Defining conservation units in a highly diverse species: A case on Arctic charr

Running title: Conservation units in a highly diverse species

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Data accessibility:

Relevant sample meta-data, environmental information, SNP VCF files, and code used in the analyses are archived on the University of Glasgow's permanent data repository, Enlighten (doi with acceptance). Demultiplexed ddRADseq data are available on NCBI Short Read Archive (SRA) (BioProjects PRJNA1061680, PRJNA607173).

Conflict of Interest:

The authors have no conflicts of interest to declare.

Abstract:

Defining appropriate conservation units is crucial to the protection and management of biodiversity. These delineations deliver further benefit when they encompass assessments of population vulnerability to extinction from pressures such as climate change. However, delineations and vulnerability assessments are particularly difficult within highly diverse species, such as the salmonid fish Arctic charr (*Salvelinus alpinus*), that show extensive phenotypic and genetic variation within and across locations, variable and complex life histories, and broad geographic distributions. As yet, the nature and scope of Arctic charr diversity has not been characterised at the scale needed to delineate key conservation units in Scotland. To identify evolutionarily significant and vulnerable populations to prioritise conservation needs, we conducted a genomic study of Arctic charr populations across Britain and Ireland with a focus on Scottish populations (N=64 populations; 24,878 SNPs; 410 individuals). We found that most lake populations represented distinct genetic clusters, with limited gene flow between them resulting in high levels of genetic differentiation. Higher level groupings of genetic similarity across catchments likely reflect historic anadromy and migration, with populations primarily grouping east or west of the central watershed divide in Scotland. Analysing genetic offset, also known as genomic vulnerability, we identified strong inverse correlations between genetic vulnerability with latitude and distance to the sea suggesting that more southern and more inland populations are more vulnerable to loss due to climate change. Additionally, patterns of vulnerability across several additional metrics identified further populations that may be at high vulnerability to loss. We further used our genetic data, along with phenotypic and geographic information, to identify populations of greatest evolutionary significance. This highlighted the most important

ones to protect are those in locations with multiple ecotypes, a key facet of functional Arctic charr biodiversity, and populations that are the only ones in their Hydrometric Area.

Keywords: Conservation units, Population genomics, Fish, Evolutionarily Significant Units, Management Units, Genomic vulnerability

Introduction:

The management and protection of biodiversity requires both the delineation of appropriate units of conservation and an assessment of their vulnerability to damage or loss. While conservation action is frequently directed at the species level, an increasing body of work has demonstrated the importance of intraspecific, within-species, diversity to ecological versatility, ecosystem recovery, and adaptation to changing environments (Des Roches et al., 2018; Le Coeur et al., 2021; Reusch et al., 2005; Schindler et al., 2010). For many species, particularly highly diverse ones, conservation management at a species level is insufficient for protecting vital variation and it is becoming increasingly common practice to define intraspecific conservation units (Forester et al., 2022; Mee et al., 2015). However, there are many different functional definitions of what constitutes a biologically meaningful conservation unit, and the criteria used to define these units can drastically impact the assessment of conservation importance (Mee et al., 2015; Minter et al., 2021; Mussmann et al., 2020). For example, the emphasis on morphological characteristics in the Kottelat & Freyhof (2007) review of the freshwater fishes of Europe led to a number of highly debated species classifications (Adams & Maitland, 2007; Barthelemy et al., 2023; Crotti et al., 2020; Denys, 2021; Etheridge et al., 2012; Gratton et al., 2014; Vavalidis et al., 2019). Given this, the delineation of intraspecific units is key to conservation policy.

With the aim of conserving the extant diversity of a species and protecting its adaptive potential (i.e. future biodiversity), definitions of intraspecific conservation units focus on identifying reproductively isolated groups, or groups showing high genetic differentiation as a proxy for reproductive isolation (Allendorf et al., 2022; Mable, 2019). Two of the most commonly applied conservation units are

Evolutionarily Significant Units (ESUs) and Management Units (MUs) (Allendorf et al., 2022; Funk et al., 2012; Moritz, 1994). Broadly an ESU can be defined as a reproductively isolated population, or group of populations, that show notable adaptive differentiation or functional diversity for the species. However, a number of variations of this definition exist, with some placing emphasis on contemporary patterns of diversity while others focus more on distinct historical and evolutionary lineages (Casacci et al., 2014; Coates et al., 2018; De Guia & Saitoh, 2007; Moritz, 1994; Waples, 1991). In contrast, MUs are generally defined as demographically independent, reproductively isolated, units without the element of adaptive differentiation used for ESUs (Funk et al., 2012; Moritz, 1994). Many studies define these units hierarchically, with broader ESUs containing multiple MUs (Forester et al., 2022; Funk et al., 2012; Shaney et al., 2020) but the applicability of this approach differs with species and context.

Several data types, for example genetic, phenotypic, and geographic data, should be combined where possible when defining units of conservation (Hoelzel, 2023). Information from different data types can provide important evidence that may reinforce the conclusion from another; this idea of concordance was a key part of the original definition of an ESU (Ryder, 1986). In addition, different types of data can provide alternative insights into what is evolutionarily significant, for example when high morphological differentiation is not matched by high genetic differentiation (Apolônio Silva de Oliveira et al., 2017; Funk et al., 2012). Nonetheless many studies defining intraspecific conservation units have used a criterion based solely on genetic data (Robertson et al., 2014). The advent of large-scale genomic datasets that use both neutral and adaptive loci have increased capacity to delineate conservation units more precisely and with greater biological depth (Allendorf et al.,

2022; Funk et al., 2012). How conservation units are delineated are highly susceptible to whether patterns of differentiation are considered at a genome-wide scale or restricted to single or several adaptive genes (Waples et al., 2022; Waples & Lindley, 2018).

Beyond the delineation of conservation units, another important requirement of conservation management is the need to identify habitats and sites that may require urgent conservation action or monitoring because they are more vulnerable to loss (Campbell et al., 2017; Sgrò et al., 2011). This can involve investigating both the population for conservation and its supporting habitat. For example, small habitats with lower carrying capacity will limit the size of a population and potentially its overall diversity, restricting its ability to respond to change (Fischer & Lindenmayer, 2007).

Increasing temperatures are a threat to many species, with increased water temperature shown to affect the development and morphology of freshwater species (Campbell et al., 2021; Esin et al., 2021; Muir et al., 2022). The threat levels are magnified for populations in smaller lakes or streams which may have less refuge from high water temperatures when compared with populations in larger habitats (Campbell et al., 2017; Isaak & Young, 2023). Thus, using the combination of population genetics and environmental information to identify the level of the threat to a population from various factors can help identify not just vulnerable species but also vulnerable sites for that species to prioritise for conservation action.

A species that aptly demonstrates the complexity of defining units of conservation is the Arctic charr (*Salvelinus alpinus*), a salmonid fish. Across its Holarctic range, the species exhibits very high variation in the expression of phenotypic traits and high levels of genetic variation (Gordeeva et al., 2015; Jonsson

& Jonsson, 2001; Klemetsen, 2010; Maitland & Adams, 2018; Recknagel et al., 2017; Wilson et al., 2004). Across its distribution, there are a number of lakes that contain multiple distinct populations, known as ecotypes, diverged to specialise in different trophic niches and living in sympatry (Elmer, 2016; Klemetsen, 2010; Maitland & Adams, 2018). These ecotypes are often distinguished by differences in their diet and foraging tactics but can be differentiated in other characters such as morphology and spawning time (Adams et al., 1998; Doenz et al., 2019; Garduño-Paz et al., 2012; Hooker et al., 2016; Jacobs et al., 2020; Kess et al., 2021; Malmquist et al., 1992; Skúlason et al., 1996). The presence of distinct sympatric ecotypes tends to be associated with local environmental features such as lake size and climate (Blain et al., 2025; Fenton et al., 2024a; Tiddy et al., 2024). The extent of divergence between co-existing sympatric ecotypes varies by location and each represents an important example of the functional diversity of the species (Adams et al., 2007a; Adams & Huntingford, 2004; Fenton et al., 2024b; Gordeeva et al., 2015; Moccetti et al., 2019; Schluter, 1996). Many of these instances of ecotype divergence are the result of secondary contact and admixture of multiple lineages that colonised post-glaciation, while others represent recent sympatric divergences from a single lineage and therefore show lower levels of genetic differentiation but frequently clear differentiation in phenotypes (Garduño-Paz et al., 2012; Jacobs et al., 2020; Kettle-White, 2001).

The high intraspecific diversity of Arctic charr has resulted in both the species and their habitats being designated as having high conservation, natural heritage, and scientific value for many countries, including the UK (Bean et al., 2018). In Britain and Ireland, the species is not anadromous, remaining in freshwater throughout the life cycle (Finstad & Hein, 2012; Jørgensen & Johnsen, 2014;

Maitland & Adams, 2018) thereby inhibiting contemporary gene flow between populations in unconnected river catchments. This effect is compounded by the existence of multiple lakes which harbour sympatric and independently evolved ecotypes with genetic, phenotypic and ecological distinctiveness (Fenton et al., 2024b; Hooker et al., 2016; Jacobs et al., 2020). Given the natural heritage value and the geographic isolation of populations, there is a particular need to identify evolutionary significant and/or vulnerable sites for Arctic charr for conservation purposes. Recent guidelines for selection of Sites of Special Scientific Interest (SSSIs) in Great Britain make a strong case for selection and protection of sites supporting within-species diversity and seeks to preserve the habitats giving rise to this diversity (Bean et al., 2018). There is also a concerted movement towards the protection of genetic diversity and future adaptive potential of species, particularly those known to threatened, both in the UK and internationally in response to Aichi Targets (2011-2020) and the Kunming-Montreal framework (2022-2030) (Department of Agriculture et al., 2025; Hoban et al., 2025; Hollingsworth et al., 2019). However, given that management resources are limited, it is important to define populations, or groups of populations, that are arguably higher priority for protection than others.

To delineate conservation units and identify vulnerable populations, we conducted a national scale genomic study of Arctic charr in Scotland, contextualised across Britain and Ireland. We evaluated multiple approaches to determine populations for conservation action using a high-density genome-wide dataset of SNPs. We analysed overall patterns of genome-wide genetic structuring and phylogenetic analysis across all populations and catchments. Further we examined patterns of variation in putatively adaptive SNPs associated with the lake

environment and climate. We inferred populations that may be of higher risk to loss via explicit vulnerability metrics such as genetic offset (genomic vulnerability). We then used our analyses in tandem with phenotypic and ecological knowledge and geographical information to delineate ESUs and MUs within the species and highlight populations of significance to protect.

Materials and methods:

Sample acquisition and collection:

Arctic charr were sampled from 49 Scottish lakes, three Irish lakes, two Welsh lakes, and one English lake (Figure 1) between 1997 and 2021 using 30 m x 1.5 m Nordic multi-panel mesh survey gill nets (CEN, 2015) except at Llyn Padarn where fry traps were used to catch fry samples. Sample numbers and locations for each lake are indicated in Table 1. Several of the sampled lakes in Scotland contain multiple sympatric ecotypes (Adams et al., 1998; Fraser et al., 1998; Jacobs et al., 2020). The dataset includes three instances of parapatric divergences (two neighbouring lakes from the same river system supporting two ecotypes): Shin-Merkland, Doine-Lubnaig, and Stack-More (Adams et al., 2006; Adams et al., 2007a). Each ecotype was considered its own population in all steps of data generation and analysis unless otherwise specified. Therefore, in this paper, we use the term population to refer to all Arctic charr individuals in the same lake of origin or a group of individuals that share morphological/ecological traits within a lake. Lochs Maree and Stack are both known to contain multiple ecotypes (Adams et al., 2008) however these were not sampled explicitly and so each of those lakes was considered as one population for the purposes of this study. In total, we had samples from 64 different Arctic charr populations covering 26 of the 27 Scottish Hydrometric

Areas known to contain extant Arctic charr populations (Maitland & Adams, 2018). The dataset included 10 individuals per population for each unimodal population and five individuals per ecotype in multiple ecotype lakes, wherever possible (Table 1). Hydrometric Areas are defined as single river catchments or several contiguous river catchments which are designated across the United Kingdom (National River Flow Archive, 2014).

DNA extraction and ddRADseq:

DNA was extracted from fin clips, muscle, gill, head kidney, or from whole fry tissue using the Mackerey-Nagel NucleoSpin DNA Extraction Kit following manufacture recommendations. DNA quality and quantity were assessed using agarose gel electrophoresis and the Qubit Fluorometer with Life Technologies dsDNA BR assay. Four libraries of 90 samples plus five technical replicates were prepared using a ddRADseq protocol for Illumina, modified from Recknagel et al., (2015), and following Jacobs et al., (2020). Briefly, double restriction enzyme digestion was performed with *MspI* and *PstI*. A size selection range of 145-295bp was isolated using Pippin Prep. Final RAD-tag enrichment was performed over 10 PCR amplification cycles. Paired end 75bp sequencing was performed on the Illumina NextSeq500 platform at Glasgow Polyomics to generate 450 M reads per library (target of 4 M reads per individual and 10% PhiX).

Processing of ddRADseq data:

Raw sequence quality was assessed using *FastQC v0.11.9* (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The *process_radtags* pipeline in *Stacks v2.60* was used for demultiplexing raw sequence data (Rochette et al., 2019). To prevent a drop in sequence quality affecting mapping, the first 6 bp of

the second read were removed using *Trimmomatic v0.39* (Bolger et al., 2014). New data were combined with previously generated data for eight Scottish lakes (Awe, Dughaill, na Sealga, Tay, Lubnaig, Merkland, Uaine, Eck) from Jacobs et al., (2020) (ddRADseq NCBI short read bioproject: PRJNA607173). Mapping was performed to the annotated *Salvelinus sp.* genome (ASM291031v2) using *bwa mem v0.7.16* using a seed length of 20 (Li, 2013). Reads with a mapping quality < 20 were filtered out using *samtools v1.15* (Danecek et al., 2021). Ten individuals with < 1M mapped reads were removed due to the high amounts of missing data. The *gstacks* module of *Stacks*, was run with the –gt-alpha set to 0.01 to ensure called genotypes were reliable when RAD-loci building. SNPs were retained in the populations module of *Stacks* if they met the following criteria: present in 66% of all individuals in each population and across all populations, a minimum minor allele frequency of 0.01, maximum observed heterozygosity of 0.5 with only the first SNP per locus retained. We used the script *filter_hwe_by_pop.pl* (https://github.com/jpuritz/dDocent) to filter any SNPs that were out of Hardy-Weinberg Equilibrium within populations, although no such SNPs were detected. *VCFtools v0.1.16* was used to identify and remove any SNPS with read depth <10x or >30x (Danecek et al., 2011).

Genotyping error:

We used an R script published by Mastretta-Yanes et al., (2015) to estimate the rate of genotyping error using 20 replicated individuals. Overall error rate was calculated to be 2.6%. The R script was then adapted to identify particular SNPs that showed a high rate of mismatching across technical replicates. SNPs that mismatched in more than 1 replicate pair (i.e. >5% genotyping error rate) were removed from the dataset for all individuals. Genotyping error was then recalculated from technical replicates and resulted in a mean error rate of 1.4%. Technical

replicate samples were then removed from the dataset, retaining the replicate with the higher coverage. After filtering we had 24,878 SNPs and 410 individuals covering 64 different populations.

Summary statistics:

Pairwise F_{ST}, observed and expected heterozygosity, number of private alleles and F_{IS} were generated for all populations and loci using the –fstats parameter in the *populations* module of *Stacks*. Allelic richness was estimated for each population using the *hierfstat v0.5-10* R package (Goudet, 2005; R Core Team, 2021).

Identification of SNPs correlated to environmental and climatic variables:

We conducted a Redundancy Analysis (RDA) using the *vegan v2.6.2* R package to identify SNPs associated with variation in environmental and climatic variables (Table S1); these represent our putatively adaptive SNP dataset to analyse patterns of adaptive differentiation (Oksanen et al., 2022). Environmental data for 19 variables was collected from WorldClim using the raster R package *v3.5-15* using latitude and longitude co-ordinates (resolution 2.5 arcmin) (Fick & Hijmans, 2017; Hijmans & van Etten, 2012). Bathymetric data on lake surface area, mean and max depth, and altitude was collected for each lake from published work (Fenton et al., 2024a; Maitland & Adams, 2018; Murray & Pullar, 1910). An additional bathymetric variable, littoral zone percentage (defined as the area of the lake shallower than 5 metres depth) was calculated using available bathymetric maps and *ImageJ v1.50i* (Murray & Pullar, 1910; Schneider et al., 2012).

To create a dataset of uncorrelated variables, as collinearity can affect genotype-environment patterns (Ratner, 2009), a collinearity matrix was made in R for all environmental and bathymetric variables (Table S2). Variables with correlation coefficient (ρ) of < 0.7 were retained as independent variables and in cases where variables showed high correlation (ρ > 0.7) only one variable was retained (Layton et al., 2021) (Table S3). This resulted in a set of 10 uncorrelated variables (mean diurnal range, isothermality, maximum temperature of the warmest month, mean temperature of the wettest quarter, mean temperature of the driest quarter, annual precipitation, altitude, mean lake depth, lake surface area and the proportion of the lake comprising littoral zone (as %)).

Genes and regions containing adaptive SNPs:

To determine if our putative adaptive SNPs were related to functional processes, we investigated the genes and regions containing these SNPs. First, we compared the position of adaptive SNPs with a database of markers for quantitative trait loci (QTL) for various phenotypes from a range of salmonid species mapped to the *Salvelinus sp.* genome (Fenton et al., 2024b). Second, we compared SNP positions to all the annotated genes in the *Salvelinus sp.* genome (ASM291031v2) to identify genes containing associated SNPs. Both of these comparisons were carried out using *BEDtools v2.27.1* (Quinlan & Hall, 2010), with a limit of the SNP being within ± 100kbp for QTLs and ± 1kbp for genes to be considered. We then ran gene ontology term overrepresentation analysis (ORA) for any genes deemed to contain adaptive SNPs using the *TopGO v2.40.0* R package (Alexa & Rahnenfuhrer, 2020) using all genes containing any RAD loci from our ddRADseq data (associated or otherwise) as the comparison dataset. Results were summarised using REVIGO (Supek et al., 2011).

Analysis of population structuring:

For analysis of population structuring, we removed any non-neutral SNPs to more accurately reflect neutral evolutionary population genomic patterns (Funk et al., 2012). These were the putatively adaptive SNPs (1,104 loci) associated with environmental variables and SNPs in the top 5% of global F_{ST} scores, inferred from *hierfstat* (Goudet, 2005). This left a dataset of 22,763 SNPs. Admixture modelling was run using *PopCluster* for K=2 to K=64 with medium scaling and 10 replicates per K (Wang, 2022). The FSTIS and DLK2 estimators were used to infer the optimum number of K (Figure S1). Principal component analyses were run for all SNPs and associated SNPs using the *adegenet R package v2.1.10* (Jombart, 2008).

Distance-based phylogenetic relationship between populations were constructed using an unrooted Neighbour-Joining (NJ) tree in the *poppr R package v2.9.3* (Kamvar et al., 2015). Any SNPs with missing data were removed to prevent an effect on the phylogenetic trees. This left a dataset of 5,949 SNPs for phylogenetic analyses. The tree was run with 1,000 bootstraps with no root.

Calculating genetic offset:

Future forecast climatic data was derived from the Coupled Model Intercomparison Project Phase 5 (CMIP5) under the Representative Concentration Pathways (RCP) scenarios RCP 4.5 and RCP 8.5. The RCP 4.5 scenario assumes CO_2 emissions begin to decline by 2045 while RCP 8.5 assumes that CO_2 levels will continue to rise throughout the 21st century. SNPs associated with climatic variables were determined using an RDA as described earlier. We used climate associated SNPs to ensure genetic offset did not simply reflect genetic drift rather than adaptive signals (Láruson et al., 2022). We removed any SNPs that showed 5 or less different

minor allele frequency values across all populations, leaving us with a dataset of 235 SNPs. Genetic offset was not calculated for Lough Finn and Coniston Water due to insufficient number (<2) of individuals. To model current genotype-environment relationships, *gradientforest R package v0.1.32* was used with 250 trees (Ellis et al., 2012). This model was then used to transform current and future climatic data based on their importance in explaining genomic variation with the Euclidean distance between these current and future values representing genetic offset. Due to the way *gradientforest* models genotype-environment relationships, a single genetic offset score was calculated for each lake meaning that there was a single score for lakes containing multiple ecotypes rather than one per ecotype.

Susceptibility metrics:

Lake sensitivity scores for each lake in Scotland were taken from Maitland & Adams (2018). To summarise, sensitivity scores were calculated based on three bathymetric and location variables: Maximum depth of the lake, surface area of the lake, and lake altitude (Winfield et al., 2010). Deeper lakes were deemed to offer more refuge from high surface water temperatures, so were given a lower score. Similarly, higher altitude was deemed to provide some protection from thermal stress, so higher altitude was given a lower score. Small lakes will support smaller populations than larger ones and are impacted more by extreme climate events as a result (Fischer & Lindenmayer, 2007) so lakes with a smaller surface area were given a higher score. Scores for each variable were combined to give a score ranging from 3 to 11 with a score of 3 being a lake of least concern for population loss while 11 is of highest concern (Table S4).

Results:

Summary statistics:

Across the genomic dataset (N = 24,878 SNPs) from 410 individuals from 64 populations of Arctic charr, observed heterozygosity ranged from 0.029 to 0.188 (Figure S2) and allelic richness from 1.027 to 1.171. The Loch Lochy benthivore ecotype population was the most genetically diverse, with the highest observed heterozygosity and allelic richness (H_O = 0.188, A_R = 1.171) while the Loch Mealt population was the least genetically diverse (H_O = 0.029, A_R = 1.023) (Table 1). The number of private alleles ranged from 0 to 164. The Loch Ard Achadh (P_A = 164), Lough Bunaveela (P_A = 127), and Loch Loch populations (P_A = 83) had the highest numbers of private alleles. The number of private alleles negatively correlated with observed heterozygosity ($P < 0.001$, $R^2 = 0.329$).

Population structuring:

A neighbour-joining tree broadly separated populations by drainage divide in Scotland (Figure 2). Nearly all populations in river systems that flow to the east coast of Scotland appear in one cluster, populations in west-flowing river systems in a second. The west flowing system cluster formed two major subgroups. All our outgroup populations from elsewhere in Britain and Ireland appear amongst the west-flowing system population cluster. Within these larger clusters, populations in the same or nearby river catchments/Hydrometric Areas were usually sister taxa but some sister taxa were also geographically distant populations such as lochs Eck and na Sealga.

A principal component analysis did not separate major groups of populations, instead each PC axis only separated out one, or a small number of populations and

explained a small amount of the total variance. Individuals from lochs Coulin, Uaine, Fannich, Maree, and the Dughaill planktivore populations in the northwest of mainland Scotland separate from all other populations along EV1 which explained 3.62% of the total variance (Figure 3A) while EV2 (explaining 2.57% of the total variance) splits the Loch Loch population, which is in central eastern Scotland, from all others suggesting high levels of population distinctiveness.

Pairwise genetic differentiation was high between most populations, with a mean pairwise F_{ST} of 0.256 across all populations (Figure 4, Table S5). The lowest genetic differentiation F_{ST} (F_{ST}= 0.045) was between Loch Doon and the Talla Reservoir population (which is a conservation refuge population derived from Loch Doon ancestors in the late 1980s (Maitland et al., 2007)). The highest pairwise differentiation was between Lough Finn in Ireland and the Loch Rannoch benthivore population (F_{ST} = 0.663), this is probably an artifact of the small number of individuals we had for each population. Several populations such as Loch Loch (mean pairwise F_{ST} = 0.384), Loch Seil (mean pairwise F_{ST} = 0.371), and Loch Tarff (mean pairwise F_{ST} = 0.357) showed particularly high pairwise F_{ST} in all comparisons (all pairwise F_{ST} > 0.2).

Admixture analysis was used to infer the number of genetic clusters (K) in the dataset. Fifty distinct clusters were identified as the most likely number in both DLK2 and FSTIS (Figure S1), with most of these clusters consisting of all individuals from a single population and evidence of admixture was rare (Figure 5). An analysis to partition the molecular variance found that most variation was explained by populations within Hydrometric Areas (35.4%). The east-west split found in the topology of the neighbour-joining tree explains <1% of the total variance in the

AMOVA model. Hydrometric Area delineations within the east-west split explains 13.6% of the total variance. (Table S6).

Patterns of putatively adaptive variation:

We used a redundancy analysis to identify SNPs strongly associated with changes in climatic and lake variables. Approximately 10% of the total variance in the whole dataset was explained by these environmental predictors. Bio3 (Isothermality (BIO2/BIO7)), Bio8 (Mean Temperature of Wettest Quarter), Bio9 (Mean Temperature of Driest Quarter) all counterposed in the opposite direction of Bio12 (Annual Precipitation) and Bio2 (mean diurnal range) on RDA1 (Figure S3). Altitude and littoral zone percentage counterposed in the opposite direction of mean lake depth and surface on RDA2.

Our analysis showed that all 10 constrained axes were significant in explaining variance and so we then identified SNPs with significant associations across all of them (Table S7). Overall, we identified a set of 1,104 SNPs as putatively adaptive to broad scale environmental conditions and lake bathymetry (Table S8). Surface area was the strongest predictor for the highest number of SNPs (N=234) followed by Bio9 (Mean Temperature of Driest Quarter) (N=166) (Table S9). This is reflected in the hierarchy of the importance of environmental variables in explaining adaptive variance with surface area the most important variable followed by Bio9 (Figure S4). When identifying the positions of the putatively adaptive SNPs to annotated genes in the *Salvelinus sp.* genome, we found that 586 known genes contained, or were proximal to, at least one of the 1104 putatively adaptive SNPs (Table S10). GO term analysis showed that amongst the known functions of these genes, a number of processes relating to formation, development, and

morphogenesis such as skeletal system development (GO:0001501) and sagittal suture morphogenesis (GO:0060367) showed overrepresentation (Table S11).

When we compared positions to a database of the known genomic location of salmonid quantitative traits (Fenton et al., 2024b), we found that 35 QTLs contained, or were found close to, those loci we identified as adaptive SNPs (Table S12). Seven of these QTLs were originally identified from genetic mapping experiments from Arctic charr while 10 were from lake whitefish (*Coregonus clupeaformis*), 13 from coho salmon (*Oncorhynchus kisutch*), and five from lake trout (*Salvelinus namaycush*) all closely related species. Over half of the QTLs associated with morphological variation, with seven QTLs related to body length, six related to body shape, three related to body length, and a further three related to Fulton's condition factor.

Exploring patterns of adaptive variation through a PCA revealed that EV1 explained 10.3% of the total variance and clearly differentiated the Loch Seil population from all other populations (Figure 3B). EV2 explained 8% of total variance and most clearly separates the Lough Bunaveela population (Ireland) from the other populations in one direction and a group including the lochs Coulin, Fannich, Uaine and the Dughaill planktivore populations in the other direction. Conducting a PCA on the environmental variables, to determine if the patterns identified resulted from distinct environmental conditions, did not show the local conditions for these populations to be outliers in any obvious way (Figure S5).

Susceptibility metrics:

We assessed the potential vulnerability of populations of Arctic charr to a range of threats using three different metrics: genetic offset, lake sensitivity, and levels of observed heterozygosity focusing primarily on the Scottish populations.

We used genetic offset, also known as genomic vulnerability, as a measure of potential fitness maladaptation to changing climate, where higher values indicate elevated predicted maladaptation to future climatic change and risk of extirpation (Fitzpatrick & Keller, 2015). A redundancy analysis was used to identify a subset of SNPs (N=235) associated with change in climatic variables to be used to generate genetic offset scores. Under the RCP 4.5 emission scenario, genetic offset scores spanned from 0.005 (lowest risk) in the Loch Nam Brac population up to lochs Uaine (0.022) and Insh (0.028) (Table 2). We did find a significant relationship between genetic offset scores and latitude, with southern populations generally showing higher genetic offset scores ($P = 0.004$, $R^2 = 0.13$) (Figure 6A). However, many of the highest offset scores were found in the more northern parts of our distribution (Figure S6). We found a much stronger relationship between offset scores and distance to sea ($P < 0.001$, $R^2 = 0.23$) with populations found in lakes at a further distance from the sea through river systems having higher genetic offset scores (Figure 6B). There was no significant relationship between genetic offset scores and observed heterozygosity ($P = 0.249$, $R^2 = 0.01$) (Figure S7). Similar results were seen when using the more extreme RCP 8.5 scenario to calculate genetic offset scores with, for example, genetic offset scores showing a strongly correlated with latitude (Figure S8).

We assessed how the local environment affects potential population vulnerability using a metric of lake sensitivity based on lake bathymetry and altitude called lake sensitivity scores from Maitland and Adams, 2018. Possible scores ranged from 3 to 11, from least to most vulnerable. Three lochs, lochs Rannoch, Ericht, and Treig, had that lowest possible score of 3 in our dataset (Table 2) whilst the highest score was 8, in lochs Seil and Ard Achadh, and the average score was 5.

While the different metrics of susceptibility were largely uncorrelated with each other (Table S13), there were some populations such as Loch Seil that performed poorly across all metrics, showing low genetic diversity, a high lake sensitivity score, and a high genetic offset score (Table 2). These populations were not found in any specific region of our dataset and were spread out across the range.

Discussion:

Our results highlight how different genetic criteria to delineate priority populations for conservation can lead to differing classification of conservation units. We found that levels of genetic differentiation and genetic distinctiveness were high between almost all populations of Arctic charr in our study. The Hydrometric Area (the catchment or cluster of adjacent catchments) of origin of the population explains a small but meaningful component of the genetic variation which is otherwise very high across all populations. An important finding of our study further identified an important component of putatively adaptive variation that is unique to particular lakes and has the potential to result in differential responses between populations to future selection forces. Given the demonstrated high levels of population differentiation and weak geographic patterns, with distant geographic populations often showing similar levels of differentiation to geographical close ones, we believe geographically large-scale groupings would be inappropriate for conservation unit delineation. We instead focused on prioritising of lakes or regions that are conservation-relevant ESUs by exploring and balancing combined information and weighing different ecological and evolutionary components.

National-scale structuring and regional genetic differentiation:

While Arctic charr is now non-anadromous in Britain and Ireland, although the species was likely anadromous when it first colonised post-glaciation (Fenton et al., 2023). At broader scales, an NJ tree largely clusters populations on either side of the hydrological central divide in Scotland forming east and west groups (Figure 2). These groupings likely represent a historic period of anadromy before the phenotype was lost with populations in nearby river systems clustering together due to the higher likelihood of contact with one another, a pattern known in freshwater fish species that do not disperse between unconnected river systems (Gómez & Lunt, 2007). These groupings influenced by drainage divide, are similar to that seen in North American populations of Brook trout (*Salvelinus fontinalis*) which can be found either side of the Eastern Continent Drainage Divide (Kazyak et al., 2022). However, this east-west grouping only explains a small proportion of the total variance (Figure S6) due to high levels of population differentiation and genetic distinctiveness.

Analysis of population differentiation showed that most populations were quite differentiated. This is reflected in many of the pairwise F_{ST} comparisons between populations were notably above the 0.15 value often used to determine significance of differentiation (Table S5) (Frankham et al., 2009). The patterns of high genetic differentiation are reflected in the admixture analysis with most populations forming their own distinct genetic clusters (Figure 5) and showing little support for clustering of these populations into larger groups. Populations showing lower genetic differentiation and some grouping at a level greater than populations were generally those found in interconnected lakes in the same catchment (e.g. lochs Doine and Lubnaig) or where populations are from the same lake (e.g. the ecotypes at lochs Awe or na Sealga). These findings are consistent with previous studies that found

non-anadromous populations of Arctic charr generally show high levels of genetic differentiation and lower levels of genetic diversity (Salisbury et al., 2023; Shikano et al., 2015). In this Arctic charr are more structured and differentiated than many other salmonids such as Lake whitefish found in North America (Graham et al., 2022) and Brown trout (*Salmo trutta*) in both England and Ireland (Moccetti et al., 2024; Rodger et al., 2021).

A number of populations in our dataset are highly geographical isolated, such as lochs Mealt and Ard Achadh, on the isles of Skye and Islay respectively. Whether such isolated populations are of higher conservation value has been debated across species and depends on whether these populations exist in differing environments and their persistence potential (Finn et al., 2009; Lesica & Allendorf, 1995). We found that many of these highly isolated populations tended to have low heterozygosity with relatively high numbers of private alleles (Table 1). These isolated populations with no contemporary gene flow with other populations have likely gained unique mutations, and their genetic differentiation may be driven more by genetic drift rather than selective pressures (Funk et al., 2016). The lack of gene flow reflected in the genetic distinctiveness of peripheral populations puts them at particular risk of extirpation due to potentially lower genetic diversity and novel variation and higher risk of accumulation of deleterious mutations. Overall, the data did not suggest these populations were inherently of higher conservation value.

Levels of differentiation between ecotype pairs was variable and ranged from 0.05 to 0.20 pairwise F_{ST}. The extent of differentiation is consistent with previously inferred differences in the divergence histories and how the separate ecotypes arose. Pairs previously inferred to have resulted from admixture after secondary contact of distinct colonising lineages showed much higher differentiation (pairwise

F_{ST} 0.16-0.20), such as the sympatric ecotype pairs in lochs Tay, Dughaill and the Rannoch planktivore versus the Rannoch piscivore/benthivore (Jacobs et al., 2020; Verspoor et al., 2010). In contrast the ecotypes that have been found to be sympatric divergences, such as lochs Awe and na Sealga, have markedly lower genetic differentiation (pairwise F_{ST} 0.05-0.06) despite clear eco-morphological distinctiveness (Garduño-Paz et al., 2012; Jacobs et al., 2020). While less is known about the lochs Ericht, Lochy, and Arkaig ecotype pair origins, because these divergences have not been studied in depth before, the levels of genetic differentiation found here was relatively high (pairwise F_{ST} 0.14-0.17) suggesting that these pairs resulted from two post glacial invasions into the same lake and secondary contact, though this needs to be investigated further (Fenton *et al.* in prep). Complex and differing evolutionary histories of sympatric ecotypes in postglacial lakes has been recently demonstrated in other areas such as Arctic charr in Russia and European whitefish in Europe (Alekseyev et al., 2002; Fang et al., 2022; Öhlund et al., 2020). This highlights the unique genetic backgrounds, conservation value and adaptive potential of these highly diverse populations.

Adaptive differentiation:

A potential route to delineating conservation units is to focus on adaptive genes (Hoelzel, 2023; Miller et al., 2024) as has been seen in Pacific salmonids in North America where conservation units are based on migration timing which is largely controlled by the *GREB1L/ROCK1* region and is an important phenotype to preserve variability in (Hess et al., 2016; Prince et al., 2017; Waples et al., 2022; Waples & Lindley, 2018). As such we identified a set of 1,104 SNPs significantly correlated with climatic and lake variables across these populations to explore this potential avenue as well as investigate the genes and regions behind local

adaptation across a broad scale. Given that previous studies on Arctic charr have highlighted that, adaptive phenotypes, like head and body shape in ecotype populations, can be highly polygenic (Fenton et al., 2024b; Jacobs et al., 2020), we did not expect to define single regions that could be used conservation unit delineation. Indeed, we ended up finding a high number of putatively adaptive SNPs across the genome (Table S8).

Over half of these environment-associated SNPs (N=624) were found within or proximal to known genes in the *Salvelinus sp.* genome. We found a high number of terms relating to morphogenesis and development as overrepresented amongst these SNPs. Fully half of the salmonid QTLs that we found contained putatively adaptive SNPs were related directly to morphology (Table S12). Further, 32 of the genes found to contain putatively adaptive SNPs were shared with a recent genome-wide association analysis that identified key genes that underlie head and body shape morphology differences between ecotypes of Arctic charr (Fenton et al., 2024b). While morphology was the most prevalent in our dataset, several QTLs identified were related to other important phenotypes such as age of sexual maturation and spawning date. Spawning date is known to vary between these specific populations, such as in Loch Awe which has spring and autumn spawning ecotypes (Garduño-Paz et al., 2012). Arctic charr is famously highly morphologically variable across populations (Klemetsen, 2013). Our results from genomics reinforce the conservation and functional importance in adaptation to local lake environments and climatic conditions (Bush & Adams, 2007).

That we identified functional processes and genomic regions associated with head and bone morphology for SNPs associated with temperature variables support previous studies which indicate that both phenotypes are influenced by temperature

across various fish species (Campbell et al., 2021; Hooker et al., 2023; Lema et al., 2019; Riesch et al., 2018). The QTLs containing environment-associated SNPs came from a number of different salmonid species, thereby indicating that key regions underlying important phenotypes are to a meaningful extent shared across species as has been previously suggested for salmonids (Fenton et al., 2024b; Jacobs et al., 2017; Salisbury & Ruzzante, 2022). Overall, adaptive variation is facilitated through a number of different processes and our results emphasis/support the role of morphology acting as a key aspect of local adaptation across populations in the British Isles. The functional role of these candidate regions would be a valuable area of future research.

Patterns of differentiation in these putatively adaptive SNPs largely identified distinct single populations, like Loch Seil and Lough Bunaveela, with differing patterns rather than larger groupings (Figure 3B) as were identified in similar studies on other species (Forester et al., 2022). These populations are not the same as the ones that appear as most distinct in the PCA using all SNPs (Figure 3A). The environmental conditions, lake bathymetry and climatic conditions, for these populations, were not obviously distinct from the rest of the populations in our dataset (Figure S5). Given that this dataset is the first time many of these populations have been explored genetically, further exploration is needed to properly understand the patterns seen. The identification of putatively adaptive SNPs located within genes and regions involved in important adaptive phenotypes despite high levels of genetic differentiation and drift is an important result for other species with highly isolated populations (Cortázar-Chinarro et al., 2017; Funk et al., 2016). Overall, we demonstrably showed how some of the intraspecific genetic variation

seen amongst populations is functional and thus adaptive and how these adaptive SNPs could be used to delineate ESUs once further evidence is gathered.

Population susceptibility:

A key part of conservation efforts is identifying populations that may be more vulnerable to loss and are therefore of higher conservation priority. Populations can be more vulnerable for a variety of reasons, from low levels of standing diversity to higher risk due to their environment, with these factors often uncorrelated with one another (Campbell et al., 2021; Fischer & Lindenmayer, 2007). The Arctic charr is adapted to cold, highly oxygenated environments and as such the species is highly susceptible to climate change, particularly increasing water temperatures, and eutrophication (Kelly et al., 2020; Winfield et al., 2008). These risks are thought to be particularly exacerbated at the southern edge of the species range for example in North America and central Europe (Kelly et al., 2020; Layton et al., 2021). However, predicting the potential of population viability and risk is a challenging endeavour impacted by its environment and its population characteristics. Genetic offset, often referred to as genomic vulnerability, is a metric for fitness maladaptation to future climatic change (Fitzpatrick et al., 2021; Layton et al., 2021).

Several previous studies on genetic offset in salmonid species such as Arctic charr and Sockeye salmon *Oncorhynchus nerka* in North America have highlighted an important correlation between genetic offset scores and latitude with more southern populations tending to be at higher vulnerability (Layton et al., 2021; Tigano et al., 2024). Given that populations in Britain and Ireland are the southern extent of the European range with the exception of those in the Alps (Tiberti & Splendiani, 2019), for the species it is perhaps unsurprising that we found more southern populations, which are already living in conditions towards the limit of the species,

may be more vulnerable to future increase in temperature, which are predicted in the RCIP scenarios (Figure 6a) (Layton et al., 2021). The vulnerability of more southern populations is corroborated by the distribution across Britain, with only a few extant populations found in the southern parts of Scotland and northern England and Wales, and most of the recorded local extinctions occurring in these same regions (Maitland & Adams, 2018).

However, the relationship had elements of inconsistency with the highest genetic offset scores found in the more northern latitudes suggesting varying conditions due to latitude is not the only driving force of this pattern. We did find a much stronger relationship between genetic offset scores and geographic distance to the sea, with populations in lakes a further to the sea having higher genetic offset scores (Figure 6B). This relationship suggests that more inland populations have had reduced levels of gene flow since colonisation, as seen in other fish species (Gouthier et al., 2023), and has resulted in them being more locally adapted but less flexible to future change. Notably, genetic offset scores were not driven by levels of observed heterozygosity (Figure S7) suggesting scores were not driven by solely by standing levels of genetic variation (Láruson et al., 2022; Tigano et al., 2024).

While the genetic offset and observed heterozygosity did not correlate in our dataset, observed heterozygosity still represents an important indicator of population vulnerability. Low levels of standing genetic variation suggests a population is less able to adapt to changes in their environment and are at higher risk of reduced population fitness due to inbreeding depression (Reed & Frankham, 2003). Similarly, populations in small lake environments may also be of concern for potential loss. The size of lake environments varied drastically with many of the more genetically diverse populations found in the deeper, larger lakes (Fenton et al., 2024a) while

small lake size seems to have limited the overall diversity of the population supported while also providing less refuge from rising water temperatures making them at higher risk to loss (Campbell et al., 2021; Fischer & Lindenmayer, 2007).

Overall, our metrics of susceptibility were largely independent of each other, allowing us to investigate vulnerability across multiple axes (Table S13) as is already done in other work focusing on determining conservation priority (Auber et al., 2022; Du et al., 2024). Looking across our metrics did not highlight any specific geographic regions of particular concern with the worst scoring populations in each metric found distributed across our range (Table 2). While we did note some populations that performed poorly across all metrics such as Loch Seil, our analyses suggest that individual populations need to be assessed individually to determine their viability and vulnerability. Our analyses do show however that the latitudinal position of a population, its distance from the sea, and overall lake size can also be used to give indications of potential vulnerability and can be used in a wider-scale analysis to identify potential regions of higher vulnerability or viability in the species distribution. Further work should look to include other potential threats to the species like lake acidification, hydroelectric work or non-native introductions to better assess population vulnerability (Knudsen et al., 2016; Maitland et al., 2007). Therefore, rather than trying to define a single definitive statistic to determine vulnerability, the investigation of vulnerability across a range of metrics can help us identify vulnerable populations in ways that may be missed by the use of a single metric.

Criterion for delineating ESUs and MUs:

Conversation units like ESUs have had numerous definitions with the definition used often reflective of the system the unit is being applied to (Coates et al., 2018; De Guia & Saitoh, 2007; Moritz, 1994). The dataset generated here for the

highly diverse Arctic charr is good example of how suitable criteria for some systems may not be suited for others and so a criterion logically for the species and system in question needs to be devised.

For example, many studies that define ESUs and MUs delineate them in a hierarchical fashion with larger ESUs containing multiple reproductively isolated populations considered to be MUs (Funk et al., 2012; Galland et al., 2021; Shaney et al., 2020). If we used the hierarchical approach often used for delineating ESUs and MUs (Funk et al., 2012), then the larger east-west groupings of populations seen in the neighbour-joining tree (Figure 2) could represent our ESUs, which largely splits population by direction of river system discharge, with the differentiated groups of populations within them representing MUs. However, the high levels of genetic differentiation we found, suggest that a hierarchical approach would be inappropriate for Arctic charr. These east-west groups only capture a small amount of the total variation and would undervalue the individual sites/populations that show substantial adaptive differentiation or functional diversity as they would only be valued as MUs.

We therefore believed a population level approach was the best given the high levels of genetic differentiation between populations. We defined ESUs as populations or groups of populations that: 1) showed high reproductive isolation to other groups using genetic differentiation as a proxy, and 2) showed substantial adaptive differentiation, important functional diversity or represented populations of geographic importance. Groups that showed evidence of reproductive isolation but did not show sufficient evidence for our second criteria were considered MUs.

To evaluate the second criterion, we explored patterns of adaptive differentiation through the identification of adaptive SNPs. While the identification of adaptive variance undeniably has importance in the delineation of conservation

units (Hoelzel, 2023), we cautioned the use of the SNPs we identified as putatively adaptive for the basis of our conservation unit delineations. Due to the high levels of population differentiation seen in the dataset and the lack of prior research on many of these populations, particularly those like Loch Seil that show distinct patterns, means it would be highly speculative to basis our consideration of evolutionary significant primarily on this information. For example, we lack a specific region or gene of interest with a known adaptive function that we could prioritise conservation units (Waples et al., 2022; Waples & Lindley, 2018). We therefore suggest further studies are required before these populations can be considered evolutionary significant. Our study does provide a wealth of information on potential genes and regions of interest for these further studies to work from.

Delineation of ESUs and MUs:

It is evident from the foregoing that high levels of genetic variation mean that clear ESU and MUs were not apparent in the genetics data. We therefore decided to merge as much data from all sources as exist when attempting to delineating conservation units. This included phenotypic information from a wealth of existing studies and environmental and geographic information (Bush & Adams, 2007; Jacobs et al., 2020; Maitland & Adams, 2018). In addition, we created a logical framework for defining reasonable population groupings. Thus, we suggest that that only populations in close proximity (<20km) and show notable gene flow, conservation translocations (Maitland et al., 2007), and populations with notable shared genetic origin, e.g. recently diverged ecotypes (Jacobs et al., 2020), should be considered within the same intraspecific conservation units due to the high levels of genetic differentiation seen between most populations. These are not exact rules, for example not all populations within 20km of each other show evidence of notable

gene flow, e.g. lochs Nam Brac and Stack. Our results strongly urge against the movement of Arctic charr between lakes in different river catchments particularly those on the opposite sides of the east-west drainage divide, in alignment with previous suggestions for the conservation of the species (Maitland et al., 2007), due to the lack of contemporary connection between populations. The general high levels of genetic differentiation suggest that conservation of most populations need to be handled independent of one another.

Given our findings we delineated ESUs based on 1) the presence of ecotype, 2) parapatric divergences, and 3) the potential for divergence. We deemed ecotype populations, either in multimodal lakes or parapatric divergences, as having high conservation value to their importance of the functional diversity of the species (Jonsson & Jonsson, 2001; Maitland & Adams, 2018). These includes ecotypes that do not show clear genetic differentiation but show clear evidence of reproductive isolation and ecological distinctiveness with Arctic charr in freshwater lakes known have very marked homing to their natal spawning sites, as seen in Lake Windermere (Frost, 1965). Populations that show notably lower genetic differentiation to an ecotype population but are not part of the system, i.e. not in the same lake or parapatric system, were placed in the same ESU. We also considered populations which may have high potential for evolutionary divergence due to geographic isolation as being of higher conservation value. For example, populations that are the only extant population in their respective HA have a higher potential to gain unique and important adaptive variation, due to their extreme isolation, and their loss would significantly affect the geographic range of the species (Lesica & Allendorf, 1995).

We attributed ESUs to populations that fulfil one of these criteria and because of high level of divergence between the remaining populations all other populations were assigned as MUs. We applied this criterion to the genetic groupings found in our study with the results listed in Table 3.

Ecotype populations in multimodal lakes like Awe and na Sealga were still considered ESUs in our system despite a lack of clear genetic differentiation from one another as there is sufficient evidence of differences in spawning habitat and timing and ecology distinctiveness to suggest these represent important incipient diverging groups (Adams et al., 1998; Adams & Huntingford, 2004; Alexander & Adams, 2000; Garduño-Paz et al., 2010; Jacobs, 2018; Jonsson & Jonsson, 2001; Kettle-White, 2001). Indeed, with their complete separation in spawning times, the ecotypes at Loch Awe arguably have the highest potential for divergence and speciation amongst ecotype pairs ecotypes and should be protected as such. Similarly, while only the Shin-Merkland parapatric divergence showed notable genetic differentiation, there is sufficient evidence of reproductive isolation in the Doine-Lubnaig and Stack-More pairs in previous works that suggests these should still be considered different ESUs (Adams et al., 2006; Adams, Fraser, et al., 2007). The known ecotypes at lochs Maree and Stack not directly studied in this work (Adams et al., 2008) should investigated more directly although these should still be considered separate ESUs. While most of these ESUs consist solely of one ecotype population, ecotypes such as the Loch Dughaill planktivore were placed in an ESU containing other populations, such as Loch Coulin and Fannich, due to patterns of genetic similarity seen in admixture and PCA analyses influenced by a shard ancestral history (Fenton *et al.,* in prep).

The populations in lochs Eck, Doon, Bruicheach, Lee, and Earn all represent the only populations in their respective Hydrometric Areas and were therefore considered important groups with high divergence potential. The Loch of Girlsta, which is the sole extant population on the Shetland Islands, represents an ESU on the same criteria but needs further investigation as it was not included in our dataset (Maitland & Adams, 2018). Lochs Eck, Doon, and Girlsta are already have some conservation status through recognition of Sites of Special Scientific Importance (SSSIs) (Mainstone et al., 2018). Lochs Doon, Lee, and Girlsta have also been flagged previously as sites that may be of higher risk of loss due to factors such as lake acidification (Maitland et al., 1991, 2007).

Other factors such as unique historical lineages (Jacobsen et al., 2022; Moore et al., 2015) and adaptively important phenotypes, for example a difference in spawning (Walker, 2007), foraging/diet (Garduño-Paz & Adams, 2010) or habitat usage (Kristjánsson et al., 2012), would also be considered evolutionarily significant and worth prioritising for protection as has been seen in our studies delineating ESUs (Zhao et al., 2020). On the basis of our current understanding, we define all remaining groups working designations of MUs. These designations should not be considered as definitive and more data on these populations as well as the many others not in our dataset should be used to further our understanding and help delineate accurate conservation units within the species. While the vulnerability of populations was not the focus of our conservation unit delineations, the vulnerability of populations is an important consideration for how to manage these populations as has been done for other species (Miller et al., 2024).

Arctic charr is listed as one of the priority fish species under the UK Biodiversity Framework (BRIG, 2007) in recognition of the need for conservation

action to protect the species and the substantial diversity seen within it. A small number of Arctic charr populations in Scotland, such as lochs Eck and Doon which are present in this study, are included as notified features within protected sites (such as Sites of Special Scientific Interest) and are therefore protected in law (Bean et al., 2018; Mainstone et al., 2018). A number of other Arctic charr populations, such as lochs Maree and Rannoch, that are located within sites classified as Special Areas of Conservation under the EU Habitats Directive and Special Protection Areas (under the EU Birds Directive) to protect other habitats and species present which also benefits Arctic charr (McLeod et al., 2009). Current protection is clearly not protecting the high levels of diversity we know from this study to be extant in Scotland and the large number of ESUs and MUs defined here indicates that many populations are defined as separate units for the conservation of diversity. While the formal designation of ESU population as a notified feature based purely on our findings is unlikely, inclusion of some ESUs populations to list of notified features in SSSIs, already protected for other purposes, is possible if their supporting habitat occurs within their boundaries as is the case for the populations in lochs Maree and Calder for example. However, inclusion with the network of protected sites this is not the only way that Arctic charr populations can be protected. Protection of supporting habitats through Water Framework Directive (WFD) River Basin Management Plans, which focus on water regulation and planning activities, and the application of clear regulation to prevent the introduction of non-native species can all contribute to the health and security of existing populations (SEPA, 2021).

Conclusions:

Populations clustered into their river catchments and on a wider scale by river system flow however levels of genetic differentiation were high, even between populations in the same river systems, suggesting low levels or a lack of gene flow and that most populations are reproductively isolated from one another. Identification of putatively adaptive SNPs highlighted a number of genes and regions with roles in local adaptation that warrant further investigation to determine the importance of their role. Our metrics of population vulnerability highlighted the value of using multiple metrics to explore vulnerability demonstrating who the geographical location and size of lake environment can affect population vulnerability. We focused conservation efforts on populations showing important phenotypes to preserve, such as ecological versatility, as well as that show important adaptive potential. Our study highlights the importance of wide-scale datasets when delineating conservation units as well as the challenging for appropriate delineations in highly diverse species. Our study demonstrates how both neutral and adaptive processes, interacting with abiotic environment, impact the conservation priority and evolutionary trajectories of a highly diverse species.

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Figures and Tables:

Table 1:

Table 1. Overview of each of our 64 populations with summary statistics describing of genomic diversity. Location is either given as country (for outgroups) or Hydrometric Area for Scottish populations (numbered and named per the National River Flow Archive (2014). Pa refers to number of private alleles, He to expected heterozygosity, Ho to observed heterozygosity, π to nucleotide diversity, Ar to allelic richness.

Pop ID	Latitude	Longitude	Sampling Year	N	Pa	Ho	He	π	Ar
a'Bhaid-Luachraich	57.81	-5.55	2012	10	32	0.093	0.075	0.080	1.080
a'Garbh-bhaid Mor	58.39	-4.95	2021	10	80	0.065	0.048	0.051	1.051
Ard Achadh	55.36	-6.16	2021	10	164	0.048	0.039	0.042	1.042
Arkaig (Benthivore)	56.97	-5.14	2007	4	0	0.116	0.094	0.110	1.110
Arkaig (Planktivore)	56.97	-5.14	2007	5	0	0.116	0.098	0.110	1.110
Awe (Benthivore)	56.30	-5.23	1997	5	0	0.119	0.108	0.125	1.124
Awe (Planktivore)	56.30	-5.23	1997	5	0	0.121	0.103	0.119	1.119
Bodlyn	52.80	-4.01	2002	2	0	0.112	0.089	0.128	1.128
Braig horrisdale	57.67	-5.67	2016	9	75	0.067	0.056	0.060	1.060
Brora	58.05	-3.95	2010	4	1	0.095	0.074	0.088	1.088
Bruicheach	57.39	-4.58	2009	8	24	0.075	0.059	0.065	1.065
Bunaveela	54.02	-9.54	2001	10	127	0.054	0.044	0.047	1.047
Calder	58.52	-3.59	2021	3	0	0.108	0.084	0.102	1.102
Conniston	54.34	-3.07	2005	1	0	0.129	0.065	0.129	1.129
Coulin	57.55	-5.35	1997	9	0	0.079	0.066	0.071	1.071
Damh	57.50	-5.57	2008	2	0	0.112	0.069	0.104	1.104
Doine	56.34	-4.48	1997	4	0	0.118	0.088	0.106	1.106
Doon	55.24	-4.37	1997	6	0	0.112	0.090	0.100	1.100
Dubh	56.88	-5.71	2021	5	12	0.124	0.091	0.108	1.108
Dughaill (Benthivore)	57.47	-5.35	2013/2014	5	2	0.102	0.087	0.098	1.098
Dughaill (Planktivore)	57.47	-5.35	2013/2014	5	0	0.105	0.093	0.105	1.105
Duntelchaig	57.35	-4.29	2010	4	0	0.122	0.089	0.113	1.113
Earn	56.38	-4.23	2016	9	27	0.102	0.083	0.089	1.089
Eck	56.08	-4.99	1997	10	59	0.060	0.049	0.057	1.057
Ericht (Benthivore)	56.82	-4.39	2007	4	1	0.127	0.106	0.126	1.126
Ericht (Planktivore)	56.82	-4.39	2007	5	0	0.145	0.121	0.136	1.136

Fannich	57.64	-4.96	2007	9	2	0.068	0.056	0.060	1.060
Finn	54.86	-8.14	2012	1	0	0.051	0.025	0.051	1.051
Garry	56.80	-4.25	2007	8	2	0.115	0.095	0.103	1.103
Insh	57.12	-3.93	2007	10	8	0.092	0.077	0.082	1.082
Kindrum	55.23	-7.71	1997	7	43	0.072	0.058	0.065	1.065
Laggan	56.92	-4.55	2007	6	0	0.115	0.099	0.110	1.110
Langavat	58.04	-6.82	1997	10	81	0.087	0.074	0.078	1.078
Lee	56.90	-2.95	1997	9	35	0.108	0.090	0.096	1.096
Loch	56.85	-3.67	2007	10	83	0.037	0.029	0.031	1.031
Lochy (Benthivore)	56.96	-4.92	2009	2	0	0.188	0.124	0.171	1.171
Lochy (Planktivore)	56.96	-4.92	2009	5	0	0.123	0.104	0.116	1.116
Lubnaig	56.30	-4.30	1997	10	2	0.103	0.078	0.100	1.100
Luichart	57.62	-4.78	2009	10	29	0.102	0.086	0.092	1.092
Maree	57.71	-5.53	2008	10	7	0.131	0.117	0.124	1.124
Meadie	58.33	-4.56	2007	5	2	0.101	0.078	0.089	1.089
Mealt	57.61	-6.18	2009	2	0	0.029	0.018	0.027	1.027
Merkland	58.23	-4.73	1997	10	26	0.068	0.052	0.065	1.065
More	58.28	-4.87	1998	4	0	0.121	0.091	0.113	1.113
Morie	57.75	-4.47	2009	3	0	0.123	0.087	0.116	1.116
Nam Brac	58.38	-5.12	2010	3	0	0.085	0.062	0.081	1.081
naSealga (Benthivore)	57.79	-5.31	2015	5	0	0.089	0.071	0.083	1.083
naSealga (Planktivore)	57.79	-5.31	2015	5	0	0.088	0.072	0.084	1.084
Naver	58.30	-4.35	2011	9	27	0.118	0.100	0.107	1.107
Osgaig	58.05	-5.32	2015	9	35	0.086	0.073	0.079	1.079
Padarn	53.13	-4.13	2020	10	65	0.095	0.082	0.087	1.087
Rannoch (Benthivore)	56.70	-4.23	2010	1	0	0.101	0.050	0.101	1.101
Rannoch (Planktivore)	56.70	-4.23	2010	6	5	0.115	0.098	0.109	1.109
Rannoch (Piscivore)	56.70	-4.23	2010	4	0	0.133	0.111	0.128	1.128
Seil	56.32	-5.55	2021	9	77	0.046	0.039	0.042	1.042
Shin	58.12	-4.57	1997	7	1	0.111	0.095	0.104	1.104
Stack	58.34	-4.92	1997	5	0	0.115	0.102	0.118	1.118
Talla	55.48	-3.40	1997	8	0	0.098	0.084	0.091	1.091
Tarff	57.15	-4.61	2012	8	57	0.049	0.038	0.042	1.042
Tay (Benthivore)	56.52	-4.14	1997	5	1	0.127	0.105	0.122	1.122
Tay (Planktivore)	56.52	-4.14	1997	5	1	0.121	0.107	0.123	1.123
Treig	56.81	-4.73	2007	7	0	0.127	0.124	0.135	1.135
Tummel	56.71	-3.95	2007	9	18	0.126	0.105	0.113	1.113
Uaine	57.52	-5.39	1997	10	0	0.067	0.060	0.064	1.064

Table 2:

Table 2: Susceptibility metric scores for each population in Scotland. Due to how each metric is generated, ecotypes in the same lake are given the same genetic offset and lake sensitivity scores. Observed heterozygosity was calculated based on all SNPs (N=24,878) while genetic offset was only calculated using SNPs associated with change in climatic variables (N=235).

Lake	Observed Heterozygosity	Genetic offset RCP4.5	Lake sensitivity score
a'Bhadid-Luachraich	0.09	0.007	5
a'Garbh-bhaid Mor	0.06	0.006	6
Ard Achadh	0.05	0.006	8
Arkaig_Bn	0.12	0.013	5
Arkaig_Pl	0.12		
Awe_Bn	0.12	0.018	6
Awe_Pl	0.12		
Braig Horrisdale	0.07	0.013	6
Brora	0.09	0.019	7
Bruicheach	0.07	0.013	5
Calder	0.11	0.012	6
Coulin	0.08	0.013	6
Damh	0.11	0.007	6
Doine	0.12	0.015	6
Doon	0.11	0.013	4
Dubh	0.12	0.009	6
Dughaill_Bn	0.10	0.014	7
Dughaill_Pl	0.10		
Duntelchaig	0.12	0.015	4
Earn	0.10	0.016	5
Eck	0.06	0.013	6
Ericht_Bn	0.13	0.018	3
Ericht_Pl	0.14		
Fannich	0.07	0.016	4

Garry	0.12	0.018	4
Insh	0.09	0.028	4
Laggan	0.12	0.020	4
Langavat	0.09	0.013	6
Lee	0.11	0.016	4
Loch	0.04	0.019	5
Lochy_Bn	0.19	0.019	5
Lochy_Pl	0.12		
Lubnaig	0.10	0.015	5
Luichart	0.10	0.013	5
Maree	0.13	0.008	6
Meadie	0.10	0.007	6
Mealt	0.03	0.012	6
Merkland	0.07	0.007	6
More	0.12	0.013	6
Morie	0.12	0.016	5
nam Brac	0.09	0.005	6
naSealga_Bn	0.09	0.017	5
naSealga_Pl	0.09		
Naver	0.12	0.007	5
Osgaig	0.09	0.006	6
Rannoch_Bn	0.10		
Rannoch_Pl	0.13	0.016	3
Rannoch_Ps	0.11		
Seil	0.05	0.016	8
Shin	0.11	0.006	5
Stack	0.11	0.006	6
Talla	0.10	0.014	5
Tarff	0.05	0.015	5
Tay_Bn	0.13	0.017	4
Tay_Pl	0.12		
Treig	0.13	0.014	3
Tummel	0.13	0.016	5
Uaine	0.07	0.022	7

Table 3:

Table 3: Conservation unit classifications for populations in our dataset as Evolutionarily Significant Units (ESUs) or management units (MUs) based on our analyses and previous findings.

Population(s)	Conservation unit type	Delineation criteria for ESU
a'Bhaid-Luachraich	MU	
a'Garbh-bhaid Mor	MU	
Ard Achadh	MU	
Arkaig (Benthivore)	ESU	Diverged ecotype pop.
Arkaig (Planktivore)	ESU	Diverged ecotype pop.
Awe (Benthivore)	ESU	Diverged ecotype pop.
Awe (Planktivore)	ESU	Diverged ecotype pop.
Braig horrisdale	MU	
Brora	MU	
Bruicheach	ESU	Only extant HA pop.
Calder	MU	Diverged ecotype pop.
Coulin, Dughaill (Planktivore), Fannich, Uaine	ESU	
Damh	MU	
Doine	ESU	Diverged ecotype pop.
Doon, Talla	ESU	Only extant HA pop.
Dubh	MU	
Dughaill (Benthivore)	ESU	Diverged ecotype pop.
Duntelchaig	MU	
Earn	ESU	Only extant HA pop.
Eck	ESU	Only extant HA pop.
Ericht (Benthivore)	ESU	Diverged ecotype pop.
Ericht (Planktivore), Garry	ESU	Diverged ecotype pop.
Insh	MU	
Laggan, Treig	MU	
Langavat	MU	
Lee	ESU	Only extant HA pop.
Loch	MU	

Lochy (Benthivore)	ESU	Diverged ecotype pop.
Lochy (Planktivore)	ESU	Diverged ecotype pop.
Lubnaig	ESU	
Luichart	MU	
Maree	ESU	
Meadie	MU	
Mealt	MU	
Merkland	ESU	Diverged ecotype pop.
More	ESU	Diverged ecotype pop.
Morie	MU	
Nam Brac	MU	
naSealga (Benthivore)	ESU	Diverged ecotype pop.
naSealga (Planktivore)	ESU	Diverged ecotype pop.
Naver	MU	
Osgaig	MU	
Rannoch (Benthivore)	ESU	Diverged ecotype pop.
Rannoch (Piscivore)	ESU	Diverged ecotype pop.
Rannoch (Planktivore)	ESU	Diverged ecotype pop.
Seil	MU	
Shin	ESU	Diverged ecotype pop.
Stack	ESU	
Tarff	MU	
Tay (Benthivore)	ESU	Diverged ecotype pop.
Tay (Planktivore)	ESU	Diverged ecotype pop.
Tummel	MU	

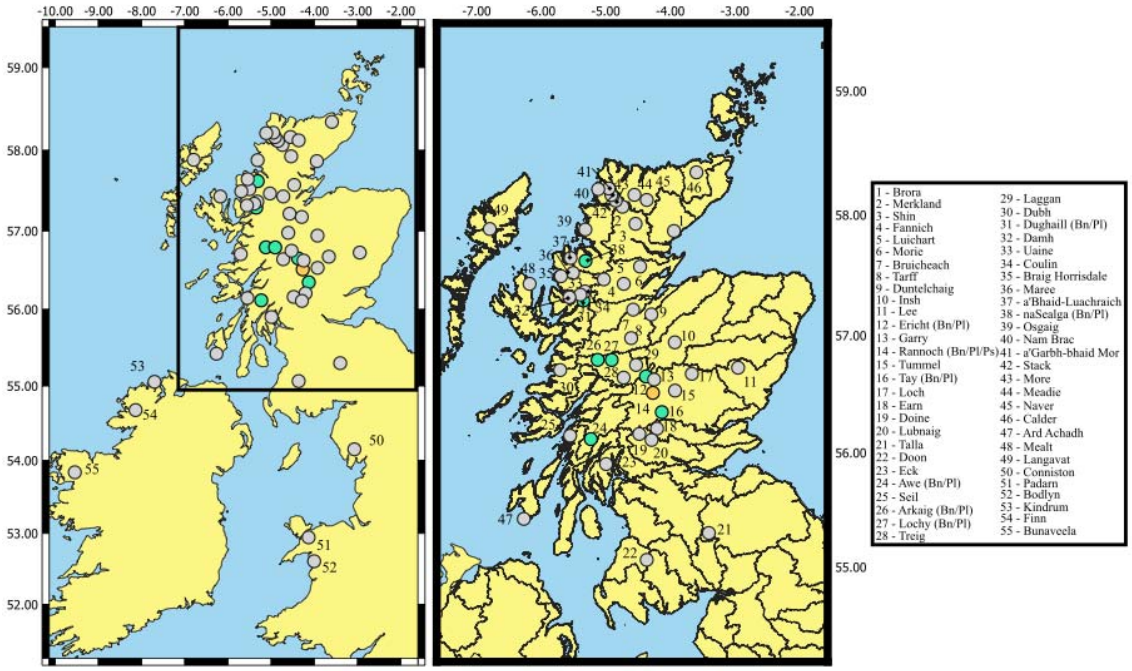

Figure 1:

Figure 1: Map showing the location of the populations from the British Isles included in this study. Lake origin is indicated on the map in ascending number order following Hydrometric Area order. Lakes with multiple ecotypes are indicated by Bn/Pl/Ps delineators in the legend and with green (bimodal) and oranges (trimodal) colours respectively. Bn refers to benthivore, Pl refers to planktivore and Ps refers to piscivore. Hydrometric Area boundaries are indicated on the Scotland specific panel on the right.

Figure 2:

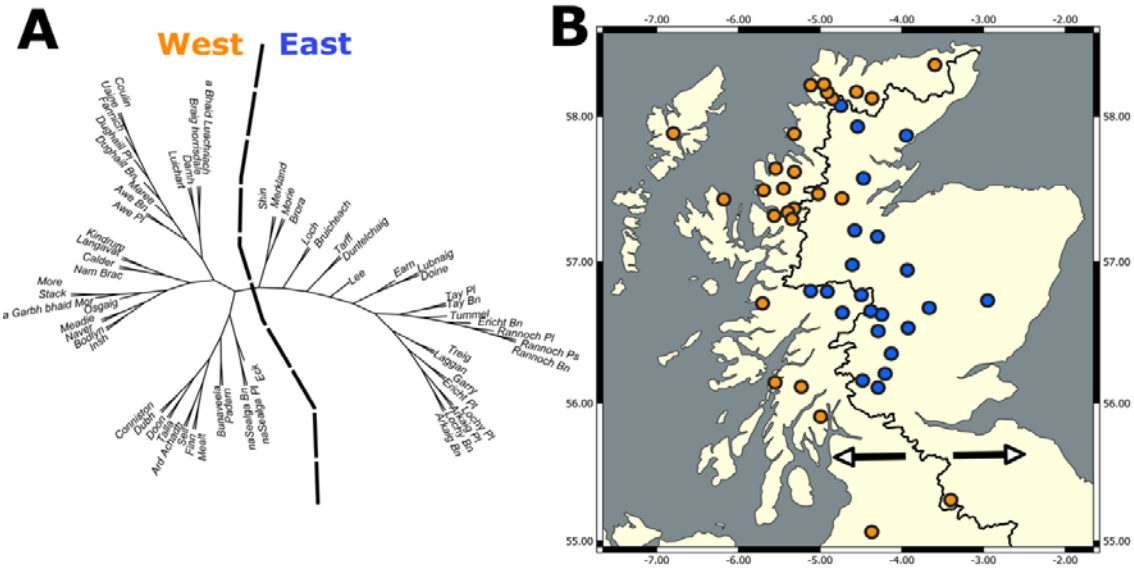

Figure 2: A) Neighbour-joining plot. NJ plot is split into largely east and west flowing river system groups and is indicated by the dotted line. B) shows the geographical distribution of this divide with the black line showing where the drainage divide occurs, populations to the left-hand side appear in west draining river catchments and those to the right in east draining. Populations are coloured by which side of the neighbour-joining tree they appear in, orange for west and blue for east. All outgroup populations are found in the west flowing group.

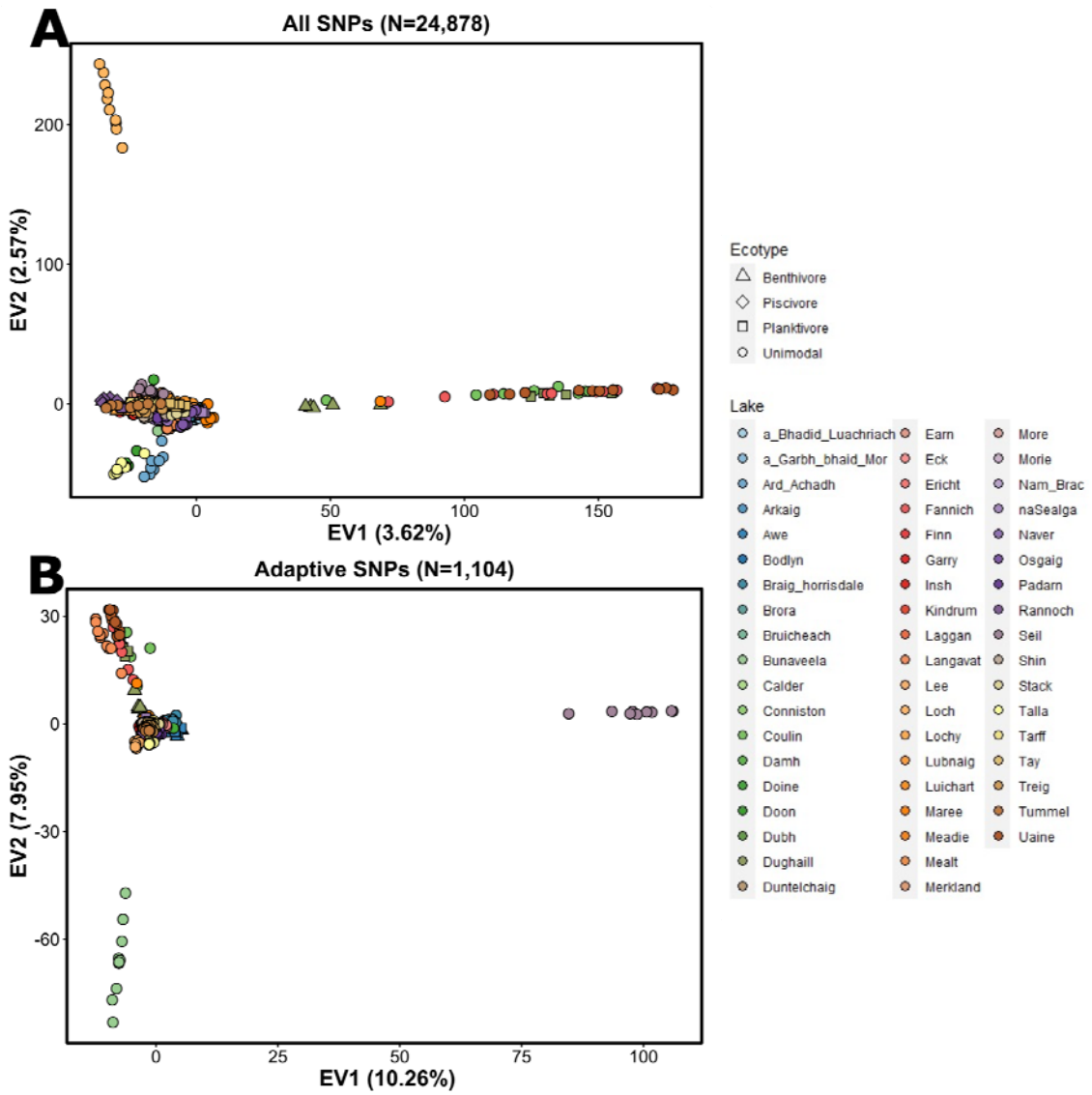

Figure 3:

Figure 3: Principal components analyses based on all SNPs (A) (N=24,878) and associated SNPs (N=1,104) (B). Individual points are coloured by lake of origin indicated in the legend. Shape is based on population ecotype, triangle for benthivores, diamond for piscivores and squares for planktivores. Unimodal populations are given circles.

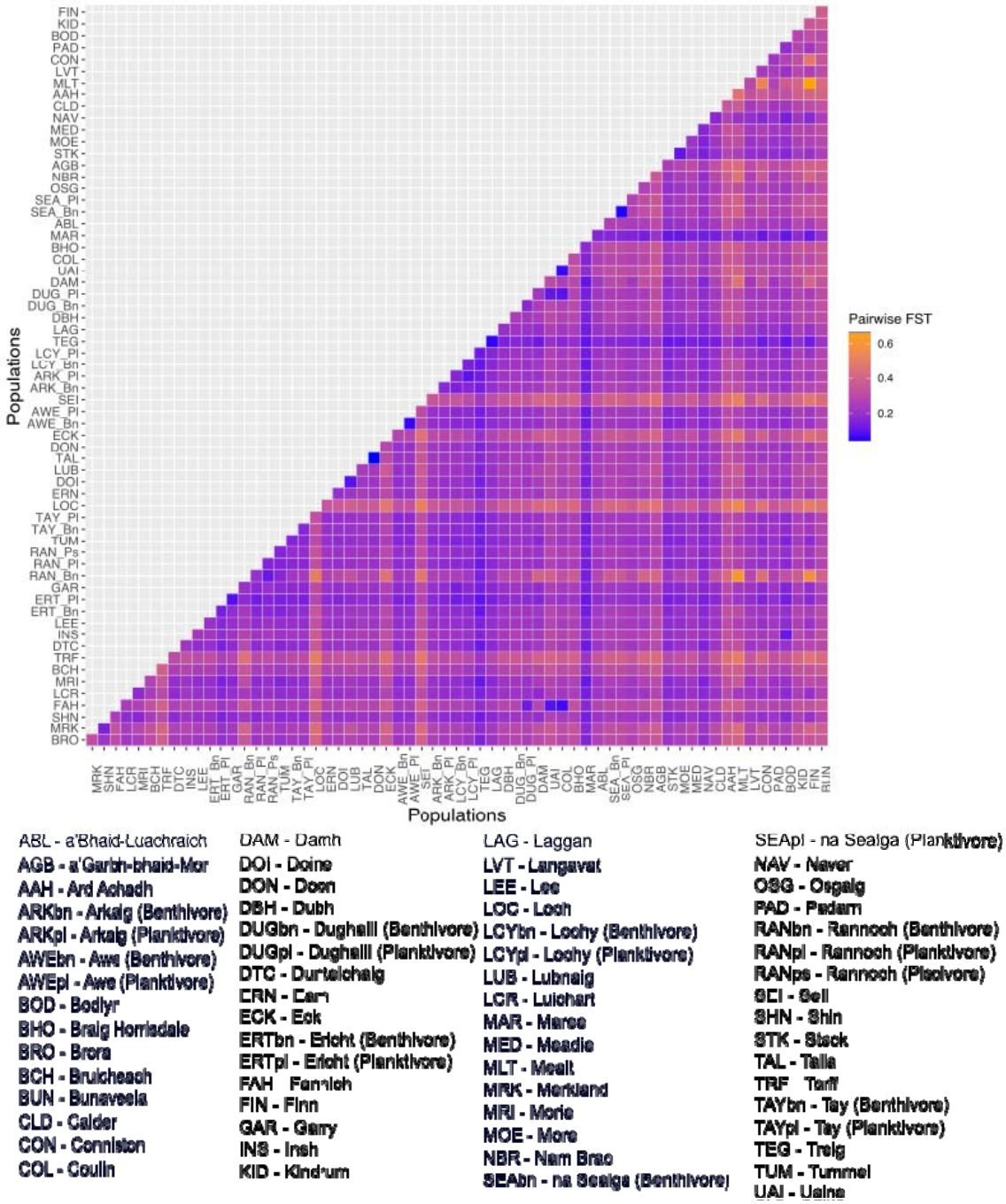

Figure 4:

Figure 4: Plot of pairwise F_{ST} comparisons. F_{ST} scores are colours in a range of blue to orange going from low to high. Populations are order in ascending Hydrometric area of origin, order as indicated in Figure 1.

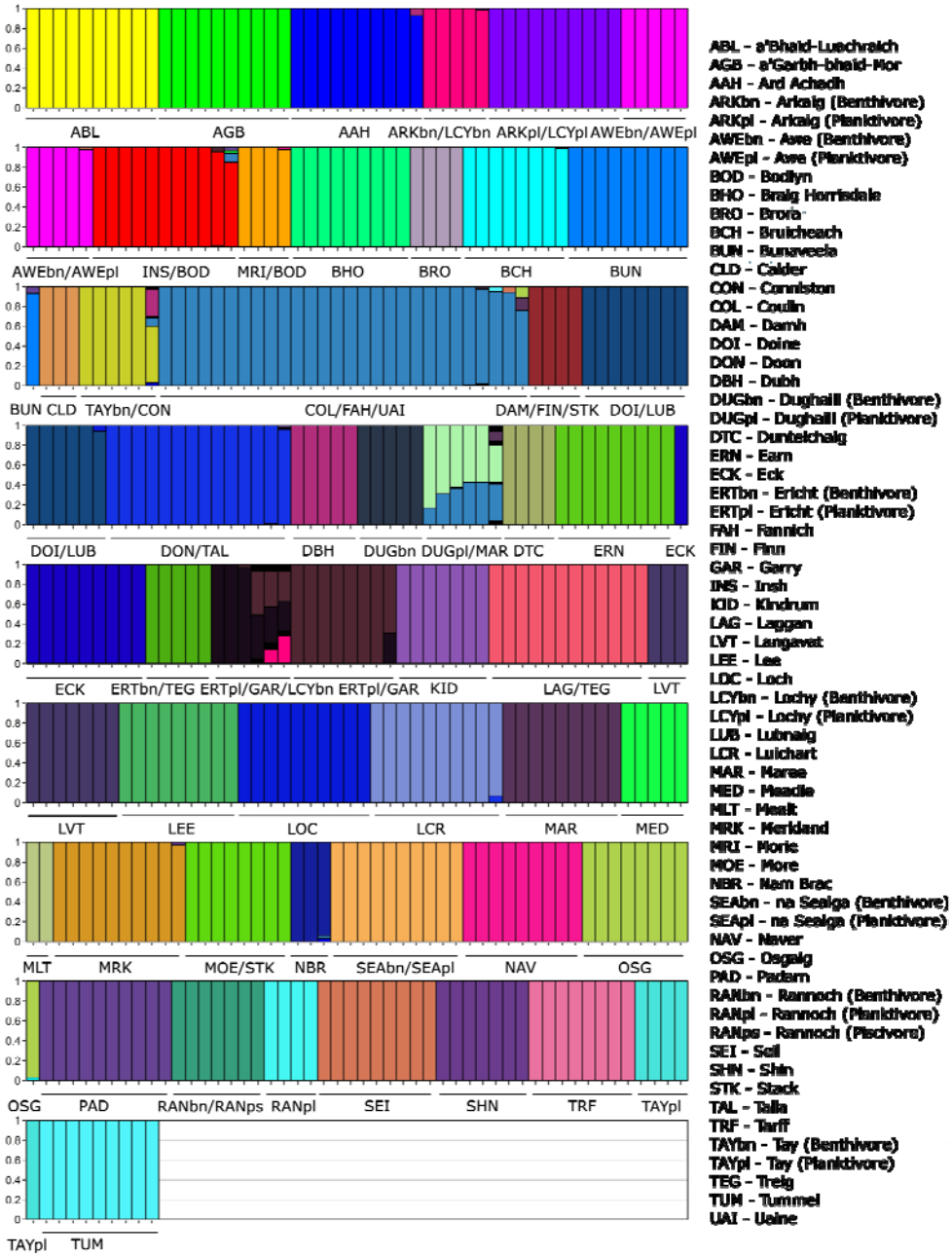

Figure 5:

Figure 5: Admixture plot for most likely number of genetic clusters (K), K=50. Individuals are ordered by their genetic cluster. Most populations split into their own distinct genetic clusters with only some proximal populations sharing the same

clusters. 3 letter codes for each population are provided. Ecotypes for relevant populations are indicated, bn for benthivore, pl for planktivore, ps for piscivore.

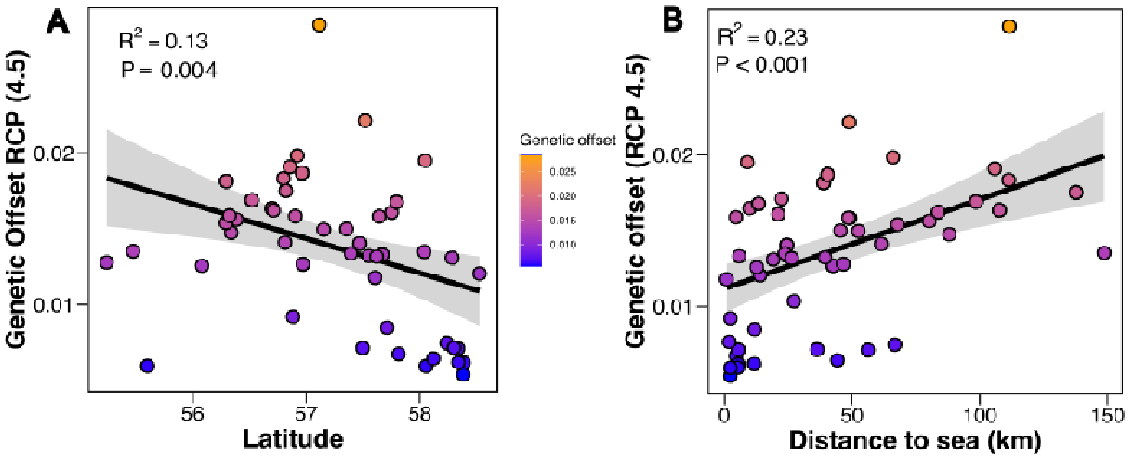

Figure 6:

Figure 6: Genetic offset scores under RCP 4.5 scenario for each lake against A) latitude and B) distance from the sea in kilometres. Each point, population, is coloured by genetic offset score going from blue to orange as they go from low to high.