

# Living in a post-industrial landscape: repeated patterns of genetic divergence in brown trout (*Salmo trutta* L.) across the British Isles

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## Abstract

**Aim:** The British Isles have been worked for millennia to extract metal ores to feed industrial development, leaving a legacy of mine water pollution that continues to impact freshwater communities in many regions. Brown trout (*Salmo trutta* L.) have long been observed to persist in these metal-impacted systems as apex predators, with previous studies showing a small number of impacted populations to be highly genetically divergent. We sought to understand the scale of genetic diversity across regions and the repeatability of genetic divergence in trout populations affected by metal pollution.

**Location:** We examined four mine water-impacted regions across the British Isles: west Wales, northeast England, southwest England and southeast Ireland.

**Methods:** We employed a panel of 95 SNP loci to screen 1236 individuals from 71 sites representing paired metal-impacted and clean sites from across the four regions. From these, we obtained diversity statistics, assessed genetic structuring of populations and modelled historical demographic scenarios to understand which factors most credibly explain genetic variation in divergent populations.

**Results:** We evidenced hierarchical population structure in the regions studied, in line with expectations from phylogeographic history. However, in a hierarchical analysis of genetic structuring the first level of differentiation was driven by the divergence of the metal-impacted trout of Cornwall in southwest England. Within regions we observed reduced genetic diversity and repeated patterns of local genetic sub-structuring between paired samples from metal-impacted and relatively clean sites. Demographic history analyses suggested the timing of these splits to be relatively recent and to be associated with periods of peak mining activity.

**Main conclusions:** Our findings demonstrate distinct patterns of genetic isolation and reduced diversity arising from legacy pollution in freshwater ecosystems, with impacts being most apparent where both chemical pollution and physical barriers are

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present. Management should focus on the amelioration of mine water wash-out and the removal of barriers to fish movement to safeguard genetic diversity in impacted populations.

#### KEYWORDS

metal tolerance, mine water, population genetics, *Salmo trutta*, SNP assay

## 1 | INTRODUCTION

Fundamental to the practice of ecological research during an epoch of unprecedented biodiversity decline is quantifying the impact of anthropogenic stressors on the viability of species and their populations. Increasingly, research has demonstrated that conserving biodiversity during the Anthropocene must look beyond species and/or functional groups, but instead must focus on the conservation of genetic diversity within populations (Hoban et al., 2021). Conservation of genome-wide diversity has been shown to be critical beyond the maintenance of functional units of interest in maintaining long-term adaptive potential and providing protection from the deleterious effects of inbreeding depression (Kardos et al., 2021). Numerous anthropogenic stressors have been associated with the loss of genetic diversity within populations, including several relevant to this study: inorganic pollution (Mussali-Galante et al., 2013; Xie et al., 2016) and habitat loss and fragmentation (Lino et al., 2019; Schlaepfer et al., 2018).

Recently bottlenecked populations are repeatedly observed to have a higher proportion of deleterious mutations due to the increased effects of genetic drift and weakened purifying selection in smaller populations (Bortoluzzi et al., 2020; Grossen et al., 2020). Furthermore, loss of genetic diversity through drift and inbreeding depression as a consequence of population contractions are predicted to have long-term effects in removing alleles which may facilitate evolutionary responses to future stressors, described as adaptive potential (Hoffmann et al., 2017). Such adaptive potential is critical to enable population persistence in the face of rapid environmental change, e.g. due to global warming (Reed et al., 2011), and a lack of adaptive genetic variation has been linked to populations showing high rates of decline, e.g. in the yellow warbler, a species experiencing major changes in climate across different parts of its range in North America (Bay et al., 2018). Similarly, a recent meta-analysis (Leigh et al., 2019) suggested a decline of ~6% in genetic diversity across 91 studied species over the last century, making these impacts widespread and pertinent to a species' future viability. Identification of populations experiencing strong patterns of genetic drift due to population declines and isolation is therefore valuable in assessing the impact of anthropogenic stressors on the viability and health of populations.

These effects are perhaps best understood in charismatic megafauna in which population sizes have fallen to hundreds or even tens of individuals (Mondol et al., 2013). Freshwater migratory fish have, however, faced the greatest rate of decline of any vertebrate group

assessed to date (Deinet et al., 2020) and it is, therefore, pertinent to understand the extent to which the genetic diversity of impacted populations has been affected by and associated with, potentially deleterious, effects. Anthropogenic activities have been shown previously to alter the population genetic structure of salmonid populations, such as the proliferation of large dams on the Columbia river selecting for a less diverse range of life history strategies and favouring variation for residency and passage by fish ladders (Waples et al., 2008) or via the loss of adaptive variation through habitat alteration (Thompson et al., 2019). These factors have shaped the evolutionary potential of these isolated populations to readapt to freshwater ecosystems after they are restored to their natural state; with longer isolation periods and more drastic bottlenecks associated with isolation reducing the rate of realised positive population outcomes from habitat restoration (Fraik et al., 2021). Given the variable scale of population genetic impact from anthropogenic stressors, conservation of impacted populations must therefore focus on accurately quantifying the scale of impact to inform best management responses. Areas of focus in elucidating the level of negative effect include genetic structure and drift in isolated populations, and historic demography to contextualise changes and the degree of local adaptation (Hoban et al., 2021). Conservation practitioners may choose to undertake genetic rescue of isolated populations, with this having the potential to introgress adaptive alleles into groups that have experienced high levels of drift and have otherwise limited evolutionary potential (Fitzpatrick & Reid, 2019). Other studies have, however, demonstrated the risk of outbreeding depression from the loss of local adaptation in experimentally outcrossed Atlantic salmon populations (Houde et al., 2011) and, as such, careful examination of the diversity of source populations and the projected impacts of inbreeding and drift should be taken into account to ensure best outcomes for ongoing population viability (Trask et al., 2021). In anadromous fishes this has particular relevance where there is commercial exploitation through a mixed-stock fishery at sea harvesting genetically distinct populations of differing natal origin and risk status; evidence of capture from such vulnerable populations should inform fishing effort and restrictions (e.g. Bradbury et al., 2016; Gilbey et al., 2021; King et al., 2016; Koljonen et al., 2014; Lehnert et al., 2023; Shaklee et al., 1999). Conversely, the study of genetic diversity and population structure may sometimes highlight how recognised taxonomic units lack evolutionary significance, as in the case of the phenotypically described and the critically-endangered Balearic shearwater (*Puffinus mauretanicus*), which has been shown to exhibit no significant evidence of genetic distinction from the less threatened Yelkouan

shearwater (*Puffinus yelkouan*) (Ferrer Obiol et al., 2022); as such, these two 'species' should be treated as a single entity. Thus, the modelling of demographic history offers insight through evolutionary time as to one of the most critical factors shaping genetic diversity: effective population size (Melis et al., 2020), and the impact of historical environmental change on population dynamics and how future anthropogenic changes are therefore likely to impact populations (Prates et al., 2016). Lastly, monitoring of genetic diversity and associated environmental changes enable the identification of factors interacting to reduce the genetic integrity of populations, thereby facilitating actions to ameliorate such threats (Nicol et al., 2017).

Here, we examine how long-term anthropogenic pollution impacts populations of an anadromous fish species, *Salmo trutta* L., native to the waters of Britain and Ireland. Specifically, we analyse fish across a broad geographical range, assessing genetic diversity, genetic drift and isolation in populations with differing origins and genetic backgrounds that have been exposed to a common stressor: metal pollution from mine water wash-out. This study system provides insight into the level of risk posed by such pollution to ongoing population viability and informs on the conservation status of local populations.

## 1.1 | Study system

The British Isles have been mined since the early Bronze age (approx. 2000–1500 years BC), with this practice being widespread across many regions, including much of Wales, Cornwall and west Devon, southeast Ireland and northeast England (Mighall et al., 2004; Timberlake, 2017). Peak periods of mining activity occurred around the Roman occupation of Britain (McFarlane et al., 2014), during the medieval period around 1150 AD (Le Roux et al., 2004) and throughout the Industrial Revolution (Mighall et al., 2002; Pirrie et al., 2002). Considerable disturbance has occurred to freshwater ecosystems within these regions, with the physical modification of rivers for hydrological extraction methods (i.e. 'tin streaming') (Hudson-Edwards et al., 2009), sediment release and often acute levels of dissolved metals. Metal pollution from mining activities (hereafter 'mine water') remains a considerable stressor despite the cessation of the majority of mining activities, due to the persistence of metals and ongoing release of mine water from adits (drainage channels) and spoil heaps from closed workings (Johnston et al., 2007). Mine water pollution has a strong impact on the ecology of polluted watercourses, with the loss of pollution-sensitive invertebrate species from acute toxicity (Dills & Rogers, 1974) and the subsequent loss of higher consumers. This remains a relevant ecological concern, with an estimated 9% of rivers in England and Wales still impacted by mine water pollution (Johnston et al., 2008).

Brown trout (*Salmo trutta*) is widely distributed across temperate European freshwater ecosystems with value as an apex predator within these environments (Jensen et al., 2008; Perälä et al., 2021), with additional high social and economic value (King, 2015). Brown trout across the British Isles display high levels of genetic diversity relating to their phylogeographic history after the last glacial

maximum (McKeown et al., 2010) and local adaptation across fine spatial scales (King et al., 2020; Meier et al., 2014) maintained by high rates of natal fidelity in migratory individuals (Andersson et al., 2017; King et al., 2016). Brown trout have long been seen to tolerate and to be early recolonisers of mine water polluted environments, with early observations of the resultant damage to chromophores producing the 'black finned' trout of western Wales (O'Grady, 1981). The genetic structure and diversity of populations of trout living in highly metal-polluted rivers in west Cornwall have been the focus of multiple studies. These have shown that metal concentrations experienced in the River Hayle, Cornwall, are lethal to metal naïve trout from nearby rivers (Durrant et al., 2011) and that trout living within the River Hayle have significantly elevated metal tissue burdens (Uren Webster et al., 2013). Additional work has shown that ion homeostasis pathways are differentially transcribed between metal-impacted and naïve fish (Uren Webster et al., 2013) and microsatellite analysis has revealed that trout living within metal-impacted rivers in Cornwall are genetically distinct from those from nearby relatively clean rivers, with demographic history modelling suggesting divergence around periods of peak mining activity (Paris et al., 2015). Genetic structure could be maintained by chemical avoidance of metal-naïve migratory individuals to adverse water chemistry, leading to reduced gene flow and genetic isolation (Van Genderen et al., 2016), or could be the result of local adaptation of resident fish to metal-rich environments and mortality of non-adapted individuals (Giardina et al., 2009; Petitjean et al., 2023).

## 1.2 | Aims of the study

In this study, we seek to understand whether patterns of genetic divergence observed previously in metal-impacted populations of *S. trutta* in Cornwall in southwest England (Paris et al., 2015) are replicated in other post-industrial landscapes across Britain and Ireland, where trout populations with distinct phylogeographic histories and differing patterns of background genetic diversity have experienced similar metal-associated stressors. We utilise a newly developed SNP panel (Osmond et al., 2023) to deliver greatly increased breadth of genomic coverage in our population genetic analyses and to provide increased reliability, precision and power to detect changes to genetic diversity and structure (Laoun et al., 2020; Morin et al., 2009); SNPs have largely superseded the use of microsatellites in other recent studies to examine similar questions (e.g. Amish et al., 2022; Jenkins et al., 2019; Ruegg et al., 2014). Specifically, we seek to understand: (i) do genome-wide SNP markers deliver consistent evidence of genetic structuring across trout populations compared to microsatellite studies and what background geographic genetic structure do we see among populations of trout in regions of the British Isles with a history of mining?; (ii) is the pattern of genetic structuring and reduced genetic diversity associated with mine water pollution repeated across different areas of Britain and Ireland beyond the small number of trout populations studied previously in west Cornwall?; (iii) does demographic history modelling credibly support observed genetic structure and does it coincide

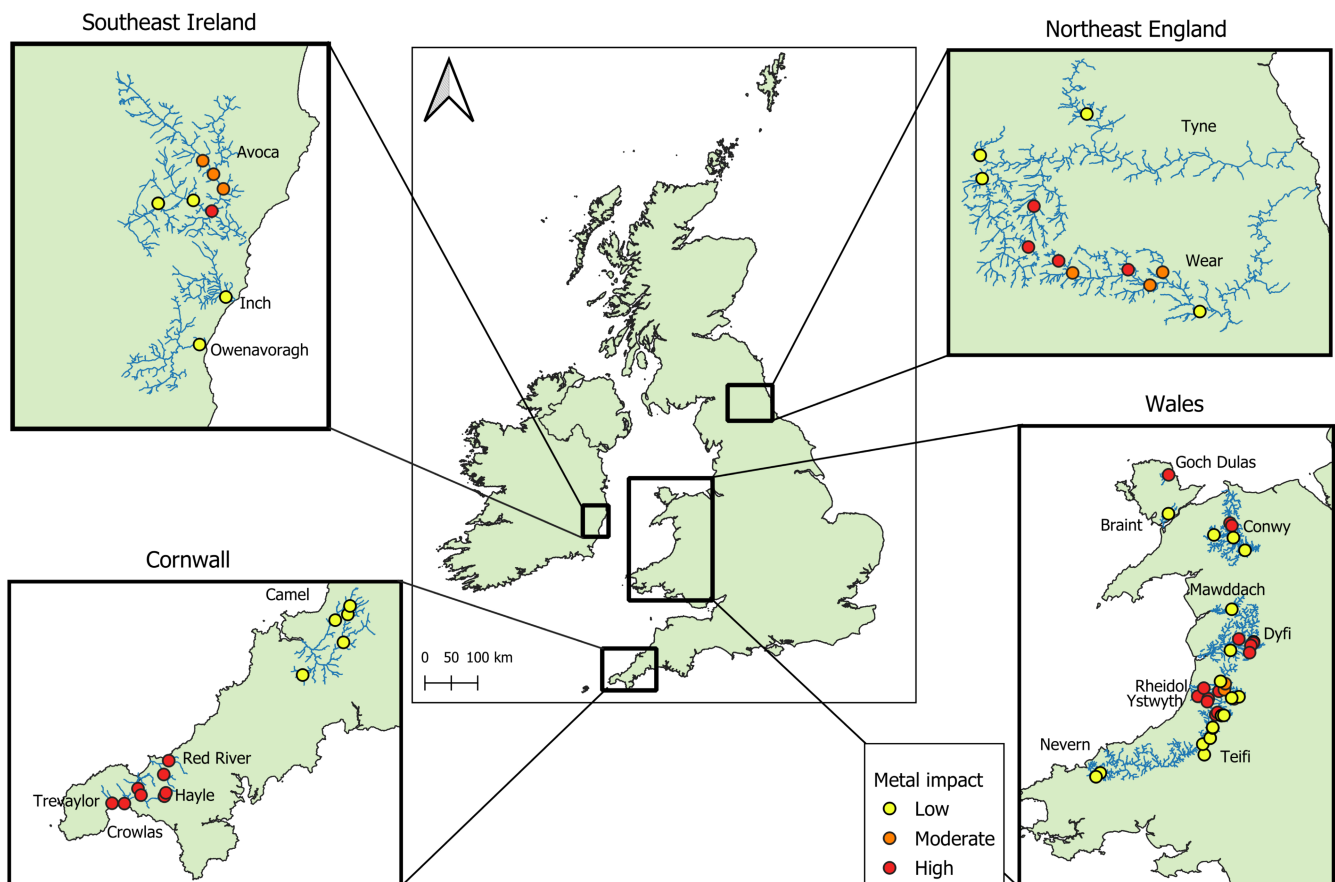
with known historical peaks in exploitation of metal resources?; and (iv) what other biological and environmental factors interact with anthropogenic metal-pollution to either confer resilience or increased susceptibility to deleterious genetic impacts? An understanding of the answers to these questions reveals a broad picture of genetic variability of trout populations in a post-industrial landscape and, in turn, what might be feasible to seek to conserve.

## 2 | METHODS

### 2.1 | Sampling

Sites were identified across four regions, covering southwest (SW) England, southeast (SE) Ireland, western Wales and northeast (NE) England (Figure 1). Water chemistry data and electrofishing reports were obtained from Natural Resources Wales, Environment Protection Agency Ireland, Inland Fisheries Ireland and the Environment Agency. Catchments were targeted within each of these regions based upon concentrations of heavy-metals and the reported persistence of trout populations. Bioavailability of Water Framework Directive relevant metals was calculated using the online Bio-met bioavailability tool v5.0 (<https://bio-met.net/>). A paired sampling strategy was employed, sampling

relatively clean tributaries (those with dissolved metal concentrations relatively unenriched by legacy pollution) within the same catchment as metal impacted sites. Where clean tributaries were unavailable, nearby relatively clean catchments within each of the regions were also sampled, being selected based upon proximity, similar physical and chemical (excluding metal-inputs) properties and previous knowledge of genetic structure (King et al., 2020; McKeown et al., 2010; Prodöhl et al., 2017). At each site, trout were captured by electrofishing over the summers of 2020 and 2021, targeting 1+ parr to reduce potential bias of sampling related individuals in young of the year, with a minimum of 12 individuals targeted per site. Adipose fin clips were collected and stored in absolute ethanol. Additional tissue samples were obtained from previous sampling in 2012 and 2013 as part of the DURESS project to complement our samples, particularly from relatively clean sites in south Wales. All samples were collected by Home Office licenced individuals, in accordance with best welfare practices. A total of 71 sites across 21 catchments were sampled; full details are given in Appendix S1. Water chemistry data for each site are given in Appendix S2. Sites are labelled in a coded format for consistency, such as YHA, whereby the first two letters (YH) indicate the catchment (Ystwyth) and the last letter (A) the individual site. For some historic sampling sites, such as HAY1, a three-letter prefix (HAY) indicates the catchment (Hayle) with the number (1) indicating the individual sampling site from this catchment.



**FIGURE 1** Sampling sites for brown trout (*Salmo trutta* L.) across four regions of the British Isles with a history of metal-extraction and ongoing legacy mine water pollution. Sampled catchments are labelled in their respective inset maps for each of the four major regions and the relative metal-impact at each sampled site is indicated by the colour of the point.

## 2.2 | Genetic analysis

DNA was extracted using the Hotshot extraction method (Truett et al., 2000). We genotyped each sample on the Fluidigm EP1 genotyping system at 95 single nucleotide polymorphism (SNP) loci, with these loci developed and found to be neutral, unlinked and highly polymorphic across populations of in *S. trutta* in the British Isles; making them effective for understanding of genetic structure and diversity, as described in detail in Osmond et al. (2023). Genotype calls were manually curated to ensure accurate calling of individuals, and any individuals with >10% missing genotypes were excluded from downstream analysis.

## 2.3 | Siblings

Each population was screened for full-sibling relationships using the software COLONY v2.0 (Jones & Wang, 2010), with the following parameters: high precision, medium length run, assuming both male and female polygamy and without inbreeding. To check for consistency, analyses were run twice using different random number seeds. If a full-sibling family was detected with a high probability of inclusion (>0.9) in both runs, the yank-2 method was employed to trim full siblings as previous work suggests that this minimises biases in population structure, whilst retaining accurate estimates of other genetic parameters (Waples & Anderson, 2017). Sites where full-sibling removal resulted in <5 individuals were removed from the downstream analysis.

## 2.4 | Estimation of genetic indices

Downstream analyses were conducted in R version 4.1.0 (R Core Team, 2021). Summary statistics of genetic diversity for each population and pairwise Weir-Cockerham  $F_{ST}$  were calculated using the *hierfstat* package (Goudet, 2005), with 1000 bootstraps run to assess confidence intervals. Inbreeding coefficients ( $F_{IS}$ ) were calculated using the *divBasic()* function of the *diveRsity* package (Keenan et al., 2013). *Genepop* v4.0 (Rousset, 2017) was used with default parameters to calculate pairwise linkage disequilibrium between loci, and heterozygosity deficiency and excess from Hardy-Weinberg equilibrium for each locus within each population.  $p$ -values for linkage disequilibrium and Hardy-Weinberg deficiency and excess were corrected using the Holm-Bonferroni method (Holm, 1979) for multiple comparisons.

## 2.5 | Discriminant analyses of principal components (DAPC)

A priori and de novo DAPC analyses (with and without sampling site priors, respectively) were conducted with the *adegenet* package (Jombart, 2008). De novo analyses were conducted using a  $k$ -means clustering method implemented in the *find.clusters()* function and selecting the value of  $k$  with the lowest Bayesian Information

Criterion (BIC) score. Where there was an indistinct pattern of change in BIC scores, multiple levels of  $k$  were investigated for biological relevance. The optimal number of PC axes to be retained, up to  $N_{pop} - 1$ , was calculated using the *optim.a.score()* function. The number of PC axes retained in DAPC followed the recommendations of Thia (2023). DAPC analyses were plotted using the *ade4* (Dray & Dufour, 2007) and *ggplot2* (Wickham, 2016) packages. All DAPC analyses, including de novo plots and individual assignment, are given in Appendix S3.

## 2.6 | STRUCTURE analysis

Genetic structuring of populations was also investigated using the program STRUCTURE (Pritchard et al., 2000). Ten runs were performed for each value of  $k$ , with  $k=1-10$  or when the number of populations ( $n$ ) < 10,  $k=n+1$ , with ploidy set at 2 (diploid) and with no population of origin prior. STRUCTURE was run for 250,000 total repetitions following a burn-in of 100,000 repetitions; all other parameters used default settings. To infer finer-levels of structure (Janes et al., 2017), hierarchical analyses were performed based on the optimum  $k$  value from analysis of the full dataset using the  $\Delta k$  method of Evanno et al. (2005). Output files were exported and analysed in R using the *pophelper* package (Francis, 2017).

Previous studies have reported biases in the  $\Delta k$  method (Janes et al., 2017; Kalinowski, 2011). Whilst inference of clusters that maximise the value of  $\Delta k$ , as implemented by the Evanno methodology (Evanno et al., 2005), can accurately reveal upper hierarchical-level structure for a given dataset, this can be biased when sample sizes representing each cluster are uneven and when hierarchical-island models of migration are assumed (Puechmaile, 2016). We therefore also analysed outputs using the Puechmaile (Puechmaile, 2016) method of  $k$  determination using the online StructureSelector application (Li & Liu, 2018). Where the  $\Delta k$  and Puechmaile metrics disagreed, individual assignment plots for the optimum  $k$  identified by each method were examined in StructureSelector for biological relevance, excluding 'ghost' clusters (Puechmaile, 2016) or equal assignment of individuals to different levels of  $k$ . Final outputs were plotted in R using the *pophelper* (Francis, 2017) and *ggplot2* package and spatial data were plotted in QGIS v3.30.1. Complete plots of hierarchical structure and test statistics are given in Appendix S4.

## 2.7 | Reconstructing demographic history – DIYABC analysis

For reconstructing the demographic history underlying the observed genetic diversity and structure, we used Approximate Bayesian Computation analyses with tree-based classification, as implemented in the DIYABC Random Forest R Shiny package (Collin et al., 2021). Scenarios were simulated for populations where strong genetic structure potentially driven by metal impact was observed; populations

were grouped based on assignment by STRUCTURE and DAPC. Fish in four regions which showed the most distinct genetic structure between metal-impacted populations and their paired controls were explored: (1) the Hayle and Camel, (2) Anglesey, (3) the Wear, and (4) the Tyne (Figure 1). For each group of populations, simple scenarios of pairwise or common divergence were initially run with wide priors of 10–4500 generations for all historical events, 10–10,000 for all current population sizes, and 10–100,000 for ancestral population sizes. Based upon the credibility of each of these simple models, more complex scenarios were run with plausible, historically informed bottlenecks or expansion events added to the most credible scenarios. A complete list of the models and parameters used, and the individuals included in each analysis can be found in Appendix S5. For each model, 20,000 simulations were run, 1000 trees were used for classification of model choice, and the posterior distribution of parameters was calculated from the best model using 100,000 simulations. The range of priors for the modelled scenarios were compared with the observed statistics using principal components analysis along the first three primary axes to ensure an appropriate range of priors and scenarios were simulated. The fit of the observed dataset was compared with the modelled scenarios using the linear discriminant analysis function in DIYABC-RF. Full details of the DIYABC-RF analyses, including samples included in the models, modelled scenarios, posterior probabilities plots of the parameters of the most credible model and LDA plots of fit, can be found in Appendix S5.

### 3 | RESULTS

A total of 1236 individuals were genotyped using Fluidigm SNP assays (Osmond et al., 2023). Of these, 38 (3.1%) failed to score reliably at >10% of loci and were removed from further analysis. COLONY identified several credible full-sibling families, with all individuals from Nant Helygog and Nant Peiran (Wales), and Sulphur Brook (southeast Ireland) belonging to a single full-sib family at each site. Thirteen other sampled sites had full-sibling families of more than two individuals, which were yank-2 (Waples & Anderson, 2017) trimmed for downstream analyses, leaving a final dataset of 1139 individuals across 68 sites genotyped at the 95 SNPs, with a median of 16 samples per site, varying between 5 (Conwy US – CWC) and 32 (Porthcollum – HAY2) individuals.

Pairwise  $F_{st}$  between sampled populations varied from 0.00 (Conwy US – CWC and Cwmrheidol mine downstream – RHC) to

0.36 (West Allen Ninebanks – TYD) and (Rheidol mine U/S – RHB). Observed heterozygosity ranged from 0.21 (Rheidol mine U/S) to 0.35 (Trehannick – CAM2) and expected heterozygosity ranged from 0.21 (Rheidol mine U/S) to 0.36 (Pencarrow – CAM5). Percentage of polymorphic loci ranged from 61.1% (Rheidol mine U/S) to 96.8% (Pencarrow). Fixation index ( $F_{is}$ ) ranged from –0.064 (Rhiw Saeson – DFE) to 0.071 (Glasffryd – TFC). Full details of  $F_{ST}$  across all sampled populations can be found in Appendix S6; summary statistics for each population are given in Appendix S7. In tests across all loci in each population, only one locus, Str\_8233 was found to be significantly in Hardy-Weinberg deficit, in Wearhead (WEB), and no loci were significantly in Hardy Weinberg excess (Appendix S8).

#### 3.1 | Patterns of population structure using the discriminant analysis of principal components (DAPC)

The first level of a priori DAPC analysis revealed separation of a Hayle cluster along the DF1 axis, and separation of the populations from northeast (NE) England along the DF2 axis (Figure 2a). The other metal-impacted rivers from Cornwall (Trevaylor (TRV), Crowlas (CRO) and Red River (RR1 and RR2)) clustered as intermediate between the Hayle and a large cluster containing fish from southwest England, southeast Ireland and west Wales. Trevaylor and Crowlas trout were separated on the DF3 axis, with fish from two Welsh sites (CWE and RHB) also placed slightly apart from the main large cluster (Figure 2b).

Within Wales, structure appeared to be driven by outlying trout populations from the upper Rheidol (RHA, RHB) and the Conwy (Caletwr and Gwyrd) which clustered separately along the first PC axis (DF1) from the main Welsh cluster and from one another (Figure A1, Appendix S3). Removal of the outlying upper Rheidol group (RHA, RHB and RHE) and Conwy group (CWE and CWF) resulted in the sites from upper Teifi catchment (TFB, TFC, TFD and TFE) separating along the first PC axis and Afon Goch Dulas (AGA) as a separate cluster along the second PC axis (Figure 2c,d).

Within NE England, the metal impacted sites of Killihope Burn (WEA), Nenthead (TYE) and West Allen (TYD) clustered separately to the main cluster of relatively clean sites, with DF1 v DF2 most strongly isolating Killihope and Nenthead, and West Allen strongly separating along the DF3 axis (Figure 2e,f). DAPC analysis of the remaining northeast England sites excluding these three outliers revealed no substructure between the Tyne and Wear catchments.

**FIGURE 2** A priori discriminant analysis of principal components (DAPC) plots (Discriminant Function (DF) 1 v DF2 and DF1 v DF3) for brown trout (*Salmo trutta*) samples genotyped at 95 SNP loci. Individual samples are indicated by points, with the centroid of populations given in the labelled boxes. Individual points and centroids are coloured by site of origin, with a legend given at the base of the figure. Populations that are highly metal impacted are indicated by a red triangle. (a, b) DAPC analysis of 1139 individual fish from four metal-impacted regions across Britain and Ireland, retaining 23 principal components (PCs). The first three discriminant functions explained 36.4%, 16.8% and 9.5% of the variance, respectively. (c, d) DAPC analysis of 435 samples from Wales (omitting five outlier isolated sites – RHA, RHB, CWE, CWF and RHE) and retaining 20 PCs. The first three discriminant functions explained 22.8%, 16.9% and 10.0% of the variance, respectively. (e, f) DAPC analysis of the 187 fish sampled from northeast England, retaining 10 axes. The first three discriminant functions explained 40%, 22.9% and 22.0% of the variance, respectively.



There was no evidence of substructure between the sites sampled in Ireland (Appendix S3). De novo DAPC analyses concur with the a priori approach; full details of these and results for all DAPC analyses are given in Appendix S3.

### 3.2 | Hierarchical genetic analysis using STRUCTURE

For the full dataset,  $\Delta k$  was highest for  $k=2$  (Figure 3), which showed strong assignment of individuals from the Hayle (HAY), Trevaylor (TRV) and Crowlas (CRO) to one cluster, intermediate assignment of the River Camel (CAM), Afon Caletwr (CWE) and Nant Gwyrdd (CWF), and individuals from all other sites assigning to the second cluster. Within Cornwall, the most likely partition of the data was  $k=2$ , with a strong assignment of individuals from the Hayle to one cluster and all other populations to the second. When the Hayle was analysed individually,  $k=2$  was found to be most credible (based on the Puechmaillie estimators) with individuals from HAY1 and HAY2 assigning mostly to one cluster and individuals from HAY3 and HAY4 to the other (Appendix S4). The  $\Delta k$  was highest for  $k=2$  for fish from the non-Hayle Cornwall sites, with fish from the Trevaylor and Crowlas assigning strongly to one cluster and those from the Red River (RR) and the Camel to another. Repeating this with fish from the two Red River sites and the five Camel sites gave  $k=2$  by the  $\Delta k$  method, with the Red River individuals assigning strongly to one cluster and the other cluster represented by those from the Camel (Figure 3). The Puechmaillie estimators corresponded with the hierarchical analysis and gave  $k=5$  and  $k=6$  for two metrics each, respectively, with each river assigning to a separate cluster (Appendix S4).

Analysis of the Ireland, northeast England and western Wales samples gave  $k=2$  using the Evanno method, with strong assignment of individuals from northeast England to one cluster and those from Ireland and western Wales to another (Figure 3). Analysis of the 11 populations from northeast England showed a relatively high  $\Delta k$  for both  $k=2$  and  $k=3$ , whereas the Puechmaillie estimators selected

$k=4$  as the most credible number of clusters. For  $k=2$ , broadly equal assignment of individuals to each cluster aside from Nenthead (TYE) was observed, with this population assigning strongly to a separate cluster (Appendix S4). At  $k=3$ , there was strong assignment of individuals from West Allen (TYD) to one cluster, Nenthead fish to another cluster and fish from all other sites to a third cluster (Figure 3). At  $k=4$  there was strong assignment of Nenthead fish to one cluster, West Allen fish to another, Killihope Burn (WEA) individuals to a third and mixed assignment, represented by a fourth cluster, to all other sites. Analysis of fish from the less metal impacted sites (excluding TYD, TYE and WEA) showed low scores for  $\Delta k$  and relatively equal assignment of all individuals to each cluster, suggesting  $k=1$  as the most credible scenario.

Analysis of samples from Ireland and Wales revealed  $k=4$  as the most credible number of clusters using the  $\Delta k$  method, whereas the Puechmaillie estimators gave  $k=8$  and  $k=9$ . For  $k=4$ , there was strong assignment of individuals from Ireland to one cluster, and the upper Rheidol (RHA and RHB) and two sites on the Conwy (CWE and CWF) to another. For the other two clusters, there was mixed assignment, though the Dyfi (DF) was dominated by one cluster and the Teifi (TF) largely by another (Figure 3). Further analysis of all individuals from Ireland showed no substructure between sampled sites. The Puechmaillie estimators gave similar results, with upper Rheidol (RHA and RHB) sites assigned to a separate cluster, Nant Glan-dwr (RHE) assigned to another cluster, two sites on the Conwy (CWE and CWF) to a distinct cluster, and the Anglesey rivers (AGA and AGB) to another group. Removal of strongly outlying sites within the Welsh dataset gave an optimum  $k$  value of 5 by Puechmaillie estimators and an optimum of 2 with the Evanno method, though the  $\Delta k$  score for  $k=4$  was only marginally lower (Appendix S4). At  $k=4$ , there was strong assignment of individuals from Anglesey (AGA and AGB) to one cluster, assignment of individuals from the upper Teifi group (TFB-TFE) to a second cluster, DFB-DFF to a third, with individuals from all other sites exhibiting mixed assignment. Separate analysis of the two Anglesey rivers (AGA and AGB) showed most

**FIGURE 3** Hierarchical STRUCTURE analysis of brown trout (*Salmo trutta*) individuals from regions of the British Isles with a legacy of mining industry, following the Evanno et al. (2005)  $\Delta k$  method. Average assignment of individual fish from each sampled river or site is given by the portion of the associated pie chart on the corresponding map. Assignment plots (right) indicate the likelihood of assignment of each individual fish to each of the  $n$  most credible genetic clusters, with individual sampling sites separated by white dashed lines. The relative metal impact of sites is denoted by shading of the site label, where populations assign to distinct groups associated with differing relative levels of metal-impact with shades matching those in Figure 1 (low = yellow; moderate = orange; high = red). In the hierarchical analyses, the populations subsampled at each level are denoted by a numbered inset panel on the map plot. The levels of hierarchical analysis were as follows: (a) The full Britain and Ireland sample set, with most credible level of  $k=2$ ; (b) All fish from Cornwall, as assigned to the first cluster [predominantly orange] in a, with most credible level of  $k=2$ ; (c) Non-Hayle Cornwall fish, with most credible level of  $k=2$  (c, map 1a), followed by an analysis of only the Camel and Red River (RR) fish (c, map 1b, as assigned to the second [blue] cluster in the former analysis); (d) Fish from Wales, Ireland and northeast England, as assigned to the second [predominantly blue] cluster in a, with most credible level of  $k=2$ ; (e) Fish from only Wales and Ireland, as assigned to the first [predominantly blue] cluster in d, with most credible level of  $k=4$ ; (f) Fish from northeast England, as assigned to the second [predominantly orange] cluster in d, with most credible level of  $k=3$ ; (g) Fish from northeast England excluding the two metal-impacted outlier populations from the Tyne (TYD and TYE), with most credible level of  $k=2$ . In e, average assignment of individuals across sites in place of catchments is plotted where there is divergence of populations at sites within rivers; the positions of these sites are given on the corresponding map plot. At the first level of analysis of the full Britain and Ireland dataset and in a and d, for clarity, regions or catchments are labelled in place of individual sites. The value of  $k$  plotted for each level of the analysis is given in the bottom right corner of each corresponding inset map. For full details of the  $\Delta k$  value for each analysis and Puechmaillie estimators, see Appendix S4.





AGA individuals assigning strongly to one cluster and AGB to a second cluster (Figure 3). Full details and all plots, including log likelihoods, values of  $\Delta k$  and Puechmaillie estimators, can be found in Appendix S4.

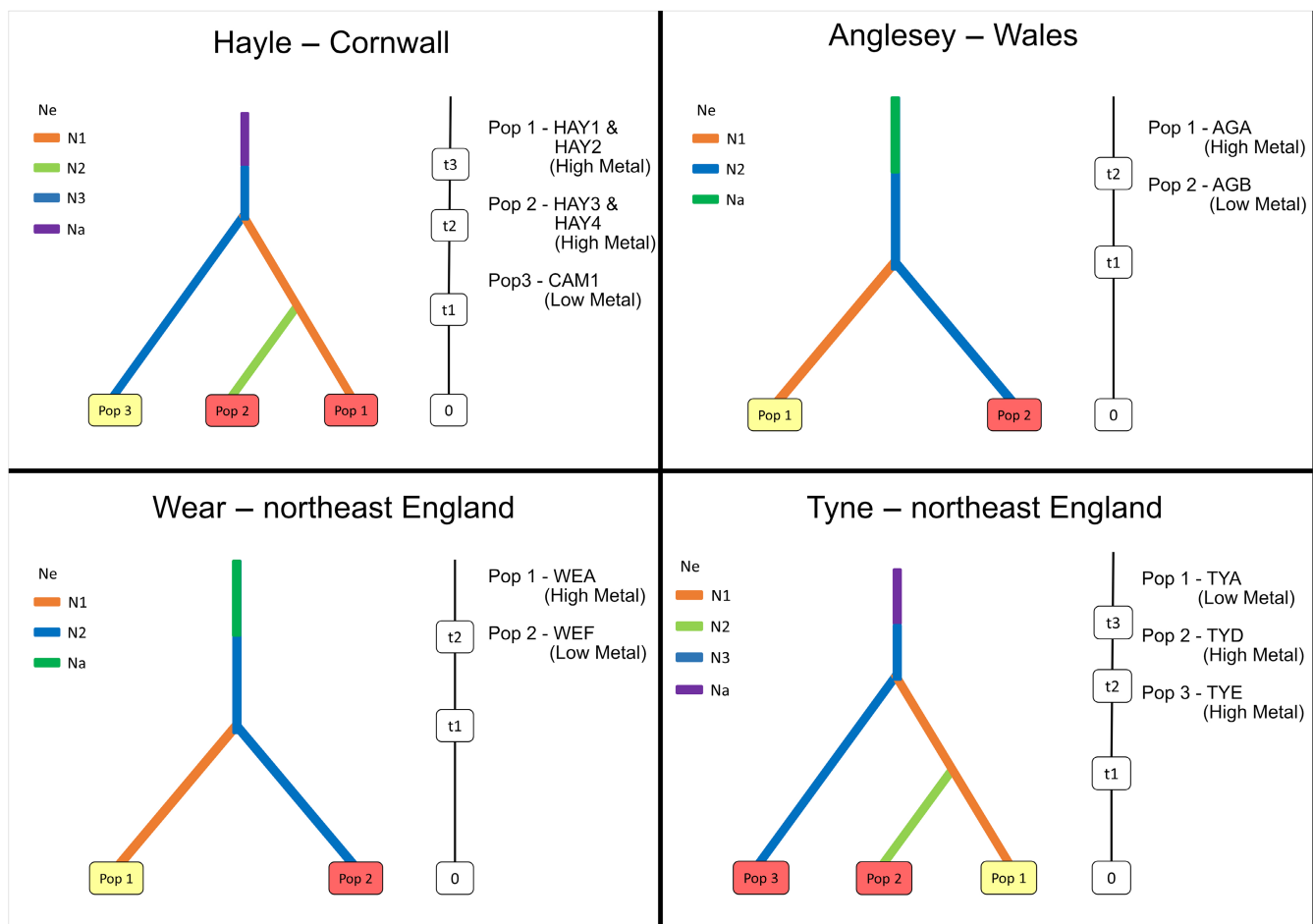
### 3.3 | Modelling of demographic history using DIYABC

Demographic modelling was carried out on the four metal-impacted catchments showing defined putatively metal-linked genetic substructuring to investigate credible historical scenarios that produced the observed population structure seen in the STRUCTURE and DAPC analyses. Firstly, within the Hayle, the most credible modelled scenario showed a split between sites in the upper and lower catchment, with a shared ancestral bottleneck event. For Anglesey, the most credible scenario was the Afon Briant population being the source of fish in the Afon Goch Dulas, again with a shared ancestral

bottleneck. For the Wear, the most credible scenario was the clean downstream population being the source of the upstream metal-impacted, with the metal-impacted population having a bottleneck event after divergence from the clean, and a shared ancestral bottleneck. For the Tyne, the most credible scenario was a more recent split between the clean Gunnerton Burn (TYA) the metal-impacted West Allen (TYD) site, converging on TYA, and an older split between the clean site and the other metal-impacted site, Nenthead (TYE). The most credible models for each analysis are shown in Figure 4, parameter posterior estimates are given in Table 1 and full details of the models chosen and compared can be found in Appendix S5.

## 4 | DISCUSSION

Here we show that populations of *Salmo trutta* L. in metal-impacted and nearby clean rivers exhibit distinctive patterns of genetic structure across multiple metal-impacted regions of the British Isles,



**FIGURE 4** Most credible models of demographic history under DIYABC analysis for four groups of populations of brown trout (*Salmo trutta*), which appear to have genetic structure related to metal impact, as identified by the DAPC and STRUCTURE analyses. For each branch plotted, effective population size is given by colour and timing of simulated demographic (convergence and bottleneck) events given by  $t_n$ . Posterior estimates of each parameter, including mean, 5th and 95th quantiles for each analysis are given in Table 1. The relative level of metal impact for each modelled population is given by the colour of the box of the population, with highly metal-impacted populations labelled in red and those from relatively clean sites in yellow.

**TABLE 1** Posterior estimates of priors for the parameters of most credible scenarios for each regional group examined by DIYABC analysis.

Scenario	Parameter	Median	$q_{0.05}$	$q_{0.95}$
Hayle	$t_1$	156	47	343
	$t_2$	651	275	1344
	$t_3$	2705	1095	4260
	$N_1$	2064	785	5928
	$N_2$	4543	712	9590
	$N_3$	4542	1991	9374
	$N_a$	79,942	40,487	99,162
Anglesey	$t_1$	511	166	1092
	$t_2$	3137	1510	4451
	$N_1$	3179	958	8455
	$N_2$	3297	1243	6354
	$N_a$	78,077	46,722	98,593
Tyne	$t_1$	411	73	1097
	$t_2$	847	330	1711
	$t_3$	2697	1208	4324
	$N_1$	7075	3406	9610
	$N_2$	1056	220	4266
	$N_3$	3145	1226	6496
	$N_a$	86,146	55,463	98,989
Wear	$t_1$	274	118	1030
	$t_2$	3173	1439	4411
	$N_1$	894	328	3853
	$N_2$	4269	2103	8080
	$N_a$	72,748	36,445	97,330

Note: For each parameter, the median value, 5th and 95th quantiles of posteriors are reported. Parameters reported are  $t_n$ ; number of generations before present sampling that the historical population event occurred. For all most credible models for each population, the most historically distant  $t$  parameter ( $t_2/t_3$ ) is for a bottleneck event, whereas all other  $t$  parameters for each population are for convergence of populations. The  $N_n$  parameters represent effective population size, with  $N_a$  being ancestral population size prior to a shared ancestral bottleneck event. Most credible models for each analysis from which these posteriors were estimated are given in Figure 4.

confirming, at a much larger scale, a trend observed previously in a study of trout inhabiting small coastal streams in metal-impacted areas of southwest England (Paris et al., 2015). The isolation of metal impacted populations appears to be greatest in those with the highest metal risk characterisation ratios (Appendix S2; Figure 2) and where physical barriers also exist (Figure 3). Genetic distances between metal-impacted populations and those from relatively clean parts of the same or nearby catchments are repeatedly significantly above zero and are comparable to levels that would be expected in salmonid populations isolated by physical barriers (King et al., 2020; Osmond et al., 2023). We also see evidence of markedly reduced genetic diversity within some of those most highly metal-impacted

populations. Moreover, whilst we observe nested population structure between metal-impacted and relatively clean sites within regions across Britain and Ireland, the first level of divergence in a hierarchy of population structure across all populations sampled is driven by the distinctiveness of trout in the highly metal-impacted region of west Cornwall, in particular, trout inhabiting the River Hayle. In addition, when modelling the demographic histories of the most distinct metal-impacted populations, we see that the most credible timescales for splits between populations are in line with peaks of anthropogenic activity and the impacts of local mining.

#### 4.1 | Population structure of trout populations across the British Isles

Both the STRUCTURE and DAPC analyses show a strong genetic split between the populations from northeast England and the rest of the sampled populations from western Britain and Ireland. The distinct genetic differentiation between trout populations in northeast England and those inhabiting rivers entering the Irish Sea (in Wales and Ireland) is consistent with previous research, with differences in the origins of post-glacial recolonising fish posited as driving this difference (McKeown et al., 2010). In the west, rivers in southwest England, and those entering the Celtic and Irish Seas were likely recolonised by migrants from refugial populations in southern England and northwest France, as observed in Atlantic salmon (Finnegan et al., 2013), whereas in northeast England migrants are thought to have a greater influence from eastern refugial populations in rivers around the North Sea (McKeown et al., 2010). Of more recent studies of genetic variation in trout, many have had a strong regional focus and have not made direct comparisons of populations from the east and west of the British Isles (e.g. Prodöhl et al., 2017), leaving a gap for future inquiry as to the drivers of this distinction. The most recent of these used a genome-wide SNP analysis of trout populations across northern Europe (including samples from northeast England) showing this region to be distinct from those in southern England and mainland Europe (Bekkevold et al., 2020). STRUCTURE analysis also shows genetic structure between Wales and Ireland, supporting expected isolation patterns related to low levels of straying of anadromous trout across deeper areas of sea (Flaten et al., 2016) and is in agreement with previous studies of trout using microsatellites across these regions (Prodöhl et al., 2017). Within geographic regions, however, we see very little genetic structure in trout populations between rivers aside from those experiencing obvious anthropogenic impacts, with the relatively unimpacted populations in the Tyne and Wear, for example, showing no obvious sub-structuring; a similar lack of genetic differentiation between neighbouring rivers in southeast Ireland and a cline of genetic structure across rivers in Wales as revealed by the DAPC analysis (Figure 2c,d). Such apparent genetic connectivity of populations from nearby rivers within regions challenges the orthodoxy of treating individual rivers as entirely separate populations for management purposes (King et al., 2016, 2020). Moreover, these results highlight the growing body of evidence

illustrating the importance of anadromous trout straying from their natal rivers (Harris, 2017; King et al., 2016) in maintaining gene flow within a larger meta-population.

## 4.2 | Metal-impacted populations repeatedly exhibit genetic structuring and reduced genetic diversity

We repeatedly saw genetic sub-structuring within geographic regions between metal-impacted and clean control trout populations across areas with differing background genetic structure, with highly metal-impacted populations amongst those with the lowest levels of genetic diversity at the SNP loci screened. Both the DAPC and STRUCTURE analyses indicated the River Hayle (west Cornwall) as driving the first hierarchical level of structure in the dataset. Previous research using microsatellite loci also identified the role of mine water pollution in driving population structure both within the Hayle and between River Hayle trout and fish in surrounding rivers (Paris et al., 2015). Likewise, our genetic data (SNP loci) show consistent patterns of divergence across the acutely toxic Godolphin mine region, confirming that the in-river sub-structuring identified in the current study is consistent with the findings of a previous study based on microsatellite loci (Paris et al., 2015), and suggests genetic divergence at a genome-wide level. The strong genetic differentiation observed between the River Hayle population and trout in all other rivers studied is indicative of reproductive isolation. However, the relatively high heterozygosity and polymorphism of screened loci in the Hayle suggests that this distinctiveness is driven not only by extreme patterns of bottlenecking and genetic drift, but may be a consequence of local adaptation to the toxic metal concentrations within the Hayle, in line with previous observations of local adaptation (e.g. Prodöhl et al., 2019), including in response to heavy metals in other salmonid species (Esin et al., 2023). The distinctiveness of trout in the River Hayle could be maintained by avoidance behaviour of anadromous individuals straying from other rivers to the distinct water chemistry of the river or by direct mortality of metal-naïve fish on entering the river (Durrant et al., 2011; Van Genderen et al., 2016). Indeed, metal concentrations within the Hayle remain at levels toxic to metal-naïve trout (as seen in laboratory exposure experiments, Durrant et al., 2011) despite mine workings having closed, with concentrations in the lower Hayle of Cadmium averaging 0.95 µg/l and reaching much higher peaks during periods of increased discharge over winter months (Environment Agency data, Appendix S2); such concentrations would be expected to cause chronic toxicity to juvenile brown trout which are developing at that time (Brinkman & Hansen, 2007). Similar patterns of genetic distinction and reduced polymorphism are also seen in the other metal-impacted populations from Cornwall, including those in the Trevaylor, Crowlas and Red River, which are genetically distinct from one another and from the Hayle.

Within the Rivers Tyne and Wear in northeast England, we observed strong assignment of individuals from the three most highly

metal-impacted sites (WEA, TYD and TYE) to individual genetic clusters in both the STRUCTURE and DAPC analyses. For two of these sites, Nenthead (TYE) and Killihope burn (WEA), natural barriers likely limit upstream gene flow, however, there are no known impassable barriers downstream of West Allen (TYD) (Gareth Pedley, Wild Trout Trust, pers. comm.). Nonetheless, genetic diversity within these metal-impacted sites is amongst the lowest of all those sampled, with a high frequency of monomorphic loci, suggestive of low gene flow and/or historic population bottlenecks.

Similarly, within Wales, we observed strong genetic distinction of the Afon Goch Dulas (AGA) population on Anglesey from fish in all other sampled Welsh rivers (Figure 2c,d), including fish in the nearby, relatively clean Afon Briant (AGB), suggesting that isolation of the trout population in this river (Afon Goch Dulas) from nearby catchments is likely to be metal-driven. The Afon Goch Dulas is one of the most heavily polluted catchments in Wales, being impacted by acid water drainage from the Parys mine, which sits at the headwaters, with an average of over 100 µg/l of dissolved copper at the region where trout were sampled for this study (Natural Resources Wales data, Appendix S2). Trout were also sampled at the tidal limit of the catchment and sea trout were observed, indicating no barriers to migration (at least in this lower stretch of the river) and suggesting that the genetic distinction of trout in this catchment may be driven by chemical avoidance or direct toxicity and mortality in any metal-naïve trout that do enter the river. The high  $F_{ST}$  between populations in the Afon Goch Dulas and Afon Briant (0.089) is comparable with other established metal-clean population comparisons, i.e. the Red River and the nearby Camel (0.055–0.072) in Cornwall.

In central Wales, both the Ystwyth and Teifi show weak genetic structure, with sites downstream of, or near, point sources of dissolved metals assigning to separate genetic clusters within each river. The replicate sampling of trout at sites on the Afon Brefi (TFA and TFF) taken approximately 10 years apart suggests that the observed genetic structure of the Teifi is also temporally stable and, thus, the observed sub-structure is unlikely to be a result of sampling at different times. The four remaining sites on the Teifi (TFB, TFC, TFD and TFE) are all geographically proximate to one another and upstream of Cors Caron, a region of bog that is subject to low pH (Proctor & Maltby, 1998). The relative genetic similarity of trout from sites with both relatively high and low metal-impact within this region (upstream of the Cors Caron bog) suggests that, though metal-toxicity may have influenced these upstream populations, gene flow persists between these four sites. Isolation likely contributes to the split within the Teifi, with relatively few anadromous trout being observed upstream of Cors Caron (Dylan Roberts, Game and Wildlife Conservation Trust, pers. comm.). The mixed assignment of individuals within YHB (Ystwyth), sampled directly below the point source of metals at Cwmystwyth mine, is potentially representative of trout from upstream migrating downstream. During the summer, with lower rainfall, dissolution of metals from spoil heaps and adit outflows is reduced, thus, potentially temporarily reducing the chemical barrier to fish movement.

Contrary to the other regions examined, in the Avoca in southeast Ireland (AV), we did not observe strong genetic distinction of trout sampled from metal-impacted waters (in particular, Woodenbridge – AVB) with those from relatively unimpacted tributaries within the river (e.g. Derry Water – AVD), nor with those from two nearby catchments, Inch and Owenavorrhagh (INC and OWE). The metal-impacted region of the Avoca is in the lower catchment and has been suggested to act as a barrier to salmonid movement, with the apparent (historical) local extinction of salmon (Sullivan & Gray, 1992), which lack a resident life history strategy and must therefore move through the high toxicity region to reach spawning areas higher up the catchment. Indeed, by the early 2000s electrofishing surveys revealed extremely low densities of salmon and trout fry within the acidic mine-drainage affected region; however, more recently, higher densities of salmon fry have been recorded upstream of this region (Inland Fisheries Ireland, 2017; D. Osmond and J. Stevens, pers. obs., 2021). This leaves open the possibility that the movement of anadromous trout is a potential source of gene flow between the Avoca and neighbouring rivers. Previous work also failed to detect any genetic structure between trout rivers in southeast Ireland (Prodöhl et al., 2017). It is unclear how long gene flow between the Avoca and the meta-population of neighbouring rivers may have been restricted, however, it is well documented that genetic signals of isolation may sometimes not be detectable for a long time period after fragmentation (Landguth et al., 2010; Ruzich et al., 2019; Waples, 1998). In addition, the effects of isolation in the Avoca population are likely to have been buffered by the large population size of resident trout upstream of the chemical barrier, thereby reducing the effects of genetic drift (Hoffman et al., 2017). These findings suggest that deleterious impacts of anthropogenic pollution can be reduced where duration of impact is limited and large population sizes are able to buffer the effects of drift.

### 4.3 | Demographic history modelling supports recent anthropogenic events

Demographic history modelling in DIYABC repeatedly placed divergences between pairs of proximal metal-impacted and clean populations within recent history, coinciding with periods of peak metal extraction, and also credibly supported population bottleneck contractions around these times. Within the Hayle, the most credible split upstream and downstream of the Godolphin mine region occurred after the split from the Camel, with tight posterior density estimates supporting a relatively recent split coinciding with the late Medieval to early Industrial Revolution period. Divergence between the Camel and Hayle populations is estimated to be around the Roman period, during which time there was extensive metal working within Cornwall (Gerrard, 2000; Pirrie et al., 2002). Similarly, within the Wear and the Tyne, while the potential influence of both physical barriers and metal pollution in driving genetic structure is apparent, again, the most credible demographic models for the populations in both these rivers identified a divergence of the metal-impacted

fish from fish in clean sites within relatively recent history, and certainly well after the likely establishment of any existing physical barriers. Indeed, the most credible models of more recent genetic separation fit with the hypothesis of metal pollution of anthropogenic origin driving genetic structure in these populations. Similarly, demographic modelling of trout populations on Anglesey, Wales also identifies relatively recent splits coinciding with anthropogenic activity, with a split between Afon Goch Dulas and Afon Briant at approximately 500 generations ago. Historical evidence suggests that the Parys Mine region (within the Afon Goch Dulas catchment) has been mined extensively from the early bronze age, with major copper workings reported in the 1500s, peaking by the late 1700s (Vernon, 1996). The most credible scenarios for all groups analysed (Tyne, Wear, Hayle and Anglesey) indicated a shared ancestral bottleneck, prior to divergence of individual populations. However, DIYABC-RF did not deliver tight posterior estimates (Table 1) for the ancestral population size and the timing of this event, though this is likely driven by recolonization after the last glacial maximum (Fenton et al., 2023). This finding is in accordance with previous studies, which have shown that while DIYABC is generally reliable in identifying the most credible scenario, it is less powerful for estimating tight posterior estimates of older events (Cabrera & Palsbøll, 2017).

### 4.4 | Barriers to gene flow increase the negative genetic impacts of metal pollution

Within Wales, several genetically distinct trout populations were identified with strong assignment to individual clusters (RHA, RHB, RHE, CWE and CWF) in the Rheidol and the Conwy. The Rheidol has many barriers to fish migration, with all three outlier sites (RHA, RHB and RHE) situated upstream of the impassable Gyfarllwyd Falls and one (RHE) also upstream of the Dinas reservoir. The strong assignment of individuals from Nant Glandwr (RHE) to a separate cluster from the other two upper Rheidol sites confirms the multiple levels of fragmentation within this catchment (Appendix S4, Figure A33). The Dinas reservoir was constructed in the late 1950s, giving approximately 18 generations of potential drift for trout isolated in this part of the catchment. Thus, given the presence of an impassable natural waterfall in this catchment, it is probable that the trout living upstream of this barrier have been isolated from gene flow from downstream migrants and anadromous individuals for thousands of generations. Similar patterns of genetic drift in trout populations upstream of natural barriers has been described previously in the rivers of Dartmoor in southwest England (Griffiths et al., 2009).

Similarly, the two outlier sites from the Conwy both have potential barriers to migration located between them and the other sampled populations within this catchment. Afon Caletwr (CWE) is upstream of Conwy Falls, however, a fish pass was installed in 1994 to facilitate the upstream movement of anadromous trout (Juvenile Salmonid Summary Conwy Catchment, 2018). The samples from this site were collected in 2012/2013 as part of a previous research project, giving ~5 generations of potentially restored genetic connectivity after the

pass installation. Recent work has demonstrated that genetic structure between populations of steelhead/rainbow trout (*Oncorhynchus mykiss*) isolated by barriers decreases rapidly after barrier removal (Fraik et al., 2021). Nonetheless, the discontinuity of genetic structure between trout either side of the Conwy Falls suggests that the installation of the fish pass has not yet effectively restored genetic connectivity, or that other barriers may exist between Conwy Falls and Afon Caletwr which are continuing to limit the movement of fish within the catchment. More broadly, barriers have long been recognised as a threat to salmonid populations (Deinet et al., 2020) – they reduce available habitat and isolate populations from gene flow from migratory individuals (Crook et al., 2015). With a barrier estimated on average every 1.3km of river distance across Europe (Belletti et al., 2020), these signatures of restricted gene flow are likely to be generally representative of highly-fragmented populations across Britain and Ireland, with a lack of gene flow acting to reduce adaptive potential within isolated populations into the future. In addition to the impact of natural barriers limiting gene flow in these isolated populations, tributaries of the Conwy upstream of the Conwy Falls were stocked with hatchery-origin trout in the early 1990s and introgression of hatchery-origin alleles has been detected within trout fry in the upper Conwy (Hauser et al., 1991). Previous work has demonstrated the genetic detection of domestically-reared salmonids where they have been stocked (Clemento et al., 2009; King et al., 2021) and genetic fitness of populations is likely to be impacted even where native broodstock are used (Hagen et al., 2019; McGinnity et al., 2009; Milot et al., 2013).

Within the northeast England cluster, for the rivers Tyne and Wear, in line with previously reported homogeneity of trout sampled from these rivers, there is little detectable genetic structure between populations from sites with relatively low metal pollution (Bekkevold et al., 2020). This is indicative of gene flow between these two adjacent catchments, likely maintained by low-frequency straying of anadromous individuals, as has been reported in other regions of the British Isles (Harris, 2017; King et al., 2016) and emphasises the importance of such gene flow between populations. Two of the metal-impacted sites in northeast England, Nenthead (TYE) and Killihope (WEA) also have natural impassable barriers to gene flow, limiting the continuity of gene flow across the local meta-population. This is reflected in our data with populations at these sites having some of the lowest levels of genetic diversity, a high proportion of fixed loci and positive inbreeding coefficients. Previous work has demonstrated the increased effect of isolation on populations of fish where population size is reduced (Coleman et al., 2018), as might be expected in isolated trout populations experiencing high levels of mortality from metal-toxicity.

#### 4.5 | Conservation implications for fragmented populations in a post-industrial landscape

Our results demonstrate widespread genetic fragmentation and deleterious levels of genetic drift and inbreeding within trout

populations living in mine water-polluted streams and across highly fragmented habitats. Consequently, conservation should focus on the amelioration of mine water wash-out to reduce the impact of localised spikes in metal pollution, coupled with the removal of in-water barriers to fish movement to restore gene flow and to safeguard genetic diversity in impacted fish populations. Minimising the duration and severity of exposure to metal pollution by remediation of historical mine workings and adits will limit the extent of deleterious bottlenecks and subsequent drift effects on these populations (Hoffman et al., 2017; Schlaepfer et al., 2018). This would also help to promote the sustained recovery of riverine communities in general, including the aquatic invertebrate fauna communities essential to a fully-functioning freshwater aquatic ecosystem suitable for salmonid fish (Williams & Turner, 2015). With regard to the issue of restoration of river navigability for migratory fish, while this issue is not specific only to the needs of salmonids impacted by metal pollution (González-Ferreras et al., 2019; Nicol et al., 2017), the double stressors of pollution and drift in isolated populations make the safeguarding and promotion of gene flow essential if these metal-impacted populations are to maintain sufficient genetic diversity to be able to cope with environmental perturbations and new selection pressures experienced in a rapidly changing world. Previous studies have demonstrated the need for translocations of individuals into populations of fish experiencing the deleterious effects of isolation and limited ability to adapt to future stressors (Pavlova et al., 2017) and, as demonstrated by Robinson et al. (2017), genetic rescue of isolated brook trout populations resulted in greatly increased allelic richness and heterozygosity and hybrid vigour in resultant offspring. However, such transplantations are not feasible for the rescue of highly metal-impacted populations, with often severe mortality observed in naïve individuals exposed to the environmentally relevant concentrations of metals found within impacted rivers (Durrant et al., 2011). In such cases, amelioration of metal impact and the restoration of natural gene flow should be prioritised, removing or by-passing barriers where feasible. The introduction of beneficial alleles into populations is also enabled by the low-level straying of anadromous trout connecting a wider meta-population (Källo et al., 2022; King et al., 2016), as observed in the relatively low genetic structure between geographically related trout populations within regions in this study. There have been widespread declines in the number of returning sea trout across the UK (Environment Agency, 2023) with populations impacted by direct bycatch and reduced feeding opportunities produced by overexploitation of marine fisheries (ICES, 2013; King et al., 2021; Thorstad et al., 2016), as well as increased mortality from exposure to parasites linked to salmon aquaculture (Serra-Llinares et al., 2020). Restoration of the genetic viability of isolated populations from natural gene flow in anadromous fishes is therefore not only dependant on conservation measures in freshwater but should seek to ensure the viability of the marine component of the trout life history by mediating these threats where possible. Lastly, these findings are of particular pertinence at a time of increased interest in reopening mine

workings in the UK to safeguard and increase the nation's supply of rare metals for battery production amid efforts to decarbonise energy production (Shaw, 2022). Future mine workings must ensure that discharges of metal-polluted waste water into the environment is minimised to prevent further damage to the genetic integrity of fauna and flora that form part of these ecosystems.

## 5 | CONCLUSIONS

Overall, our results demonstrate repeated patterns of genetic structuring around metal-impacted regions of the British Isles in populations of a native anadromous fish. While we observe genetic structure attributable to reduced gene flow driven by physical barriers, the genetic distinctiveness of multiple highly metal-impacted populations with no known physical barriers to movement suggests a key role of metal pollution in driving this genetic divergence. Demographic modelling supports this proposition, suggesting that the observed patterns of genetic diversity and structure between populations are likely to have arisen around peak periods of anthropogenically-driven metal extraction activities in Britain and Ireland. Within the available water chemistry data compiled for the sampled sites, there are no other apparent conflating chemical factors such as pH or hardness which are divergent between the metal-impacted and relatively clean sample sites within each region studied. Thus, we suggest that it is credible to assume that chemical avoidance by metal-naïve migratory trout – as exhibited by other salmonids (Van Genderen et al., 2016)– and direct, acute metal-toxicity, might both be acting to limit gene flow into or across highly polluted regions. Adaptation within metal-impacted populations could also lead to this observed genetic structure, with metal-naïve fish being unable to occupy metal-polluted environments or breed successfully with putatively adapted populations. With habitat fragmentation caused by barriers, and mine water pollution being widespread in rivers across the British Isles, we might expect these patterns of reduced genetic diversity and fragmentation to be represented across the full range of these stressors, with these being of particular relevance in a period of renewed interest in mining to enable the renewable energy transition. Indeed, genetic fragmentation of populations appears to be highest where both metal toxicity and physical barriers have acted to reduce genetic diversity and where smaller population sizes cannot act to buffer the effects of drift. Within these doubly impacted populations, their adaptive potential is likely to be severely limited if gene flow is not restored. We consider the limited feasibility of genetic rescue with the interaction of these two stressors but suggest that the acute toxicity of metal concentrations within highly impacted rivers to metal-naïve fish makes such translocations unlikely to succeed. The removal of migratory barriers and reduced pollution gradients are therefore essential if these metal-impacted populations are to maintain sufficient genetic diversity to be able to cope with environmental perturbations and new selection pressures in a rapidly changing world.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## PEER REVIEW

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## DATA AVAILABILITY STATEMENT

Data and R analysis scripts are available from <https://doi.org/10.5061/dryad.hqbzkh1p2>.

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## BIOSKETCH

**Daniel Osmond** is a freshwater ecologist with an interest in applying molecular techniques to better understand fish populations and applying this towards conservation efforts. This paper constitutes work undertaken during the author's PhD based at the University of Exeter.

Author contributions: DRO carried out the field sample collection, lab working, analyses and writing of this manuscript. RAK assisted with lab working, data analysis and improved this manuscript. IRMR provided additional samples, assisted with ABC analyses and improved this manuscript. MWB conceived the researched questions and sampling design. JRS conceived the research questions and study design, helped with field work and assisted in writing the manuscript.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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