma cells of the gut (Walker, 1977; Walker et al., 1975). It is by forming insoluble granules that the barnacles are able to tolerate such high metal burden (Reis et al., 2011). The amphipod crustacean *O. gammarellus* accumulated lower concentrations of metals than barnacles since it accumulates dissolved metals in solution from water and from feeding on detrital material like decaying seaweed, and is therefore unlikely to ingest particulate metals from the water column (Rainbow and Luoma, 2011). Furthermore, *O. gammarellus* is more sensitive to metal accumulation than barnacles. While *O. gammarellus* has been considered as a useful metal bioindicator (Moore et al., 1991), in some cases there was no difference in metal uptake rate between polluted and control sites, suggesting that it may be able to acquire tolerance to metal pollution (Rainbow et al., 1999). However, previous laboratory studies found that *O. gammarellus* from Dulas estuary were more metal sensitive than those from uncontaminated sites (Mouneyrac et al., 2002). Additionally, concentrations of Cu and Zn measured in this study in *O. gammarellus* from 2016 were ~2 to 3-fold lower than in samples collected from past surveys of the Dulas estuary by Weeks (1992) and are in line with background levels (Moore et al., 1991).

An increase in estuarine species richness was able demonstrate the recovery of the Dulas estuary ecosystem following the diversion of AMD away from the southern Afon Goch. The analysis showed that at both the Dulas rocky shore and sandy shore locations, there was an increase in the number of species in 2016 compared to 1993, and an increased number of metal-sensitive species. In contrast, the total number of species and species richness at the uncontaminated Alaw estuary between the two time points were equivalent. This also gave confidence that the sampling methods used in 1993 and 2016 could be compared.

In 1993, in the soft sediments of Dulas estuary, only two species were recorded in any number, the polychaete *H. diversicolor* (ragworm) and the amphipod *C. volutator*. These were also consistent with earlier observations at Dulas estuary by Foster et al. (1978). *H. diversicolor* has previously been demonstrated to be resilient to high trace metal concentrations (Bryan and Hummerstone, 1971), while *C. voluntator* has been shown to tolerate high Cu (Icely and Nott, 1980). A notable species that was present in 2017 but not in 1993 is the lugworm *Arenicola marina* which is highly sensitive to elevated levels of Cu and Zn (Bat and Raffaelli, 1998). Numerous studies have focused on the use of polychaetes such as *H. diversicolor* and *A. marina* as bioindicators of metal pollution, including from Dulas estuary (Packer et al., 1980; Zhou et al., 2003). The presence of *A. marina* and the edible cockle *C. edule* at Dulas estuary in 2016 are also indicative of a reduction in bioavailable Cu and Zn levels. Although *C. edule* seems to be sensitive to metal contamination from sediments, it bioaccumulates metals and hence is potentially a risk to humans and other animals consuming cockles from polluted sites (Veiga et al., 2019). The Dulas rocky shore community in 1993 also showed limited biodiversity and was principally composed of barnacles and the rough periwinkle *Littorina saxatilis*, while in 2016, there were more species of periwinkle and abundant crustacea, most notably *O. gammarellus*.

4.3. Implications of AMD diversion on the northern Afon Goch

The AMD from the Parys Mountain mine was diverted away from the southern Afon Goch to prevent a flood risk to the nearby town. It was simply relocated into a smaller stream channel known as the northern Afon Goch where it subsequently drains into the Irish Sea (Chalkley et al., 2019). The implication of this diversion is that while the ecology of the southern Afon Goch and Dulas estuary has improved, invertebrate biodiversity is poor in the northern stream. Benthic invertebrate data for the northern Afon Goch prior to 2003 is not available, but a comparison of the communities upstream and downstream of the AMD input reveals a marked reduction in both the number of individuals and biodiversity at the downstream sites (Fig. 7B). However, the biodiversity in the upstream sites (N1 and N2) was still relatively poor, and most likely a consequence of the channelised nature of the northern stream, with steep sides, a linear course, and little geomorphological diversity. Channelised rivers are characterised by low macroinvertebrate diversity, and are considered a poor habitat (Paetzold et al., 2008). This contrasts with the southern Afon Goch, which mostly retains its natural course and is morphologically diverse, and so has much greater potential for ecological recovery once the AMD is removed.

It could be argued that if AMD cannot be treated it is better to transfer it to an already degraded environment, such as the northern Afon Goch, to allow a potentially ecologically important site to recover. This may result in much greater impact to the already degraded site and a more appropriate route would be to divert the AMD to a location that allows treatment, although this would be expensive. However, due to the toxic levels of AMD pollution present within the northern Afon Goch and the amounts of metals entering the Irish Sea via this route (Chalkley et al., 2019), there is an incentive to ultimately remediate this stream (Todd et al., 2024). Remediation trials have been successfully investigated in the past (Younger and Potter, 2012), although discontinued due to cost, while more recently, laboratory-based passive remediation using contaminated water from the northern Afon Goch have shown success (Millán-Becerro et al., 2023). However, full natural recovery as seen with the southern river is unlikely as further restoration of the physical and morphological characteristics of the northern stream will be required (Palmer et al., 2014).

5. Conclusion

The data presented in this study shows that within 10–12 years, an estuarine and river system can recover from AMD pollution if there is no other degradation to the physical habitat. This is despite the source pollution from the abandoned mine remaining unchanged and large amounts of metal pollution entering the Irish Sea by a different route. The redirection of AMD into a 'lower value' stream channel has allowed biodiversity to almost fully recover in the previously contaminated river. Diversion has also allowed Dulas estuary to recover, while the

AMD which now enters the Irish Sea directly results in the rapid dispersal and dilution of the metal-contaminated effluent. However, the ecological recovery of this southern Afon Goch catchment did not involve any direct restoration apart from the original diversion of AMD, but it is unknown whether recovery would be more substantial if additional restoration and remediation had been implemented. The recovery of the lower river was also assisted by the natural wetland, which we have shown to effectively remove metals in the water column. Sites closest to the AMD source, such as those upstream of the wetland, or where metals may persist for long periods of time such as in the river and estuary sediments may still be recovering although it is likely that the improvements in water quality and biodiversity will continue. AMD is a major problem worldwide, however, this study has shown that where contaminating AMD is removed or treated, in a short timescale river and estuarine ecosystems can show substantial recovery.

CRediT authorship contribution statement

Andrew P. Dean: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Jennifer Nelson:** Investigation, Formal analysis, Data curation. **Andrea P. Jones:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrew Sykes:** Investigation, Formal analysis, Data curation. **Frederick Child:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christopher J. Sweeney:** Investigation, Formal analysis, Data curation. **Khalil Al-Thaqafi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Keith N. White:** Writing – review & editing, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Jon K. Pittman:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.178726.

Data availability

Data will be made available on request.

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