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Original Article

Genomic vulnerability to climate change of a poorly dispersing and threatened fish, the southern pygmy perch (*Nannoperca australis*)

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Abstract

Small and isolated populations often have low levels of standing genetic variation, which limits their capacity to evolutionarily adapt to climate change. In freshwater ecosystems, habitat fragmentation caused by instream barriers and water regulation is a prominent global issue impacting on connectivity among populations. We investigated genomic vulnerability to climate change in a small-bodied and poorly dispersing species, the southern pygmy perch, in Australia's Murray–Darling Basin. We used a genome-wide dataset for 467 individuals from 30 sites covering the range of the species in the Murray–Darling Basin. This included temporal sampling of a population in the Lower Lakes region, prior to its extirpation during the Millennium Drought and after its re-establishment through a captive breeding and reintroduction program. Southern pygmy perch exhibited high levels of population structure, with 11 distinct genetic clusters mainly delineated by river catchments. Genetic diversity was low, especially in small and isolated headwater populations. Genomic vulnerability, assessed via a genomic offset approach, correlated positively with elevation, being generally higher in upland and lower in lowland populations. We suggest that elevation could potentially serve as a proxy for climate change vulnerability in dendritic freshwater systems, particularly for species with limited dispersal capacity. The signal of low genomic vulnerability for the Lower Lakes population was consistent both before and after ex situ captive breeding and reintroduction. This highlights the importance of downstream populations as sinks of diversity. It also shows that integrating genetic management into captive breeding programs can help maintain climatic adaptive potential in threatened populations.

Keywords endangered biodiversity, freshwater fish, genomic offset, conservation genomics, genetic rescue, Murray–Darling Basin

Introduction

Genetic adaptation can play a crucial role in the persistence of species in novel and changing environments, particularly when opportunities for dispersal or phenotypic plasticity are limited (Nogués-Bravo et al. 2018). In this era of anthropogenic climate change and habitat disruption, understanding the need and capacity for species to respond via genetic adaptation is a pressing topic in conservation biology (Hoffmann et al. 2021). Although there is mounting evidence that some species are capable of rapid genetic adaptation to environmental change (Geerts et al. 2015; Hof et al. 2016; Niu et al. 2019), there is concern that many will be unable to keep pace with the current rate of change (Radchuk et al. 2019). This variability in adaptive potential is influenced by a number of species characteristics such as ecological specialization, generation time, and mating system (Thompson et al. 2023).

Resilience to climate change is not only a species-level trait but is also expected to differ among populations due to spatial variation in environmental conditions, genetic factors, and demography (Bay et al. 2018; Razgour et al. 2019). Local adaptation, the process through which populations evolve to have greater fitness in their home environment compared with elsewhere, can particularly influence how a population

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responds to climate change (Savolainen et al. 2013). Populations currently adapted to warmer conditions might possess traits that offer advantages compared with cool-adapted populations (Razgour et al. 2019). Climate change is disrupting patterns of local adaptation as environmental conditions shift beyond those that populations have previously occupied (Aitken and Whitlock 2013; Bay et al. 2018; Capblancq et al. 2020). This genotype-environment mismatch can be assessed using genomic vulnerability models, also known as genomic offset, which use contemporary genotype–environment associations (GEAs) to predict the genetic composition required under future climate scenarios (Fitzpatrick and Keller 2015; Bay et al. 2018). These ecological forecasting approaches have become increasingly popular for identifying populations at risk of climate-driven declines in fitness (Capblancq et al. 2020; Fitzpatrick et al. 2026; Lind and Lotterhos 2026). However, as with many emerging tools in ecological genomics, genomic vulnerability models require more empirical validation and are subject to several limitations. These include reliance on assumptions of local adaptation and fitness decline, potential biases arising from genetic drift and existing maladaptation, and uncertainty regarding the functional relevance of the adaptive genomic regions used in the analyses (see Section 4.4 for details).

The evolutionary potential of a population is fundamentally determined by its standing genetic diversity, or the total variation in alleles present among individuals (Barrett and Schluter 2008). Recently declining populations of small size often have reduced genetic diversity and an accumulation of mildly deleterious mutations caused by strong genetic drift (Lynch et al. 1995; Gaitan-Espitia and Hobday 2021). Such populations are therefore at an increased risk of fitness declines, loss of evolutionary potential, and extirpation under climate change (Gaitan-Espitia and Hobday 2021). Gene flow between populations can, however, enhance adaptive capacity through the introduction or supplementation of beneficial alleles (Frankham 2015; Aguirre-Liguori et al. 2021). Predicting which populations are most susceptible to climate change through genomic vulnerability modeling can be an important tool for prioritizing conservation resources and directing management strategies, such as assisted gene flow and genetic rescue (Borrell et al. 2020; Beheregaray et al. 2026).

Freshwater species are often extremely vulnerable to climate change because key features of their habitats, such as water quality and streamflow, are sensitive to global warming (Barbarossa et al. 2021; Capon et al. 2021). Additionally, because obligate freshwater species are confined to lakes and river systems, they often have a limited ability to respond to climate change through dispersal (Radinger et al. 2017). Habitat fragmentation caused by damming and water extraction is exacerbating this issue worldwide, leaving many freshwater populations that were once connected with reduced or entirely restricted gene flow (Barbarossa et al. 2020; Rosenthal et al. 2024). As a result, isolated populations are experiencing reductions in genetic diversity and adaptive potential (Shaw et al. 2025). These pressures are particularly concerning for small-bodied fishes, which often have specialized niches and low dispersal (Kopf et al. 2017). Small-bodied fishes are essential links in aquatic food webs and contribute to ecosystem functioning through roles such as controlling insect populations, stirring sediment, and grazing vegetation (Guo et al. 2023; Lintermans 2023). Despite their critical role in freshwater ecosystems, small-bodied fishes often receive less conservation attention than larger, commercially important species (Saddlier et al. 2013).

The study species is the southern pygmy perch (*Nannoperca australis*), a small-bodied (<85 mm) freshwater fish endemic to southeastern Australia, including parts of the Murray–Darling Basin (MDB) (Unmack et al. 2013; Lintermans 2023). It is a habitat specialist that prefers slow flowing or still streams and wetlands with dense aquatic vegetation (Tonkin et al. 2008). Individuals are short-lived (typically ≤3 yr) and reach sexual maturity within their first year (Humphries 1995). They generally spawn in spring to early summer when temperatures exceed 16°C, and lay demersal, nonadhesive eggs (Llewellyn 1974). The species is well studied compared with many other small-bodied fishes in the region, with previous work indicating that southern pygmy perch is highly susceptible to climate change (Hammer et al. 2013). Several reproductive traits of the species, including maternal investment in egg size and fecundity, and nuptial coloration, are known to vary in response to contrasting hydroclimatic conditions (Morrongiello et al. 2010; Morrongiello et al. 2012). Spatial and temporal variation in water quality related to flow regime also impacts its growth and survival (Morrongiello et al. 2013), and there is genomic and transcriptomic evidence for local adaptation to hydroclimatic variation across the species' range (Brauer et al. 2016; Brauer et al. 2017).

Southern pygmy perch has undergone recent demographic declines because of colonial disturbances including introduced species, riverscape fragmentation, and habitat degradation (Lintermans et al. 2024). The species is listed as endangered in the states of South Australia (Hammer et al. 2009) and New South Wales (Fisheries Management Act 1994), and nationally under the Environment Protection and Biodiversity Conservation Act 1999. In the MDB, southern pygmy perch now occupies only a fraction of its former range, existing in small and patchy populations (Lintermans 2023). Studies based on microsatellite and single nucleotide polymorphism (SNP) markers show that the species has a hierarchical pattern of population structure mainly delineated by river catchments (Brauer et al. 2016; Cole et al. 2016). A lack of strong phylogeographic structure in mitochondrial DNA, however, indicates that populations were likely to have been more connected historically (Cole et al. 2016). Coalescent models additionally suggest that population differentiation has been largely driven by environmental modifications occurring after European colonization (Cole et al. 2016). In addition, eco-evolutionary simulations indicate that the genetic signal of isolation for several southern pygmy perch populations in the MDB is consistent with the ~160-yr time frame since the construction of in-stream barriers began (Brauer and Beheregaray 2020). From the perspective of ecologically relevant genetic diversity, a landscape genomics study revealed adaptive divergence among southern pygmy perch populations in relation to temperature and precipitation gradients across the MDB (Brauer et al. 2016). Notably, evidence for adaptive divergence was strongest among headwater sites across the elevational range of the species (Brauer et al. 2016). This MDB upland region holds the smallest estimated effective population sizes (*Ne*) of southern pygmy perch, including isolates that recently experienced local extirpation (Brauer and Beheregaray 2020; Buckley et al. 2024).

Climate change, and particularly alteration to rainfall patterns, poses a major threat to southern pygmy perch. Due to its specialized habitat requirements and poor dispersal ability, southern pygmy perch is highly susceptible to extirpation during droughts in an altered system (Hammer et al. 2013). A notable example occurred during the Millennium Drought (1997 to 2010),

when a genetically distinct population in the terminal Lower Lakes of the MDB faced complete habitat desiccation (Hammer et al. 2013). Conservation intervention was required and, just prior to extirpation, 65 individuals were rescued for an ex situ captive breeding program (Attard et al. 2016). This program was genetically informed using microsatellite markers to determine breeding groups that would minimize inbreeding and maintain genetic diversity in the F1 generation (Attard et al. 2016). When the wetland habitat returned, approximately 1,350 first generation progeny from the breeding program were reintroduced to the Lower Lakes (Hammer et al. 2013). This reintroduced population has exhibited ongoing recruitment and maintained low inbreeding and similar levels of neutral and putatively adaptive genetic diversity compared with the original pre-drought population (Attard et al. 2016; Beheregaray et al. 2021; Marshall et al. 2022). However, it is unknown if this population has maintained genomic resilience to future climates, a knowledge gap common to most conservation reintroductions.

In this study, we assessed genomic vulnerability to climate change in a threatened freshwater fish with small, declining, and fragmented populations. First, we used SNP data to characterize population structure and genetic diversity in southern pygmy perch across its range in the MDB. Next, we ran a GEA analysis to identify SNPs putatively involved in climate adaptation and to assess adaptive divergence among populations. Finally, we used gradient forest to model future genotype-environment mismatches for southern pygmy perch under projected climates. We also capitalized on a rare empirical opportunity to investigate whether ex situ genetic management and reintroduction of an extirpated population (i.e. the Lower Lakes) was successful in maintaining adaptive potential related to climate change. Notwithstanding the role of the MDB's steep hydroclimatic gradient in shaping adaptive genetic diversity and plasticity in this species (Brauer et al. 2016; Brauer et al. 2017), we hypothesized that smaller upland populations with lower standing genetic diversity would show higher genomic vulnerability to future climates. Additionally, we empirically tested and explored the potential for elevation to serve as a proxy for climate change vulnerability in river systems. This study adds to the very few reported assessments of genomic vulnerability in freshwater fishes (Brauer et al. 2023; Tigano et al. 2023), a highly threatened, culturally important, and understudied group that provides ecosystem services to billions of people worldwide (Sayer et al. 2025).

Materials and methods

Sampling and genomic data

A total of 521 southern pygmy perch were sampled from 30 sites across the southern MDB between 1992 and 2019 (Fig. 1). This sampling encompassed 11 river catchments across the entire contemporary distribution of the species in this drainage basin (Cole et al. 2016; Brauer and Beheregaray 2020). Fish were caught via netting, box trapping, or electrofishing, and preserved either as frozen specimens or fin clips in 99% ethanol. In the Lower Lakes region, sampling targeted the local population before and after habitat desiccation during the Millennium Drought (Hammer et al. 2013). Samples from 2002 to 2008 are wild-caught from the original Lower Lakes population prior to its extirpation during the drought. Samples taken between 2013 and 2019 are from the reintroduced population that was established by F1 individuals from the captive breeding program (details in Attard et al. 2016; Marshall et al. 2022).

DNA was extracted either using a Qiagen DNeasy Blood and Tissue Kit, or a modified salting out protocol (Sunnucks and Hales 1996). The quality and concentration of DNA extractions were assessed using a Qubit fluorometer (Thermo Scientific), NanoDrop 1000 spectrophotometer (Thermo Scientific), and gel electrophoresis. Double-digest restriction site-associated DNA sequencing libraries were prepared using a protocol adapted from Peterson et al. (2012). Libraries were sequenced paired-end, either as 100-base-pair reads on an Illumina HiSeq2000 or as 150-base-pair reads on an Illumina HiSeq4000.

Bioinformatics

Raw sequences were demultiplexed using the process_radtags program from Stacks (Catchen et al. 2013). Residual adapter sequences and low-quality bases were trimmed using AdapterRemoval (Schubert et al. 2016). The resulting sequences were aligned to a *N. australis* reference genome (Sandoval-Castillo and Beheregaray 2026) using Bowtie 2 (Langmead and Salzberg 2012). Sequence alignment map (SAM) files were converted to binary alignment map (BAM) files, and duplicate reads were marked using SAMtools (Danecek et al. 2021). To improve mapping accuracy, indels were realigned using the Genome Analysis Toolkit (GATK) (McKenna et al. 2010). SNPs were subsequently called from the reference genome using BCFtools (Danecek et al. 2021) and filtered using VCFtools v.0.1.16 (Danecek et al. 2011). We retained only biallelic loci with $<$20% missing data and a minor allele frequency $>$1%. Individuals with $>$50% missing data were removed. Indels were discarded, as were SNPs with a mapping quality $<$30 and a depth $>$82. To account for linkage disequilibrium (LD), we thinned the SNPs to one every 500 bp. This LD pruning choice reduces the inflation of false positives in GEA analyses and prevents overstated confidence in genomic offset predictions.

Population structure and genetic diversity

We used the program fastStructure (Raj et al. 2014) to characterize population genetic structure in southern pygmy perch. We tested for 1 to 20 genetic clusters (K), with ten replicates per K value. FastStructure outputs were visualized using the R package *pophelper* (Francis 2017). To additionally explore the genetic structure, we performed a principal component analysis (PCA) using the R package *vegan* (Oksanen et al. 2024). For the PCA, missing genotypes were imputed based on the mode of allele frequencies within each genetic cluster, as estimated using the *snmf* function in the R package *LEA* (Frichot and François 2015). Finally, global and pairwise F_{ST} were calculated after the approach of Weir and Cockerham (1984) to estimate levels of population differentiation. This was done using the R package *hierfstat*, with the significance of pairwise estimates assessed through 100 bootstrap replicates using the R package *dartR* (Gruber et al. 2018).

We used *hierfstat* (Goudet et al. 2022) to assess levels of genetic diversity (observed heterozygosity, Ho; expected heterozygosity, He) and population level inbreeding (F_{IS}). These statistics were evaluated for each sampling site and for the population clusters revealed by fastStructure. For the Tookayerta Creek/Lower

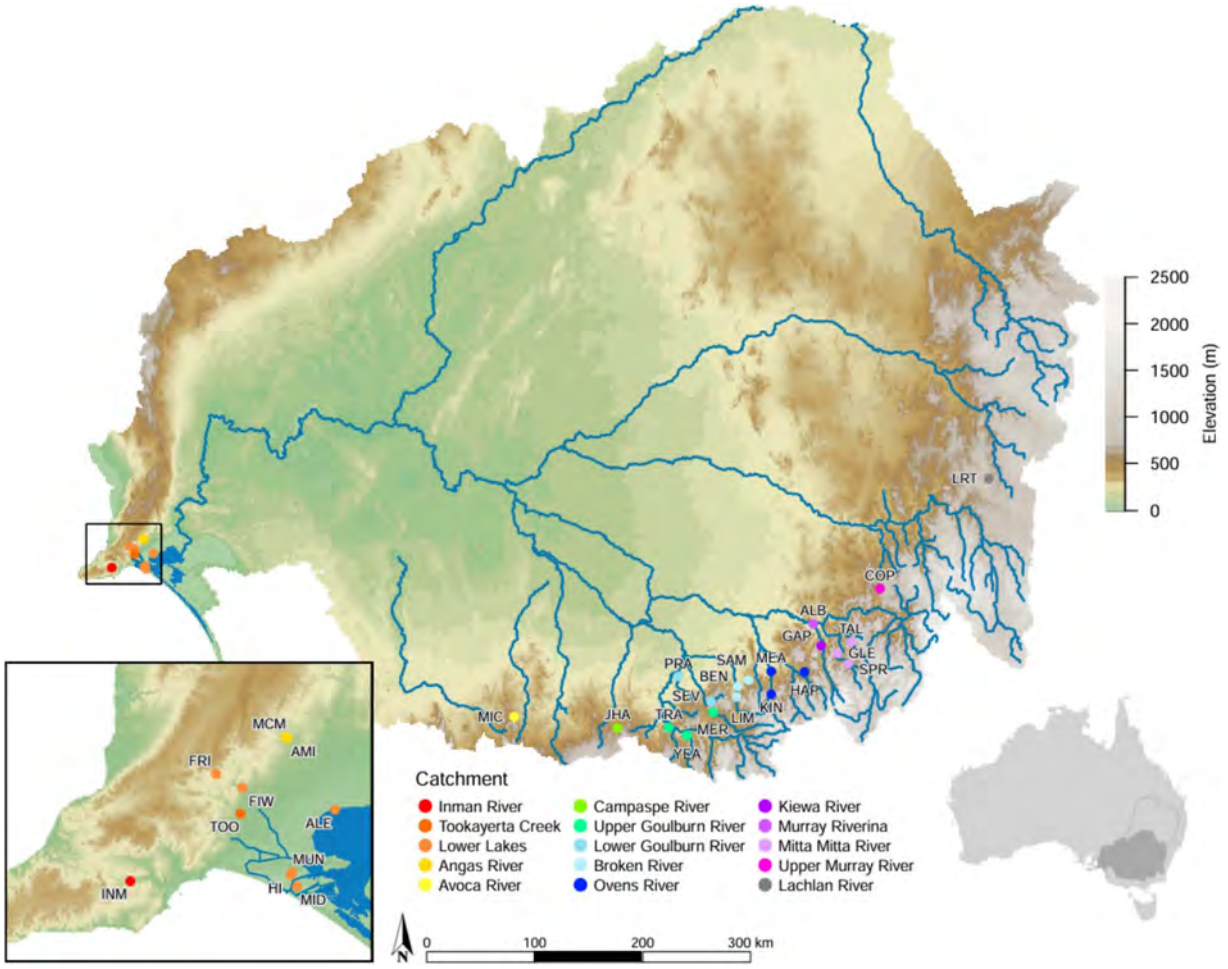

Fig. 1 Sampling locations for southern pygmy perch across an elevational gradient in the Murray–Darling Basin, Australia. Inset shows the location of the Inman River, Tookayerta Creek, Lower Lakes, and Angas River catchments. Full site names are listed in Table 1.

Lakes cluster, we assessed diversity separately for the original and reintroduced populations. We also used the *beta.dosage* function in *hierfstat* to estimate individual inbreeding coefficients according to the Weir and Goudet (2017) beta estimator, calculated relative to allele frequencies in each population. For the individual level inbreeding, we dropped 25 individuals from the reintroduced Lower Lakes population that had a higher amount of missing data (average 15% missing) than others in that population (average 1.2% missing).

Genotype–environment association analysis

To characterize the hydroclimatic environment across the MDB, we downloaded 19 bioclimatic variables from the WorldClim database (Fick and Hijmans 2017). These temperature- and precipitation-based variables are valuable for understanding climate adaptation in aquatic species, including the southern pygmy perch (e.g. Brauer et al. 2016; Brauer et al. 2018). We acquired data based on the contemporary climate, i.e. conditions averaged over 1970 to 2000, and also data projected by the CNRM-CM6-1 (Voldoire et al. 2019) model for three future time points: 2050 (averaged over 2041 to 2060), 2070 (2061 to 2080), and 2090 (2081 to 2100). In addition to these bioclimatic variables, we obtained elevation data from Geoscience Australia (2008), as the southern pygmy perch's range encompasses an elevational gradient. We used the R package *raster* (Hijmans 2023) to extract values of these variables at our sampling sites.

We used redundancy analysis (RDA) to characterize adaptive divergence among southern pygmy perch populations and identify genomic loci putatively involved in climate adaptation. Our explanatory variables were the bioclimatic and elevation parameters, and our response variables were sampling site level SNP allele frequencies (missing data imputed). To ensure reliable allele frequency estimates, sampling sites with less than 8 individuals were either removed (INM and YEA) or combined with sites in close proximity (the six Lower Lakes sites combined into one). We also combined the two Angas River sites due to their close proximity (<1 km). Prior to RDA, variance inflation factor (VIF) analysis was performed using a backward stepwise selection procedure to reduce collinearity between environmental predictors. We removed variables one at a time until all remaining variables in the model exhibited VIFs less than 10. We subsequently removed one variable from each pair of variables with a Pearson's correlation higher than 0.7, retaining the variable that minimized correlations with the remaining variables. To mitigate the influence of neutral population structure, we estimated a population allele frequency covariance matrix using the program BayPass (Gautier 2015). This matrix was reduced to XY coordinates using nonmetric multidimensional scaling implemented in the R package *MASS*

Table 1 Details of localities and sample sizes for southern pygmy perch in the Murray–Darling Basin, Australia.

Catchment	Site	Location	*N*	Latitude	Longitude	Year sampled
Inman (INM)	INM	Inman R	7	−35.538	138.507	2018, 2019
Tookayerta (TOO)	TOO	Tookayerta Ck, Black Swamp	24	−35.403	138.777	2000, 2018, 2019
Lower Lakes (LL)	ALE	Milang Drain, L. Alexandrina	9	−35.395	139.008	2000, 2008
	FIW	Finniss R, waterfalls	2	−35.351	138.781	2004, 2010
	FRI	Finniss R, Ashbourne	2	−35.324	138.717	2000, 2011
	MID	Mundoo Is., L. Alexandrina	7	−35.549	138.915	2006
	MUN	Drain off Mundoo Channel	6	−35.520	138.904	2003
	HI*	Hindmarsh Island	19	−35.526	138.899	2008,
			145	−35.526	138.899	2013, 2014, 2015, 2017, 2018, 2019
Angas (ANG)	AMI	Angas R., Strathalbyn	10	−35.252	138.891	2000, 2010, 2018, 2019
	MCM	Middle Ck	9	−35.250	138.887	2001
Avoca (AVO)	MIC	Trib to Middle Ck, Warrenmang	12	−37.028	143.338	1999
Campaspe (CAM)	JHA	Jews Harp Ck, Sidonia	13	−37.139	144.578	2000
Upper Goulburn (UGO)	MER	Merton Ck	17	−36.981	145.725	2009
	TRA	Trawool Ck	10	−37.135	145.193	2002
	YEA	Yea R., Yea	7	−37.213	145.414	1992
Lower Goulburn (LGO)	PRA	Pranjip Ck	9	−36.623	145.309	2002
	SEV	Trib to Seven Creeks	12	−36.875	145.701	Unknown
Broken (BRO)	BEN	Swanpool Ck, Swanpool	10	−36.723	146.022	1994
	SAM	Sam Ck	10	−36.661	146.152	2009
	LIM	Unnamed Ck, Lima South	17	−36.827	146.006	2009
Ovens (OVE)	KIN	King R., Cheshunt	15	−36.802	146.424	2009
	HAP	Happy Valley Ck	10	−36.579	146.824	2009
	MEA	Meadow Ck, Moyhu	8	−36.573	146.423	1999
Kiewa (KIE)	GAP	Gap Ck, Kergunyah	12	−36.317	147.022	1999, 2009
Murray Riverina (MUR)	ALB	Murray R. lagoon, Albury	12	−36.098	146.928	1994
Mitta Mitta (MIT)	SPR	Spring Ck	10	−36.499	147.349	2009
	GLE	Glencoe Ck	11	−36.393	147.221	2009
	TAL	Tallangatta Ck	8	−36.281	147.382	2009
Upper Murray (UMR)	COP	Coppabella Ck	16	−35.746	147.729	1999
Lachlan (LAC)	LRT	Blakney Ck	8	−34.657	149.032	2003

*Individuals from Hindmarsh Island sampled between 2013 and 2019 represent the reintroduced Lower Lakes population, whereas all other Lower Lakes samples belong to the original wild-caught population prior to extirpation.

(Venables and Ripley 2002), and the RDA was conditioned on these coordinates. We assessed the significance of the RDA model using the *anova.cca* function of *vegan*, with 1,000 permutations. Candidate adaptive loci were identified as those with scores more than three SDs from the mean locus scores on the first RDA axis.

Genomic vulnerability to climate change

We used gradient forest, a regression tree-based machine-learning algorithm (Ellis et al. 2012), to estimate genomic vulnerability to climate change for the southern pygmy perch. Using the R package *gradientForest*, we first modeled the spatial turnover of allele frequencies to identify which environmental variables are important in driving genetic differentiation between populations. Our inputs were sampling site level allele frequencies for candidate loci identified by the RDA, and the same environmental variables used in the RDA. We then used the *predict.gradientforest* function to transform the environmental variables into "genetic importance values," which reflect their relationship with allele frequency turnover. Genomic vulnerability was estimated as the Euclidean distance between current and future predicted genetic importance values (Fitzpatrick and Keller 2015). For the future predictions, elevation was excluded from the model because it will remain constant. We assessed genomic vulnerability for three future time points: 2050, 2070, and 2090. For each time point, we tested four Shared Socioeconomic Pathways (SSPs): SSP126 (best-case scenario), SSP245 (business-as-usual), SSP370 (intermediate scenario), SSP585 (worst-case scenario). Genomic vulnerability was spatially interpolated across the MDB using inverse distance weighting (*idw* function) from the R package *gstat* (Pebesma 2004; Gräler et al. 2016) and smoothed with a thin plate spline (*Tps* function) from the R package *fields* (Nychka et al. 2021). To investigate if the Millennium Drought extirpation and subsequent reintroduction of the Lower Lakes population might have impacted patterns of genomic vulnerability, we repeated the RDA and gradient forest with samples from either the original or reintroduced Lower Lakes population.

To explore whether genomic vulnerability could be partially explained by elevation, Ho, or their combined effects, we fitted three generalized linear models (GLMs) and performed an ANOVA

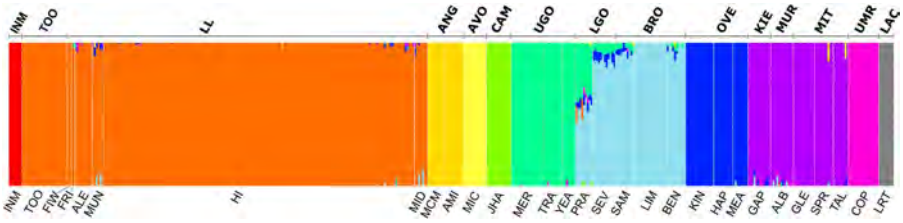

Fig. 2 fastStructure results based on 7,832 SNPs for southern pygmy perch in the Murray–Darling Basin, Australia. Depicted is $K = 11$ clusters, which are largely delineated by river catchments. Codes above and below the plot refer to catchment and site names, respectively (Table 1).

to compare the proportion of deviance explained by each model using the R package *stats* (R Core Team 2023). Additionally, we performed a GLM to test the relationship between Ho and elevation.

Results

Sampling and genomic data

After applying our quality filtering, 7,851 SNPs remained in the final data set. We removed 54 individuals that had >50% missing data. For the 467 individuals retained, the average missing data was 10% (0.03% to 50%).

Population structure and genetic diversity

We found high levels of population structure in southern pygmy perch. Our fastStructure analysis revealed 11 genetic clusters, which are mainly delineated by river catchments (Fig. 2). Exceptions to this pattern include the clustering of Tookayerta Creek individuals with those from the Lower Lakes, and the clustering of sites from Kiewa, Mitta Mitta, and Murray Riverina (Fig. 2). Sites in the Upper Goulburn formed a separate group to those of the Lower Goulburn, which clustered with the neighboring Broken River (Fig. 2). One site (PRA) in the Lower Goulburn showed admixture with the Upper Goulburn (Fig. 2). Our PCA supports the fastStructure results, with the first two axes accounting for 18.93% of the total variation and capturing the major catchment-level population structure (Supplementary Fig. S1). Pairwise F_{ST} between sampling sites ranged from 0 to 0.73, with a global F_{ST} of 0.44 (Supplementary Fig. S2). All pairwise F_{ST} comparisons were significant except between two Lower Lakes sites (MID and MUN; $F_{ST} = 0$, $P = 0.67$). Pairwise F_{ST} between fastStructure clusters ranged from 0.148 to 0.697 (Supplementary Fig. S3). Genetic diversity was generally low; for sampling sites, Ho ranged from 0.035 to 0.145, He ranged from 0.038 to 0.155, and F_{IS} ranged from −0.026 to 0.257 (Table 2). Individual level inbreeding was generally high, with an average of 0.124, ranging from −0.692 to 0.739 (Supplementary Fig. S4). For fastStructure clusters, Ho ranged from 0.035 to 0.138, He from 0.038 to 0.153, and F_{IS} from −0.011 to 0.188 (Supplementary Table S1).

Table 2 Diversity statistics for southern pygmy perch at the level of sampling sites based on 7,832 SNPs.

Catchment	Site	Ho	He	F_{IS}
Inman (INM)	INM	0.059	0.059	−0.011
Tookayerta (TOO)	TOO	0.078	0.103	0.202
Lower Lakes (LL)	ALE	0.098	0.148	0.257
	MID	0.097	0.145	0.234
	MUN	0.11	0.147	0.177
	HI*	0.145	0.155	0.056
Angas (ANG)	AMI	0.065	0.063	−0.026
	MCM	0.06	0.064	0.035
Avoca (AVO)	MIC	0.066	0.066	0.002
Campaspe (CAM)	JHA	0.057	0.059	0.021
Upper Goulburn (UGO)	MER	0.051	0.052	0.018
	TRA	0.049	0.051	0.028
	YEA	0.051	0.056	0.066
Lower Goulburn (LGO)	PRA	0.098	0.125	0.159
	SEV	0.103	0.118	0.092
Broken (BRO)	BEN	0.105	0.123	0.11
	SAM	0.115	0.127	0.07
	LIM	0.062	0.069	0.079
Ovens (OVE)	KIN	0.06	0.068	0.102
	HAP	0.067	0.072	0.041
	MEA	0.088	0.096	0.05
Kiewa (KIE)	GAP	0.101	0.107	0.047
Murray Riverina (MUR)	ALB	0.114	0.131	0.104
Mitta Mitta (MIT)	SPR	0.085	0.095	0.078
	GLE	0.079	0.088	0.081
	TAL	0.089	0.096	0.054
Upper Murray (UMR)	COP	0.074	0.078	0.051
Lachlan (LAC)	LRT	0.035	0.038	0.056

Ho, observed heterozygosity; He, expected heterozygosity; F_{IS}, population inbreeding coefficient.

Genotype–environment association analysis

After VIF analysis and accounting for collinearity, five environmental variables were retained for the RDA. These were maximum temperature of the warmest month (bio05), mean temperature of the driest quarter (bio09), precipitation seasonality (bio15), precipitation of the warmest quarter (i.e. summer rain, bio18), and elevation. The RDA model that included the reintroduced Lower Lakes population was significant ($P = 0.015$), with the environmental data explaining 29% of the variation in allele frequencies (Fig. 3). We identified 135 candidate adaptive SNPs with RDA locus scores outside 3 SDs of the mean. The RDA that included individuals from the original Lower Lakes population returned similar results, with 123 candidate adaptive SNPs identified (Supplementary Fig. S5).

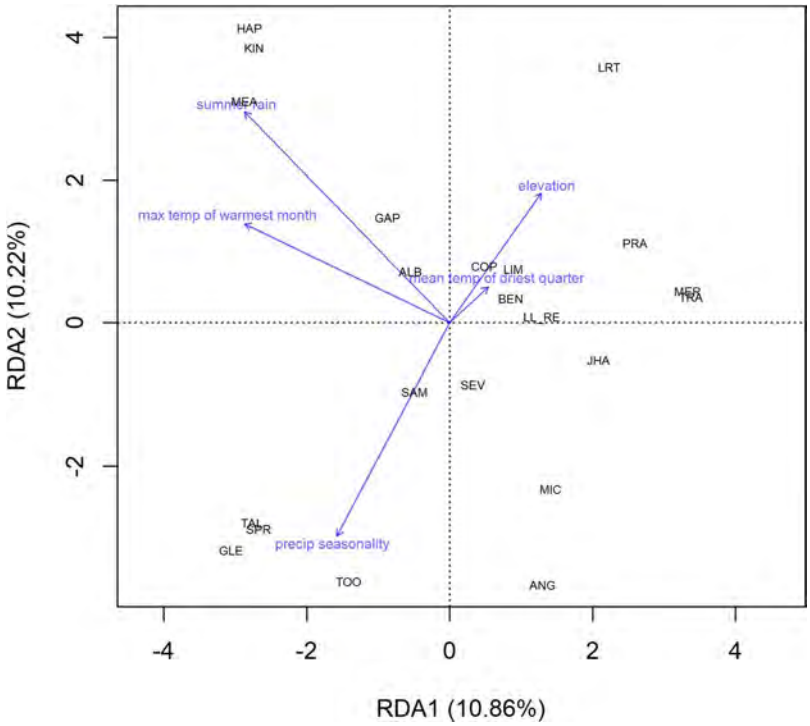

Fig. 3 Redundancy analysis (RDA) showing genotype–environment associations for southern pygmy perch in the Murray–Darling Basin. Based on sampling site level allele frequencies at 7,832 SNPs and five uncorrelated environmental variables. The model was conditioned on a population allele frequency covariance matrix calculated with BayPass. Codes for sampling sites are provided in Table 1.

Genomic vulnerability to climate change

Summer rain was identified as the most important environmental predictor of allele frequency turnover in the gradient forest model (Fig. 4). Our projections of genomic vulnerability indicate that future mismatches in GEAs will vary substantially among southern pygmy perch populations. Because genomic vulnerability estimates are dimensionless and represent relative projected genotype–environment mismatch, we interpret these values comparatively rather than as absolute measures of vulnerability. Relatively high genomic vulnerability was projected for the Avoca, Campaspe, Upper Murray, and Lachlan catchments, alongside certain sites in the Lower Goulburn (SEV), Broken (LIM), and Ovens (KIN) catchments. The lowest genomic vulnerability was projected for the Lower Lakes, Angas, and Tookayerta catchments. These patterns were consistent across different future time points and SSPs (Fig. 5). Results were also similar regardless of whether the original or reintroduced Lower Lakes population was used, suggesting that adaptive potential was maintained throughout the captive breeding program during the Millennium Drought (Supplementary Fig. S6). Elevation was a reasonable predictor of genomic vulnerability ($R^2 = 0.479$). We found a weaker negative linear relationship between genomic vulnerability and Ho ($R^2 = 0.159$) (Fig. 6, Supplementary Tables S2 and S3) and a moderate negative relationship between Ho and elevation ($R^2 = 0.305$) (Supplementary Table S2). The combined effect of Ho and elevation explained a similar proportion of the variation in genomic vulnerability as the model with elevation alone ($R^2 = 0.479$) (Supplementary Tables S2 and S3). Our ANOVA revealed that the addition of elevation to the model with heterozygosity significantly reduced the residual deviance by 0.348 ($P = 0.00063$) (Supplementary Table S3).

Discussion

Freshwater fishes are one of the most threatened animal groups across the globe (Reid et al. 2019; Garnett et al. 2022; Lintermans et al. 2024; Sayer et al. 2025), yet remain understudied in terms of climate adaptation. We used genome-wide SNPs, environmental data, and statistical modeling to investigate genomic vulnerability to climate change in a threatened, small-bodied freshwater fish, the southern pygmy perch. We confirm that this poorly dispersing species exhibits low genetic diversity and high population isolation across its fragmented range in the MDB. Gradient forest models revealed that upland populations will require larger allele frequency changes to maintain current GEAs under future climates compared with lowland populations. Populations in the lowest elevation catchments have relatively low genomic vulnerability, representing a potentially important genetic refuge for the species under climate change. Additionally, capitalizing on a rare opportunity to include samples from both before and after a conservation reintroduction, we show that genetically informed captive breeding and reintroduction programs can maintain climatic adaptive potential in imperiled populations.

Fragmented populations with low genetic diversity

Our results corroborate previous findings that southern pygmy perch exist in a series of genetically differentiated and largely isolated populations throughout the MDB (Brauer et al. 2016; Cole et al. 2016). Given the low dispersal potential of the species (Dexter et al. 2014), it is unsurprising that nearly every river catchment supports a distinct genetic cluster. The genetic

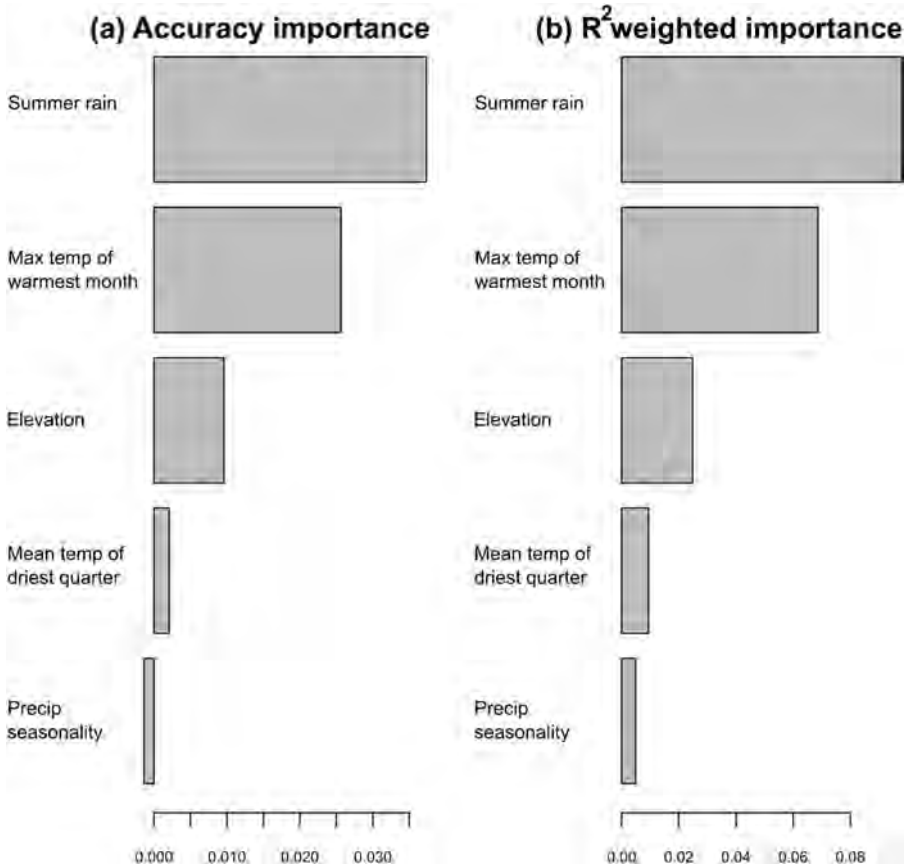

Fig. 4 Accuracy and R^2-weighted importance of environmental predictors in explaining allele frequency turnover across southern pygmy perch populations in the gradient forest model.

structure of southern pygmy perch follows expectations of the Stream Hierarchical Model (Meffe and Vrijenhoek 1988), with greater population differentiation among catchments than between sites within catchments (Supplementary Fig. S2). We observed overall low levels of genetic diversity, consistent with earlier studies based on mtDNA, microsatellites, and SNPs (Cook et al. 2007; Brauer et al. 2016; Cole et al. 2016). These values are especially low when compared with more dispersive and largely codistributed fishes such as golden perch (*Macquaria ambigua*) (Booth et al. 2024), Murray cod (*Maccullochella peelii*) (Harrisson et al. 2017), and Murray River rainbowfish (*Melanotaenia fluviatilis*) (Brauer et al. 2018). The Australian landscape is known for having extreme temporal variability in hydrological regimes, with many native terrestrial and freshwater species adapted to "boom-bust" population cycles (Balcombe and Arthington 2009; Arthington and Balcombe 2011; Attard et al. 2022; Stringer et al. 2024). Although this natural process can lead to low levels of population genetic diversity during bust phases, given sufficient habitat connectivity, genetic diversity can be recovered through gene flow during boom phases (Attard et al. 2022; Stringer et al. 2024). Habitat fragmentation arising from the construction of dams and weirs, water diversion to agriculture, and general habitat modification has contributed to recent isolation and loss of genetic diversity of southern pygmy perch (Cole et al. 2016; Brauer and Beheregaray 2020), and other threatened Australian fishes (Brauer et al. 2013; Pavlova et al. 2017). Coalescent simulations have indicated that the majority of divergence among southern pygmy perch populations between the upper and lower MDB likely occurred after European colonization, and that populations were much larger, had a greater spatial distribution, and were more connected historically (Cole et al. 2016; Buckley et al. 2021).

A common paradigm seen in riverine species is greater genetic diversity in lowland compared with upland populations (Paz-Vinas et al. 2015). Three main processes are predicted to contribute to this pattern: 1) downstream-biased dispersal driven by the unidirectional flow of water, 2) greater habitat variability downstream, supporting larger and more stable populations, and 3) upstream-directed colonization (Paz-Vinas et al. 2015). Although downstream-biased dispersal is often assumed the most important factor, simulations have shown that a combination of these processes is more likely to produce greater genetic diversity downstream (Paz-Vinas et al. 2015). We observed that populations of southern pygmy perch at lower elevations tend to have greater genetic diversity than those upland. Notably, we found that the Lower Lakes region contains the most genetically diverse population of southern pygmy perch, even though this population went through a period of ex situ captive breeding and reintroduction during the Millennium Drought. This population is known to have a larger effective size compared with those in many other catchments (Brauer and Beheregaray 2020) and this is supported by estimates of high adaptive genomic variation (Brauer et al. 2016) and adaptive plasticity (Brauer et al. 2017) for this population. More broadly, the Lower Murray has been shown to harbor more genetically diverse populations than

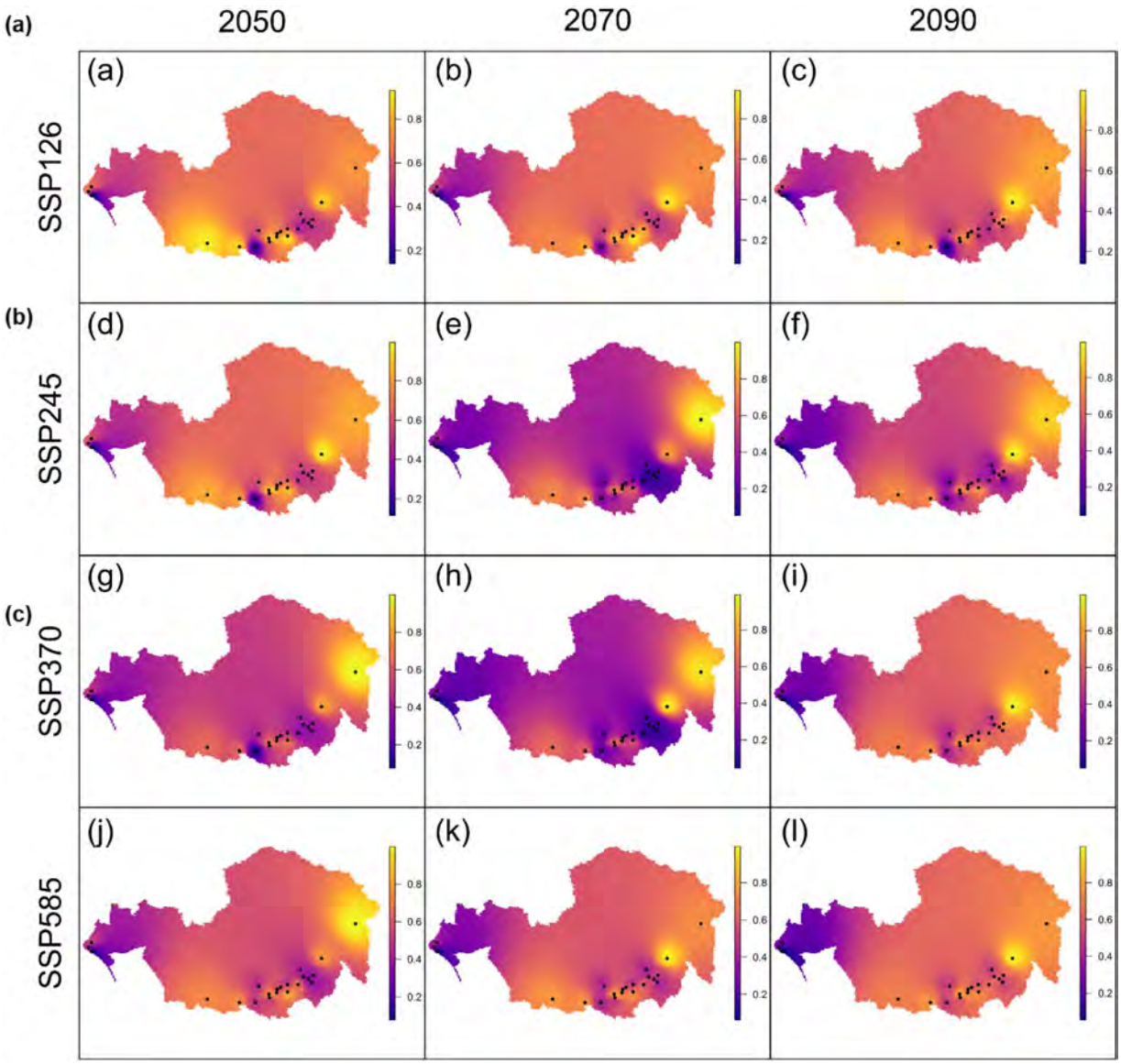

Fig. 5 Genomic vulnerability to climate change estimated for southern pygmy perch in the Murray–Darling Basin, Australia. The gradient forest model was based on 135 candidate adaptive loci. Black dots represent sampling sites. Warmer colors indicate areas with greater projected genotype–environment association mismatches in the future. Estimated for three future time points (2050, 2070, and 2090) and four Shared Socioeconomic Pathways (SSP126, SSP245, SSP370, SSP585).

upstream sites in other fish species (Sasaki et al. 2016; Brauer et al. 2018; Thiele et al. 2020). This suggests that the wetland habitats currently available in the Lower Lakes are vital for maintaining stronghold populations, especially in the face of physical vulnerability to extirpation due to cumulative catchment change as has been recently witnessed (Hammer et al. 2013; Marshall et al. 2022). Nevertheless, upstream populations may also play an important role in maintaining genetic diversity across the metapopulation. Even where standing genetic diversity is lower, upper-reach populations likely act as sources of occasional downstream migrants, and low levels of gene flow can reduce the loss of genetic diversity through drift in recipient populations. This highlights that conserving populations across the broader elevational gradient is important, not only for protecting local genetic variation, but also for maintaining the metapopulation processes that sustain genetic diversity in lower-reach populations.

Genomic vulnerability to climate change

Understanding how vulnerability to climate change might vary among populations is crucial for conservation planning and prioritization (Bay et al. 2018). Our finding that headwater populations of southern pygmy perch are most susceptible to climate change highlights the strength of genomic vulnerability analyses for clarifying spatial variation in complex interactions between genetic diversity, environmental variation, and climate predictions. In freshwater settings, climate regime is a key determinant of habitat quality, with temperature and precipitation influencing elements such as streamflow, nutrient content, and dissolved oxygen concentrations (Carpenter et al. 2011; Knouft and Ficklin 2017). As ectotherms, fishes rely on the thermal conditions of ambient water to regulate their body temperature (Agarwal et al. 2024). Thus, increases in water temperature under

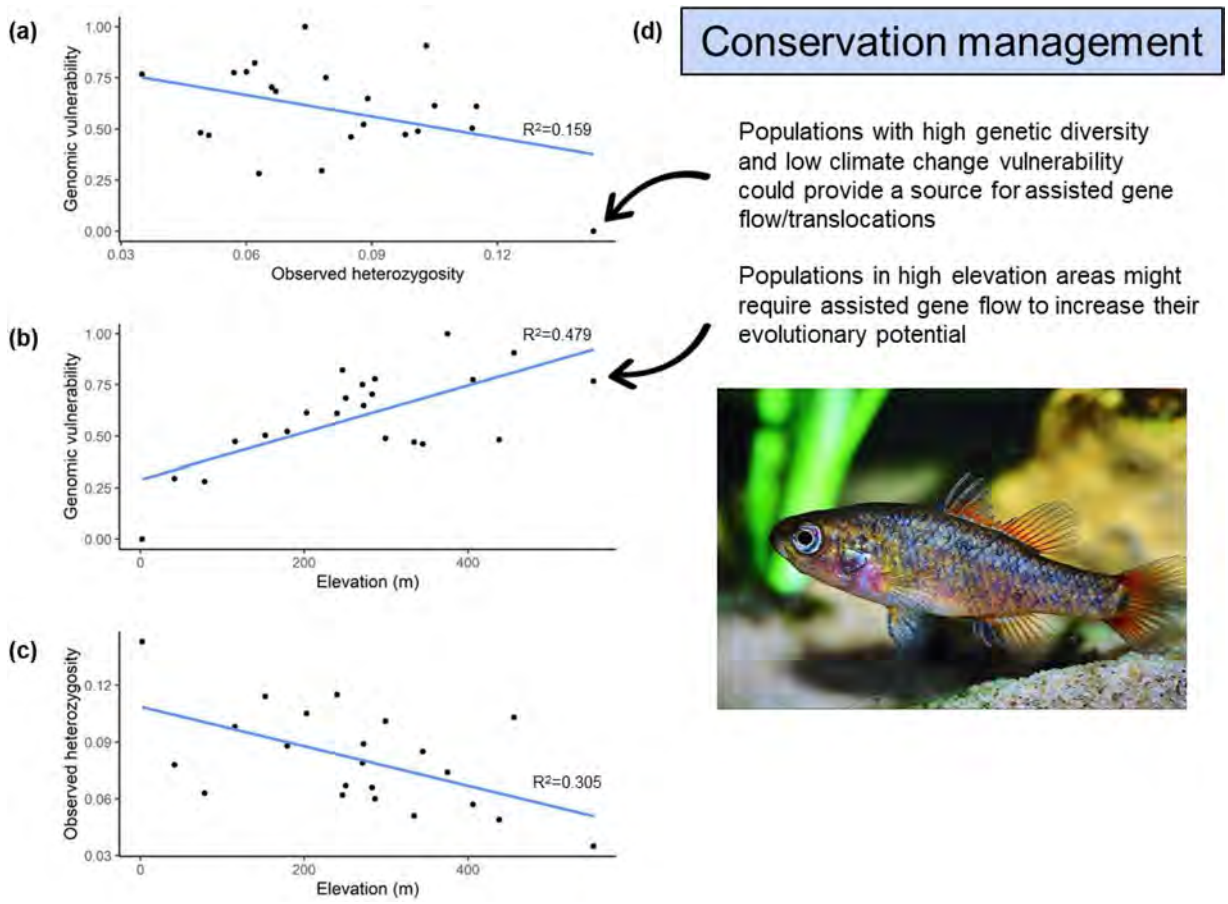

Fig. 6 Genomic vulnerability projected for the worst-case climate change scenario (2090, SSP585), plotted against (a) observed heterozygosity and (b) elevation. (c) Relationship between observed heterozygosity and elevation. (d) Simplified hypothetical conservation management framework and general recommendations for southern pygmy perch. Photo credit: Michael P. Hammer.

climate change could have implications for many physiological processes that affect fitness, including immune responses, growth, and fertilization (Agarwal et al. 2024). The MDB has considerable spatial and temporal variability in temperature and precipitation, which drives adaptive divergence in many fish species (Harrisson et al. 2017; Attard et al. 2018; Brauer et al. 2018). This includes the southern pygmy perch, which appear particularly sensitive to predictability of seasonal rainfall and variation in temperature extremes (Morrongiello et al. 2010; Morrongiello et al. 2012, 2013; Brauer et al. 2016). For example, southern pygmy perch exhibit spatial variability in reproductive bet-hedging strategies, with those in more hydrologically unpredictable environments producing more and smaller eggs (Morrongiello et al. 2012). The species also displays variation in traits associated with other hydrological properties (e.g. light and leachate concentration), which could have an adaptive or plastic basis (Morrongiello et al. 2010; Morrongiello et al. 2013). Climate projections indicate that the southern MDB will experience higher temperatures, lower winter rainfall, and declines in streamflow in the future (Whetton and Chiew 2021). Furthermore, climate variability is set to increase, including more frequent and intense droughts (Whetton and Chiew 2021). Summer rain and maximum temperature of the warmest month were the top two predictors of allele frequency turnover in our gradient forest model, and we expect that changes in these climatic factors will affect southern pygmy perch populations (Fig. 4). We found that populations in the Avoca, Campaspe, Goulburn, Broken, Ovens, Upper Murray, and Lachlan catchments have the highest genomic vulnerability to climate change (Fig. 5). On the other hand, populations in the Lower Lakes, Tookayerta, and Angas catchments have low genomic vulnerability and will likely be the most resilient under future conditions (Fig. 5), provided that their habitats and metapopulations can be physically protected as drying conditions threaten the persistence of their habitat.

Genomic vulnerability of the Lower Lakes population was low in all our models, whether based on the original population (prior to extirpation during the Millennium Drought) or the reintroduced population (re-established after a generation of ex situ captive breeding). This suggests that genetically informed captive breeding and reintroduction programs can successfully maintain climate resilience in small populations. The Lower Lakes region experiences lower annual rainfall and warmer winter temperatures than elsewhere in the MDB, and as such this population might possess traits that will be beneficial under climate change.

Elevation as a proxy for climate change vulnerability

Up to one-quarter of freshwater species worldwide, and more than one-third of Australia's freshwater fishes, are currently threatened

with extinction, and climate change is recognized as a key stressor (Lintermans et al. 2024; Sayer et al. 2025). Given the magnitude of conservation efforts required, establishing which spatial features of river systems could serve as proxies for climate change vulnerability would be valuable for guiding the prioritization of limited management resources. We found that elevation was a good predictor of genomic vulnerability (Supplementary Figs S2 and S3), with populations at upland sites projected to experience larger genotype-environment mismatches under future climates than those at lowland sites.

A portion of our elevation-related pattern of genomic vulnerability can be explained by genetic diversity, which is lower in the more vulnerable upland sites. Standing genetic variation is fundamental for climate change resilience as it provides the raw material for adaptation. Southern pygmy perch populations in lowland sites of the MDB, particularly the Lower Lakes, are likely more resilient to climate change due to having higher levels of standing genetic variation. Layton et al. (2021) similarly found a strong negative correlation between nucleotide diversity and genomic vulnerability in another fish species, Arctic charr (*Salvelinus alpinus*). Given that genetic diversity often follows an elevational pattern in freshwater species (Paz-Vinas et al. 2015), we suggest that elevation could potentially serve as a useful proxy for climate change vulnerability in these systems.

Implications for conservation management, study limitations, and future directions

As anthropogenic activities continue exerting pressure on biodiversity, captive breeding and reintroduction programs are becoming increasingly vital for the conservation of threatened species (Harding et al. 2016). Such programs have played a major role in the conservation of small-bodied fishes facing environmental stress and habitat loss in the MDB (Hammer et al. 2013). For southern pygmy perch, this includes the re-establishment of the Lower Lakes population after extirpation during the Millennium Drought, as well as the restoration of populations at previously extirpated sites within the Campaspe and Avoca catchments (Attard et al. 2016; Marshall et al. 2022; Buckley et al. 2024). A limitation of captive breeding is that genetic diversity can be readily lost through founder effects, inbreeding, genetic drift, and adaptation to captivity (Fraser 2008). The captive breeding program for southern pygmy perch utilized genetic data to establish breeding groups that minimized inbreeding and relatedness (Attard et al. 2016). This resulted in the reintroduced population retaining similar levels of genetic diversity to the original population (Marshall et al. 2022; Buckley et al. 2024). Although minimizing inbreeding and maintaining genetic diversity are common goals in captive breeding programs, few empirical studies have demonstrated that such efforts can also preserve adaptive potential under climate change. Our finding of low genomic vulnerability in the reintroduced Lower Lakes population demonstrates that careful genomic management of captive populations can indeed help to maintain adaptive capacity. With climate change expected to exacerbate the frequency and intensity of droughts in the MDB and many other parts of the world, captive breeding and reintroductions will continue to be important strategies for conserving vulnerable freshwater populations.

In river systems, populations are becoming increasingly fragmented due to instream barriers (e.g. dams, weirs, culverts), altered flow regimes, and areas of poor water quality (Barbarossa et al. 2020; Brauer and Beheregaray 2020; Watson et al. 2024). This fragmentation restricts the movement of fish and is leading to increased isolation of populations (Brauer et al. 2013; Pavlova et al. 2017; Rosenthal et al. 2024). Small and isolated populations are particularly prone to stochastic processes that can erode their genetic diversity, and ultimately, their adaptive potential to cope with environmental change (Frankham 2005). Assisted gene flow is a management strategy that has been successful in improving the long-term viability of small and imperiled populations across many taxa, with outcomes such as reductions in inbreeding and inbreeding depression, increases in fitness, and demographic rescue (Weeks et al. 2017; Onorato et al. 2024). Although there are issues that can potentially arise from assisted gene flow, such as outbreeding depression, swamping of locally adapted alleles, and spread of disease, these risks are likely outweighed by the benefits (Frankham 2015; Liddell et al. 2021). This is especially the case when considered against the risks of doing nothing (i.e. maintaining small, increasingly inbred populations in isolation) (Weeks et al. 2017; Pavlova et al. 2025). Genomic vulnerability models are practical for identifying populations where assisted gene flow could help enhance resilience to climate change (Borrell et al. 2020; Rhone et al. 2020). Southern pygmy perch currently exist in small, genetically depauperate and isolated populations in the higher elevation and headwater streams in the MDB. Freshwater fishes in this region have already been severely impacted by climate change (Silva et al. 2020), and populations are expected to decline more in the coming decades (de Oliveira et al. 2019). We advocate for the increased use of proactive genetic management tools such as assisted gene flow for southern pygmy perch and other freshwater species, especially those with poor dispersal abilities. Based on our results and a large body of previous work (Attard et al. 2016; Brauer et al. 2016; Cole et al. 2016; Marshall et al. 2022; Buckley et al. 2024), the more diverse and less genomically vulnerable Lower Lakes, Angas, and Tookayerta populations appear as ideal source populations for the translocations of individuals into the more vulnerable upland sites, including reintroduction sites matched to future climate suitability. In the MDB, assisted gene flow is already being applied in the conservation of Macquarie perch (*Macquaria australasica*), with hopes to restore genetic diversity in a small, inbred population (Pavlova et al. 2025). Reinstating natural connectivity among populations through more environmentally focused water management would also be important for the conservation of southern pygmy perch and many other aquatic species.

There is ongoing debate in the literature about the appropriateness of using genomic vulnerability models to guide conservation management (Lind et al. 2024; Lotterhos 2024). This is because there are a number of assumptions underlying genomic vulnerability models that might not necessarily hold true in empirical systems (Lotterhos 2024). Two core assumptions are that populations are currently locally adapted to their environment and that genomic vulnerability estimates therefore represent a decline in population fitness (Lotterhos 2024). In the case of southern pygmy perch, these assumptions may be violated given the small population sizes. In small populations, strong genetic drift can drag allele frequencies away from the local optimum (Brady et al. 2019). Our results might therefore underestimate genomic vulnerability

if populations are already experiencing some maladaptation to their local environments. There is experimental evidence from plant studies that genomic vulnerability models based on random or all SNPs can sometimes provide a better or similar prediction of fitness compared with models based on adaptive SNPs (Capblancq and Forester 2021; Fitzpatrick et al. 2021; Lachmuth et al. 2023; Lind et al. 2024). We chose to use GEA candidate loci to inform our genomic vulnerability models, as we assume these SNPs are more likely to be relevant to climate adaptation than a random selection of SNPs. Despite these limitations, the overall conservation implications derived from our genomic vulnerability models (e.g. the use of the Lower Lakes population as a source for translocations) support the recommendations that would be given based on the population genomic data alone. Even without the climate projections, it is clear that the less genetically diverse upland populations require conservation attention, and that the Lower Lakes population is a good source for genetic diversity, which requires a strong on-ground management focus to continue to serve this conservation potential. We argue that our genomic vulnerability models provide more clarity about which populations are most under threat from climate change and are an important addition to the conservation framework.

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Author contributions

Emily J. Booth (Formal analysis, Writing—original draft, Writing—review & editing), Chris J. Brauer (Data curation, Formal analysis, Investigation, Methodology, Writing—review & editing), Jonathan Sandoval-Castillo (Data curation, Formal analysis, Investigation, Methodology, Writing—review & editing), Scotte D. Wedderburn (Investigation, Methodology, Resources, Writing—review & editing), Nick S. Whiterod (Investigation, Methodology), Peter J. Unmack (Investigation, Methodology, Resources), Michael P. Hammer (Investigation, Methodology, Resources, Writing—review & editing), and Luciano B. Beheregaray (Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing)

Supplementary material

Supplementary material is available at *Journal of Heredity* online.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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