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Article

Riverscape genetics of nonnative Brook Trout to inform native cutthroat trout conservation

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ABSTRACT

Objective: Understanding how riverscape features influence gene flow is critical for managing population connectivity in freshwater species. We examined how landscape and stream characteristics shape the spatial genetic structure of nonnative Brook Trout *Salvelinus fontinalis* in a headwater stream network proposed for reintroduction of federally threatened Greenback Cutthroat Trout *Oncorhynchus virginalis stomias*. Brook Trout were studied to evaluate the suitability of this habitat for supporting a native trout metapopulation.

Methods: We genotyped 757 Brook Trout from 22 sites across a 60-km stream network using 12 microsatellite loci. Spatial genetic structure was assessed using clustering analysis (program STRUCTURE) and pairwise differentiation metrics (F_{ST} and Jost's D). A spatial network modeling approach was used to quantify the effects of riverscape features (e.g., stream gradient, stream order, waterfalls, and flow direction) on trout gene flow.

Results: Genetic clustering identified four distinct tributary groups, while estimates of pairwise genetic differentiation indicated some genetic connectivity across the network (mean $F_{ST} = 0.04$; mean Jost's $D = 0.06$). Trout gene flow was impeded by waterfalls, steep stream gradients, and increased hydrologic distance. Higher stream order and downstream flow direction were associated with stronger gene flow, and stream segments containing waterfalls and steeper gradients showed greater asymmetries between upstream and downstream gene flow.

Conclusions: Brook Trout populations in this stream network are spatially structured, but gene flow persists and is mediated by physical riverscape features and hydrologic distance. The observed patterns of genetic connectivity suggest that this habitat can support connectivity among populations of reintroduced Greenback Cutthroat Trout. In future native trout reintroduction efforts, prioritizing habitats with gradual stream gradients and fewer waterfalls would promote population connectivity.

KEYWORDS: fish movement, gene flow, native trout, riverscape genetics, spatially structured populations

LAY SUMMARY

Riverscape features influence patterns of connectivity among invasive Brook Trout populations in a future native trout reintroduction area. Current levels of gene flow suggest that this habitat could support connected populations of reintroduced native trout.

INTRODUCTION

There is a broad consensus among conservation biologists that population connectivity promotes resilience and viability in many species (Allendorf et al., 2022; Christie & Knowles, 2015; Correa Ayram et al., 2016; Hodgson et al., 2011; Keeley et al., 2018; Shao et al., 2019). In freshwater systems, genetic connectivity is shaped by numerous factors, including habitat heterogeneity, species life history, and the presence of natural or

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anthropogenic barriers to movement (Davis et al., 2018). Gene flow of riverine species is further influenced by the linear structures of stream networks, where dendritic architectures and asymmetric streamflow create patterns of isolation and connectivity based on network positioning. For instance, upstream ends of network branches are often genetically isolated, while regions farther downstream near network confluences experience greater connectivity (Thomaz et al., 2016; White et al., 2020). The field of riverscape genetics seeks to understand how riverscape characteristics influence gene flow and shape the genetic structure of aquatic populations (Davis et al., 2018). By identifying the effects of specific riverscape features on population connectivity, riverscape genetics can provide insights into the processes driving genetic diversity, local adaptation, and population viability (Balkenhol et al., 2015; Davis et al., 2018). This knowledge is crucial for managing genetic connectivity in stream networks to promote population resilience and adaptive potential, particularly in the face of ongoing habitat fragmentation and environmental change.

Riverscape genetics is particularly relevant for informing conservation strategies for native inland salmonid species, which have become increasingly confined to small headwater catchments due to habitat fragmentation and nonnative species introductions (Fausch et al., 2009; Gresswell, 2011; Roberts et al., 2017; Nordberg et al., 2021). Recovery efforts for these species often involve the reintroduction of native fish into headwater habitats that are devoid of nonnative competitors and isolated from invasion by barriers (Novinger & Rahel, 2003). Such efforts have successfully restored populations of several native trout species, including Apache Trout *Oncorhynchus apache* (U.S. Fish and Wildlife Service, 2009), Lahontan Cutthroat Trout *O. henshawi* (Lahontan Cutthroat Trout Coordinating Committee, 2019), Colorado River Cutthroat Trout *O. virginalis pleuriticus* (Young, 2008), and Greenback Cutthroat Trout *O. v. stomias* (Greenback Cutthroat Trout Recovery Team, 2019). However, reintroductions may be less successful when recovery habitats are small and fragmented (Harig et al., 2000; Hayes & Banish, 2017). Smaller habitats are less likely to have sufficient habitat complexity to provide resilience to stochastic disturbance events such as wildfires, floods, and droughts (Harig & Fausch, 2002), and isolation by barriers, while necessary to prevent encroachment by nonnative competitors, has the deleterious effect of preventing the demographic rescue of declining populations through immigration. Furthermore, low genetic diversity in available broodstocks of some native species could limit the success of reintroduction efforts due to inbreeding depression (Bell et al., 2024; Biermann & Havlick, 2021; Rogers et al., 2022). Thus, selecting reintroduction habitats with larger patch sizes and sufficient population connectivity could help buffer against genetic drift and improve the success of native fish recovery efforts (Dunham et al., 2014; Pennock et al., 2024).

Identifying suitable reintroduction areas for target species relies on understanding the factors that influence population connectivity in the species of interest. However, directly studying riverscape-scale connectivity may be impossible for narrowly distributed species for which occupied habitats of sufficient size do not exist (Wenger, 2008). For example, Greenback Cutthroat Trout, a salmonid native to Colorado's South Platte River basin, experienced a dramatic decline in the late 1800s due to factors including mining pollution, stream dewatering for agriculture, angler harvest, and introductions of nonnative salmonids (Harig et al., 2000; Young & Harig, 2001; Greenback Cutthroat Trout Recovery Team, 2019). As a result, Greenback Cutthroat Trout were reduced to a single population occupying 7 km of first-order stream by the early 2000s (Metcalf et al., 2012). Despite this near extinction, hatchery-reared individuals have since been reintroduced into fewer than 10 reclaimed habitat patches (Rogers et al., 2022), and managers aim to establish additional viable populations to promote redundancy (Greenback Cutthroat Trout Recovery Team, 2019). However, the habitat patches that harbor current extant Greenback Cutthroat Trout populations are small and geographically isolated, providing no opportunities to directly study the factors that influence riverscape-scale population connectivity in this species. In the absence of large, extant metapopulations of Greenback Cutthroat Trout, other salmonid species that are more widely distributed and have similar dispersal abilities can be studied as surrogate species to generate working hypotheses about factors influencing population connectivity in Greenback Cutthroat Trout. For instance, proposed reintroduction habitats for Greenback Cutthroat Trout are often occupied by nonnative Brook Trout *Salvelinus fontinalis*, which could be studied to help predict future patterns of genetic connectivity among reintroduced native trout.

The value of surrogate species in conservation has been debated because even closely related species may not respond identically to environmental stressors or management actions (Che-Castaldo & Neel, 2012; Henry et al., 2019; Reemeyer et al., 2024). A fundamental requirement of surrogate species approaches is that the surrogate exhibits sufficient similarity to the target species in the specific biological processes being examined (Lindenmayer & Likens, 2011). Despite differences in seasonal spawn timing, Brook Trout and Rocky Mountain Cutthroat Trout *O. virginalis* share key characteristics relevant to riverscape-scale population connectivity. The two species are morphologically similar and display overlapping ranges of swimming performance (Katopodis & Gervais, 2012) and similar swimming speeds among wild specimens (Castro-Santos et al., 2013; Blank et al., 2020). Additionally, upstream-biased spawning movements have been observed in western North American populations of both species (Peterson & Fausch, 2003; Young, 2011). Although differences in spawn timing could cause seasonal variation in how these species interact with certain riverscape features (e.g., due to differences in stream discharge during seasonal prespawn movements), available data suggest no strong evidence of substantial differences in movement capacity or dispersal potential between Brook Trout and Rocky Mountain Cutthroat Trout (Katopodis & Gervais, 2012; Castro-Santos et al., 2013; Blank et al., 2020). Thus, we consider Brook Trout a useful surrogate for predicting patterns of spatial genetic structure in reintroduced Greenback Cutthroat Trout and for understanding how physical habitat features influence genetic connectivity in native trout reintroduction areas.

In this study, we examined the spatial genetic structure of nonnative Brook Trout occupying a 60-km headwater stream network proposed for future reintroduction of Greenback

Cutthroat Trout. A long-term management goal is to establish a metapopulation of Greenback Cutthroat Trout in this watershed, but the degree of genetic connectivity across the stream network was unknown prior to our study. Using Brook Trout as a surrogate species, we studied salmonid gene flow throughout this riverscape to evaluate its potential to support a metapopulation of native trout. We applied a recently developed statistical framework for riverscape genetics (White et al., 2020) to quantify the effects of specific riverscape features on salmonid gene flow and estimate levels of migration throughout the network. This information is critical for assessing the feasibility of establishing a robust metapopulation of Greenback Cutthroat Trout in the reintroduction area. Our results can also inform the management of Brook Trout in their native range, where populations have declined due to habitat loss, climate change, and nonnative species introductions (Poplar-Jeffers et al., 2009; White, Rash, & Kazyak, 2023). Current native Brook Trout habitats are highly fragmented (Hudy et al., 2008; Torterotot et al., 2014; Whiteley et al., 2013), making it difficult to study riverscape-scale connectivity among native Brook Trout populations (but refer to Petty et al., 2012 and White et al., 2020). Thus, our study represents a rare opportunity to characterize the genetic structure of Brook Trout in a large, unfragmented habitat network.

METHODS

Study area

This study was conducted in a dendritic stream network comprising the uppermost portion of the Cache la Poudre River watershed in Colorado, located in Rocky Mountain National Park and Arapaho and Roosevelt National Forests. The network encompasses 60 km of headwater stream habitat that was historically occupied by Greenback Cutthroat Trout and is now targeted for reintroduction of this important conservation species. Major tributary streams to this section of the upper Cache la Poudre River include Corral Creek, Willow Creek, Hague Creek, and La Poudre Pass Creek (Figure 1). Long Draw Reservoir is an impoundment on La Poudre Pass Creek that was constructed for water supply in 1929, and the dam at the terminal end of the reservoir blocks upstream fish passage. Water release from the reservoir typically occurs between June and October and ceases in the autumn, causing the section of stream below the reservoir to experience a cycle of augmented flow during the summer and dewatering from November to May each year. Our study streams are occupied primarily by Brook Trout, with the occasional occurrence of hybridized nonnative cutthroat trout (e.g., Colorado River Cutthroat Trout × Yellowstone Cutthroat Trout *O. v. bouvieri*) of hatchery origin (Harris et al., 2022). According to historical stocking records, Brook Trout stocking occurred on the main stem of the Cache la Poudre River within and downstream of the study area beginning in 1892, with one single stocking event documented in the Hague Creek tributary (Colorado Parks and Wildlife, unpublished data; U.S. Fish and Wildlife Service, unpublished data). Available records show no evidence of Brook Trout stocking after 1955.

Field sampling and genotyping

Between July and October in 2018 and 2019, we collected Brook Trout genetic samples from 22 sites by backpack electrofishing (Figure 1; Table S1 [see online Supplementary Material]).

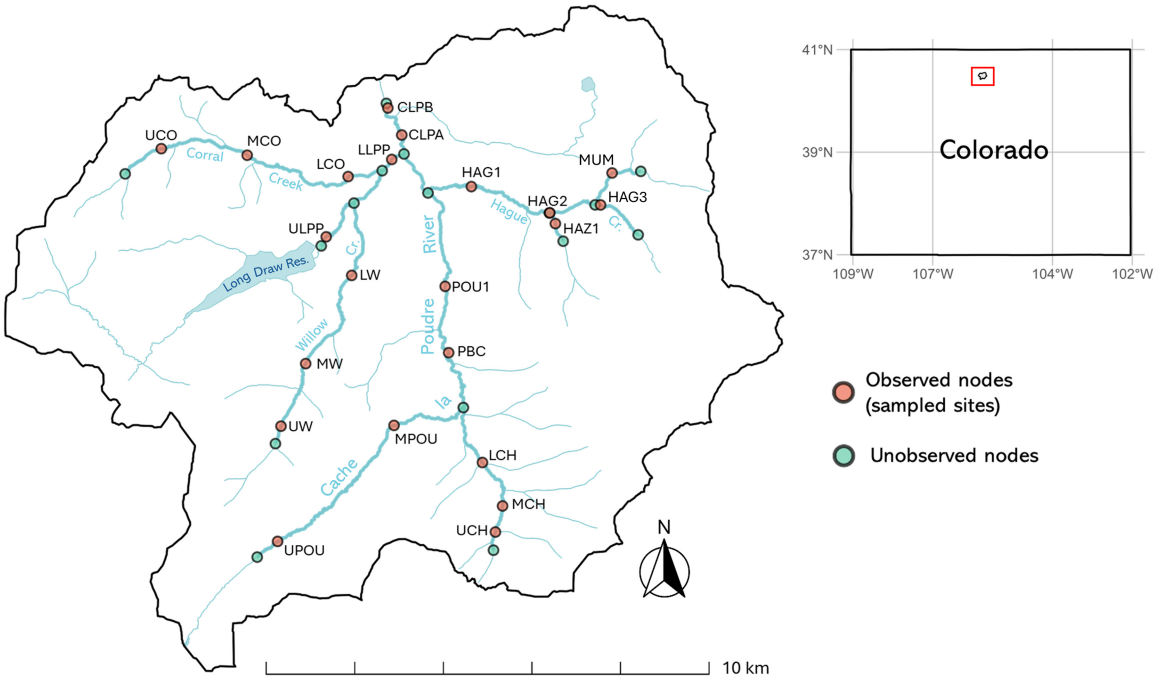

Figure 1. A map of the upper Cache la Poudre River watershed (flow direction: north) showing locations of nodes in the spatially structured ecological network. Observed nodes represent the locations of 22 Brook Trout sampling sites (refer to Table S1 [see online Supplementary Material] for definitions of site abbreviations). Unobserved nodes were placed at perennial tributary confluences and upstream of observed nodes.

Sampling sites were approximately 100 m in length. Multiple sites were sampled along each major tributary drainage to characterize potential within-tributary genetic variation and ensure broad spatial coverage across the network. All fish were measured for total length, and anal or caudal fin clips were collected before releasing fish alive to the site of capture. Fin clips were dried on chromatography paper and stored individually in coin envelopes. To minimize sampling of closely related individuals (Whiteley et al., 2012), we avoided collecting fin clips from age-0 individuals.

Brook Trout were genotyped at 12 microsatellite loci: *SfoC113*, *SfoC115*, *SfoC129*, *SfoC38*, *SfoC88*, *SfoD91*, *SfoB52*, *SfoC24*, *SfoC28*, *SfoC79*, *SfoC86*, and *SfoD75* (King et al., 2012). We randomly subsampled 35 individuals per site across size-classes for genetic analysis; if fewer than 35 individuals were captured, all individuals were genotyped (minimum sample size = 29). Prior to subsampling, length frequency histograms were visually inspected to ensure the exclusion of age-0 individuals. Genomic DNA was extracted from Brook Trout fin clips using Qiagen Dneasy Blood and Tissue Kits following the manufacturer's protocol. Microsatellite markers were amplified using polymerase chain reaction (PCR) in two 10-µL multiplexes, each containing 2 µL of genomic DNA, 5 µL of Qiagen Multiplex PCR Mastermix, 0.04–0.1 µL of each forward and reverse PCR primer (10 µM), and 2.18–2.2 µL of nuclease free water. The thermal profile for PCR amplification consisted of denaturing at 95°C for 15 min, 35 cycles of denaturation at 95°C for 45 s, annealing at 56°C for 45 s, and extension at 72°C for 2 min, followed by a final extension of 60°C for 30 min. Following amplification, PCR products were treated with a solution of formamide and GeneScan 600 LIZ size standard (Thermofisher Scientific) and visualized using an Applied Biosystems 3500 genetic analyzer. Alleles were scored using GeneMapper version 6.

Genetic diversity and spatial population structure

Because deviations from Hardy–Weinberg expectations can bias analyses of genetic structure and may indicate the presence of null alleles, genotyping errors, or underlying substructure (Hao & Storey, 2019; Pritchard et al., 2000; White et al., 2020), we tested for site-level deviations from Hardy–Weinberg equilibrium with 1,000 Monte Carlo permutations using the R package "pegas" (Paradis, 2010) and applied a Bonferroni correction for multiple comparisons across 264 tests in R (R Core Team, 2023). The R package "hierfstat" (Goudet, 2005) was used to calculate site-specific expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), and rarefied allelic richness (A; rarefied to 58 alleles). We measured genetic differentiation between all pairs of sampling sites using pairwise F_{ST} (Weir & Cockerham, 1984) and D (Jost, 2008) using the R packages "hierfstat" for F_{ST} and "mmod" (Winter, 2012) for D. The use of F_{ST}, which is widely used in landscape genetics (Davis et al., 2018; Funk et al., 2005; Kanno et al., 2011; White et al., 2020), quantifies genetic structure as a function of heterozygosity and nearness to fixation, whereas Jost's D provides a measure of actual allelic differentiation among populations (Jost, 2008; Jost et al., 2018). Thus, we opted to use both metrics to capture complementary aspects of population structure.

To investigate the spatial distribution of genetic groups throughout the stream network, we used the program STRUCTURE version 2.3.4 (Pritchard et al., 2000), a Bayesian clustering algorithm that groups genetically similar individuals based on multilocus genotypes. Across the entire study area, we evaluated $K = 1$–22 and determined the number of genetic clusters best supported by the data using the highest values of the likelihood of K (L[K]; Pritchard et al., 2000) and ΔK (Evanno et al., 2005). To investigate hierarchical population structure in the study area, we performed additional STRUCTURE runs with subsets of study sites separated into the four largest tributary drainages in our study area: Corral Creek, Willow Creek, Hague Creek, and the upper Cache la Poudre River (Figure 1). All STRUCTURE runs consisted of 20,000 burn-in iterations and 100,000 subsequent iterations with five replicates for each K, using the admixture model, correlated allele frequencies, and no location prior. We merged replicates of each K using the R package "pophelper" (Francis, 2017) and visualized individual cluster assignment probabilities using "ggplot2" (Wickham, 2016). To further visualize spatial structure, we calculated site-level group membership proportions as the mean assignment probability for each STRUCTURE cluster across individuals within a site and overlaid resulting pie charts on a map of the study area.

Riverscape genetic analysis

We applied the bidirectional gene flow in riverscapes (BGR) model (White et al., 2020) to examine the influences of six riverscape variables (Table 1) on Brook Trout gene flow. This approach models gene flow in stream networks with a genetic distance matrix as the response variable and riverscape variables as predictors, accounting for spatial autocorrelation within the dendritic network using a graph-based spatial autoregressive model (White et al., 2020). The BGR framework involves discretizing study streams into a spatially structured ecological network (SSEN), a graph made up of nodes (points on the stream network) and edges (linear stream segments connecting nodes). The SSEN consists of both observed nodes (sampling sites) and unobserved nodes, which are positioned at tributary confluences to prevent network edges from overlapping (White et al., 2020; Nakajima et al., 2023). Under this framework, riverscape covariates are measured on each edge, and a matrix of genetic distances between sampling locations is modeled as a generalized Wishart distribution parameterized by a covariance matrix, which is dependent on measured riverscape covariates and their regression coefficients (Hanks, 2017; McCullagh, 2009; Peterson et al., 2019; White et al., 2020).

To construct our SSEN, observed nodes were placed at all sampling locations, and additional unobserved nodes were placed at every perennial tributary confluence and upstream of each observed node, following White et al. (2020). Riverscape covariates (Table 1; Figure S1 [see online Supplementary Material]) were quantified on each edge of the SSEN using the R package "sf" (Pebesma, 2018), with elevation data derived from a 1/3 arc-second digital elevation model from the 3D Elevation Program (U.S. Geological Survey, 2023) and stream layers from the National Hydrography Dataset (U.S. Geological Survey, 2022). Stream order, a measure of a stream's size based on its position in a branched network, was calculated using two

Table 1. Descriptions of riverscape covariates tested for their effects on Brook Trout gene flow using the bidirectional gene flow in riverscapes model (White et al., 2020). Ranges of covariate values are shown before scaling.

Riverscape covariate	Description	Range of values	Hypothesized effect on trout gene flow
Barriers	The presence (1) or absence (0) of waterfalls with vertical drops ≥1.5 m occurring on edge *i, j*.	Binary	The presence of complete or partial barriers to trout movement will reduce gene flow. Vertical drops block the upstream movement of Brook Trout (Kondratieff & Myrick, 2006; Gomez-Uchida et al., 2009; White et al., 2020), restricting gene flow and increasing genetic differentiation.
Mean stream gradient	The mean stream gradient of edge *i, j*, calculated as the ratio of elevation change (m) to distance (m).	0.003–0.257	Higher stream gradients will reduce gene flow. Steep stream gradients create resistance to upstream fish movement, leading to genetic differentiation (Davis et al., 2018; Nakajima et al., 2023).
Dam regulation	Whether edge *i, j* is (1) or is not (0) a dam-regulated stream segment.	Binary	Dam-regulated streams will reduce gene flow. Flow intermittency can isolate fish populations (Labbe & Fausch, 2000), and annual water release patterns from Long Draw Reservoir (Figure 1) result in seasonal dewatering and a large annual flow fluctuation downstream of the dam.
Flow direction	Whether node *i* is upstream (0) or downstream (1) of node *j*.	Binary	Gene flow will be stronger in the downstream direction. Trout dispersal is often biased in the downstream direction (Morrissey & Ferguson, 2011; Lamphere & Blum, 2012; White et al., 2020).
Shreve's stream order	Shreve's stream order (Shreve, 1966) of the stream segment containing edge *i, j*.	1–8	Gene flow will be stronger on higher-order streams. High-order main-stem streams can serve as movement corridors that facilitate connectivity between intervening tributaries (White et al., 2020; Thomaz et al., 2016).
Strahler's stream order	Strahler's stream order (Strahler, 1957) of the stream segment containing edge *i, j*.	1–3	Gene flow will be stronger on higher-order streams. Refer to hypothesis for Shreve's stream order (above).
Barriers × direction	Interaction between barriers and flow direction.	Binary	Barrier presence will restrict upstream movement while allowing downstream movement, whereas barrier absence will reduce this directional disparity. Waterfalls are known to be more restrictive to fish passage in the upstream direction while allowing downstream movement (Nathan et al., 2019).
Gradient × direction	Interaction between stream gradient and flow direction.	0.003–0.257	Steeper stream gradients will impede upstream movement while allowing downstream movement, whereas gradual gradients will reduce this directional disparity. Steeper stream gradients might be more restrictive to upstream movement due to the presence of step falls and a greater energy cost to gain versus lose elevation (Adams et al., 2000).

common methods introduced by Shreve (1966) and Strahler (1957). In deriving Shreve's and Strahler's stream order, only streams on which we had sampling sites were included. Notable waterfalls (vertical drop height > 1.5 m) were identified at several locations in the stream network and defined as barriers (however, mark–recapture studies have demonstrated that at least two of our identified barriers do allow occasional upstream passage by Brook Trout [Myrick & Kondratieff, 2004; M. P. Fairchild, unpublished data]). Because vertical drops are known to impede fish passage in the upstream direction while allowing downstream movement (Nathan et al., 2019), we also tested an interaction term between barriers and flow direction. Similarly, high-gradient stream segments often contain vertical step falls that may disproportionately impede upstream movement, so we tested a plausible interaction between stream gradient and flow direction. Stream segments immediately downstream of the Long Draw Reservoir dam were defined as dam regulated. In this stream network, the hydrological impact of the Long Draw Reservoir dam diminishes farther downstream due to the increasing influence of free-flowing tributaries on the overall stream discharge. Therefore, we defined dam-regulated stream segments only as those upstream of the confluence point between the Long Draw Reservoir outflow and the Cache la Poudre River (Figure S1) because this is a major confluence that greatly reduces the effect of dam regulation on downstream hydrology. To ensure that riverscape covariates were on the same scale, nonbinary covariates were standardized from 0 to 1 by dividing each value by the maximum value for that covariate. All covariates were symmetric (i.e., the covariate value from node *i* to *j* is equal to the value from node *j* to *i*), with the exception of flow direction and its associated interaction terms.

We fit separate BGR models with two distinct genetic distance metrics as response variables: F_{ST} (Weir and Cockerham's θ) and Jost's *D*, allowing us to assess consistency of results across multiple measures of differentiation. To address potential collinearity among covariates, we calculated Pearson correlations between all covariates and excluded covariate pairs with a correlation magnitude >0.3 from being included in the same model. In our data set, the only two covariates found to be collinear were Strahler's and Shreve's stream orders (Figure S2). Following White et al. (2020), BGR models were run in R using a Markov chain–Monte Carlo (MCMC) sampler as implemented in the "rwc" package (Hanks, 2025) to estimate posterior distributions

of the regression coefficients for each covariate (i.e., their effects on trout gene flow). We implemented best subsets model selection (i.e., testing all possible combinations of noncollinear covariates), and models were ranked using deviance information criteria (DIC). Models with fewer than four variables were run for 50,000 MCMC iterations with 25,000 burn-in, and models with four or more variables were run for 100,000 iterations with 50,000 burn-in. Model convergence was verified by visual inspection of MCMC trace plots. To derive regression coefficients from the best models, we considered models within 4 DIC values of the model with the lowest DIC as competing models (separately for F_{ST} and Jost's D) (Anderson, 2008; Cain & Zhang, 2019) and averaged covariate effects across competing models using model weights (Wagenmakers & Farrell, 2004). Model-averaged posterior distributions for covariate effects were obtained by randomly sampling values from the posterior samples of competing models in proportions according to the models' weights. For covariates present in only some of the competing models, model averaging was conditional, meaning that model weights were scaled so that the weights for models containing each covariate summed to 1.

Edge weights $w_{i,j}$, representing the relative rate at which Brook Trout move from node i to node j, were quantified following White et al. (2020) as a function of K riverscape covariates and their corresponding effects on trout gene flow:

$$w_{i,j} = \exp\left(\beta_0 + \sum_{k=1}^{K} \beta_k x_{i,j,k}\right),$$

where β_0 is the intercept term, β_k is the model-averaged mean of the posterior distribution of the effect of covariate k, and $x_{i,j,k}$ is the value of covariate k for the edge connecting nodes i and j. Covariate effects were considered statistically significant if their 95% credible intervals did not overlap 0, and only statistically significant covariate effects were included in the estimation of edge weights. Edge weights are directed, such that $w_{i,j}$ need not equal $w_{j,i}$, to accommodate potential asymmetries in migration rates based on flow direction and its interaction terms. The estimated resistance to gene flow for each edge was the inverse of the corresponding edge weight, and pairwise resistance distances between sampling sites were quantified as the cumulative sum of the resistance values of edges connecting pairs of sites. Isolation by resistance (IBR; McRae, 2006) was investigated using Mantel tests to assess correlations between pairwise genetic distances (both F_{ST} and Jost's D) and resistance distances derived from edge weights. Because edge weights were directionally asymmetric, cumulative resistance distances between site pairs were averaged across both directions for the IBR Mantel tests. Isolation by distance (IBD; Wright 1943) cannot be readily incorporated into the BGR model as a covariate (Hanks, 2017; White et al., 2020), so we evaluated IBD using Mantel tests to assess correlations between pairwise genetic distances and waterway distances between sampling sites. All Mantel tests were performed using the R package "vegan" (Dixon, 2003) with 9,999 permutations. To visualize IBD and IBR relationships, we created scatterplots comparing pairwise genetic distance with waterway and resistance distances between all site pairs and fit simple linear regression lines.

RESULTS

Genetic diversity and differentiation

Across 22 sites, a total of 757 individual Brook Trout were genotyped at 12 microsatellite loci. All loci were polymorphic, and deviations from Hardy–Weinberg equilibrium were minimal, with only one locus–site combination showing significant deviation (locus *SfoD91* at site HAG2; Table S2), which did not warrant the exclusion of any markers. Site-level H_O ranged from 0.51 to 0.69 with a mean of 0.61, H_E ranged from 0.53 to 0.69 with a mean of 0.62, and A ranged from 3.9 to 6.9 with a mean of 5.7 (Table S1). Measures of genetic diversity were lowest at sites in the Corral Creek drainage compared with the rest of the study area (mean values from Corral Creek sites: $H_O = 0.54$, $H_E = 0.54$, $A = 4.1$), suggesting possible genetic isolation of this tributary. Across all sites, mean pairwise F_{ST} was 0.04 with a maximum value of 0.12, and mean Jost's D was 0.06 with maximum value 0.17 (Table S3), indicating substantial gene flow throughout the stream network. The two measures of pairwise genetic distance, F_{ST} and D, showed similar patterns of differentiation among sites (Figure 2). Pairwise genetic distances were generally highest for comparisons with sites in the Corral Creek drainage, providing additional evidence for the genetic isolation of this tributary.

Genetic clustering

Based on L(K) and ΔK, STRUCTURE results indicated that the number of genetic clusters best supported by our data was $K = 4$ (Figure S3). Although the largest value for ΔK occurred at $K = 2$, we concluded that $K = 4$ provides the best explanation because (1) the ΔK method frequently identifies $K = 2$ even when more genetic groups are present (Janes et al., 2017; Cullingham et al., 2020) and (2) $K = 4$ is supported in our data by the L(K) method and a peak in the ΔK plot at $K = 4$ (Figure S3). The four genetic clusters identified by STRUCTURE corresponded with four major tributary drainages in our study area (Corral Creek, Willow Creek, upper Cache la Poudre River, and Hague Creek), with sites near the downstream confluence of these tributaries showing mixed representation from all four clusters (Figure 3). Assessments of hierarchical population structure within these tributary groups provided minimal evidence of further genetic subdivision (Figure S4).

Effects of riverscape covariates on trout gene flow

The DIC model selection between BGR models using F_{ST} and Jost's D as response variables identified four competing models for both response variables (Table 2). Five covariates consistently appeared in all top competing models across both response variables: (1) flow direction, (2) stream gradient, (3) barriers, (4) dam regulation, and (5) the interaction term between barriers and flow direction (Table 2). Additional covariates present in only some competing models included Shreve's stream order, Strahler's stream order, and the interaction term between stream gradient and flow direction.

Using pairwise F_{ST} as the response distance matrix, we found Brook Trout gene flow to be significantly reduced by barriers

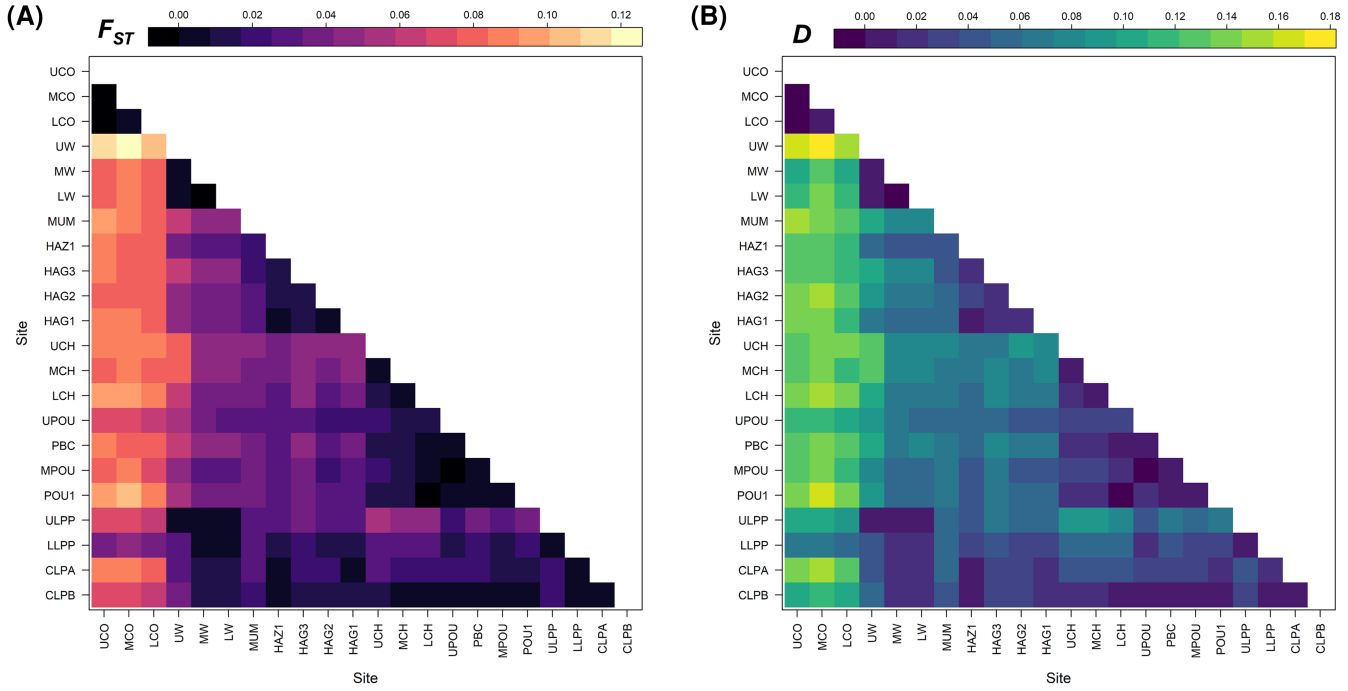

Figure 2. Heat maps of pairwise genetic distance between 22 Brook Trout sampling sites in the upper Cache la Poudre River watershed (refer to Table S1 for definitions of site abbreviations), with (A) pairwise F_{ST} (Weir & Cockerham, 1984) and (B) pairwise D (Jost, 2008). Refer to Figure 1 for site locations.

(posterior mean = −4.96, 95% credible interval [CRI] = −7.39 to −3.12) and steeper stream gradients (posterior mean = −1.97, 95% CRI = −4.21 to −0.71). In contrast, gene flow was significantly stronger in the downstream direction compared with upstream (posterior mean = 0.39, 95% CRI = 0.16–0.57) and in stream segments with higher Shreve's stream orders (posterior mean = 0.56, 95% CRI = 0.00–1.18). Contrary to our hypotheses (Table 1), gene flow was heightened in dam-regulated stream segments (posterior mean = 0.49, 95% CRI = 0.18–0.78) (Table 3). A statistically significant interaction between barriers and flow direction was also found (posterior mean = 4.86, 95% CRI = 2.95–7.34), suggesting that the asymmetry between upstream and downstream movement was more pronounced in stream segments with barriers, while upstream and downstream movement were more equal on segments without barriers. No significant effects were detected for Strahler's stream order (posterior mean = 0.36, 95% CRI = −0.19 to 0.89) or the interaction between stream gradient and flow direction (posterior mean = 0.87, 95% CRI = −0.57 to 2.46).

Results were similar from models using Jost's D as the response variable (Table 3), which showed significant negative effects of barriers (posterior mean = −5.00, 95% CRI = −7.41 to −3.15) and stream gradient (posterior mean = −3.25, 95% CRI = −5.35 to −1.30) and positive effects of downstream flow direction (posterior mean = 0.25, 95% CRI = 0.04–0.46) and dam regulation (posterior mean = 0.49, 95% CRI = 0.18–0.78), along with a significant interaction between barriers and flow direction (posterior mean = 4.95, 95% CRI = 3.06–7.39). However, there were some differences in results between the two response variables; models using Jost's D showed no effects of Shreve's stream order (posterior mean = 0.05, 95% CRI = −0.49 to 0.62) and Strahler's stream order (posterior mean = −0.26, 95% CRI = −0.87 to 0.34). Unlike F_{ST}, Jost's D models showed a significant interaction between stream gradient and flow direction (posterior mean = 1.50, 95% CRI = 0.09 to 3.06), indicating that differences between rates of upstream and downstream movement were more pronounced on steeper gradient streams.

Relative migration rates

Edge weights derived from β estimates, representing relative migration rates on SSEN edges (White et al., 2020), consistently indicated stronger migration in the downstream direction compared with upstream (Figure S5). When averaging across all SSEN edges, the relative rate of downstream migration was 16.8 times greater (using F_{ST}) and 26.0 times greater (using Jost's D) than upstream migration. Due to the significant interaction between barriers and flow direction, the discrepancy between upstream and downstream migration rates was greatly amplified by the presence of barriers. Relative downstream migration rates on SSEN edges with barriers were, on average, 190.3 times greater (F_{ST}) and 301.7 times greater (Jost's D) than upstream rates. Additionally, relative migration rates from Jost's D models showed a mediating effect of stream gradient on directional asymmetries in movement. Downstream edge weights on high-gradient edges (those with gradients greater than 0.16, the 75th percentile of edges in our SSEN) were, on average, 53.4 times greater than upstream weights, while downstream edge weights on low-gradient edges (those with gradients lower than 0.07, the 25th percentile in our SSEN) averaged only 20.3 times higher than upstream weights. Model results reflected that sites on dam-regulated edges were more genetically connected than those on free-flowing edges. When averaging upstream and downstream edge weights,

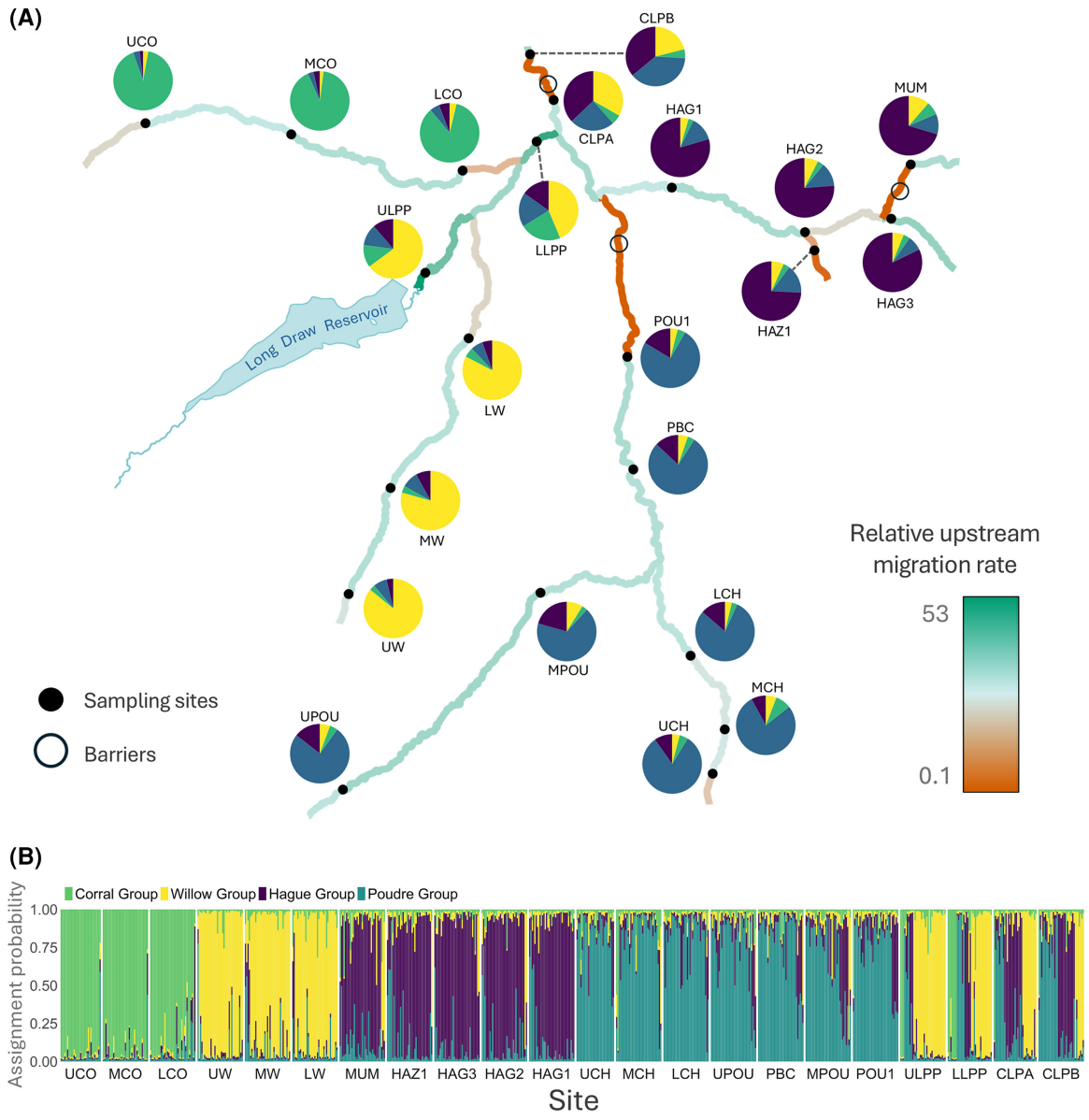

Figure 3. (A) Estimated relative upstream migration rates on spatially structured ecological network edges, quantified from the estimated effects of riverscape covariates on Brook Trout gene flow from top-ranked bidirectional gene flow in riverscapes models using pairwise Jost's *D* as the response genetic distance matrix. Pie charts show mean site-level assignment proportions to four genetic clusters from the program STRUCTURE. The lower panel (B) shows individual STRUCTURE assignment probabilities to four genetic clusters across 22 Brook Trout sampling sites (refer to Table S1 for definitions of site abbreviations).

Table 2. Deviance information criteria (DIC) values for competing riverscape genetics models (ΔDIC < 4) with F_{ST} and Jost's *D* as response variables. Interaction terms are expressed in parentheses with abbreviated covariate names (bar. = barriers, dir. = flow direction, grad. = stream gradient).

Variables	DIC	ΔDIC	Model weight
F_{ST}			
Direction + gradient + barriers + dam regulation + Shreve + (bar. × dir.)	−1,388.3	0	0.35
Direction + gradient + barriers + dam regulation + (bar. × dir.) + (grad. × dir.)	−1,387.9	0.4	0.27
Direction + gradient + barriers + dam regulation + Shreve + (bar. × dir.) + (grad. × dir.)	−1,387.8	0.5	0.23
Direction + gradient + barriers + dam regulation + Strahler + (bar. × dir.)	−1,386.5	1.8	0.15
Jost's *D*			
Direction + gradient + barriers + dam regulation + (bar. × dir.) + (grad. × dir.)	−1,249.6	0	0.44
Direction + gradient + barriers + dam regulation + Strahler + (bar. × dir.) + (grad. × dir.)	−1,249.1	0.5	0.31
Direction + gradient + barriers + dam regulation + Shreve + (bar. × dir.) + (grad. × dir.)	−1,247.0	2.6	0.16
Direction + gradient + barriers + dam regulation + (bar. × dir.)	−1,246.4	3.2	0.09

Table 3. Estimated β-values (posterior mean) and 95% credible intervals (CRIs) obtained through model-averaging top-ranked bidirectional gene flow in riverscapes models, representing the effects of riverscape covariates on Brook Trout gene flow. Values with 95% CRI overlapping zero are considered statistically not significant and are marked with asterisks.

	F_{ST}		Jost's *D*	
Variable	**β**	**95% CRI**	**β**	**95% CRI**
Intercept	3.67	3.24–4.11	3.73	3.34–4.26
Flow direction	0.39	0.16–0.57	0.25	0.04–0.46
Shreve's stream order	0.56	0.00–1.18	0.05*	−0.49 to 0.62
Strahler's stream order	0.36*	−0.19 to 0.89	−0.26*	−0.87 to 0.34
Dam regulation	0.49	0.18–0.78	0.39	0.07–0.71
Barriers	−4.96	−7.39 to −3.12	−5.00	−7.41 to −3.15
Stream gradient	−1.97	−4.21 to −0.71	−3.25	−5.35 to −1.30
Gradient × direction	0.87*	−0.57 to 2.46	1.50	0.09–3.06
Barriers × direction	4.86	2.95–7.34	4.95	3.06–7.39

estimated migration rates on dam-regulated edges were 1.78 times higher (using F_{ST}) and 1.66 times higher (using Jost's *D*) than those on free-flowing edges.

Isolation by distance and isolation by resistance

Mantel tests revealed significant relationships between genetic differentiation and both waterway distance and estimated riverscape resistance. Across all site pairs, pairwise waterway distance between sites was significantly correlated with genetic distance as measured by F_{ST} (Mantel statistic $r = 0.41$, $P = 0.0009$) and Jost's *D* ($r = 0.44$, $P = 0.0002$). Additionally, pairwise resistance distance derived from BGR model results showed significant correlations with both F_{ST} ($r = 0.43$, $P = 0.0006$) and Jost's *D* ($r = 0.48$, $P = 0.0001$). Visual inspection of the scatterplots showing IBD and IBR relationships (Figure 4) revealed two distinct clusters of points, reflecting elevated genetic differentiation in comparisons involving Corral Creek sites. To investigate this grouping structure, we generated additional plots (Figure S6) in which site pairs were divided into two categories: those including Corral Creek sites and all others. Separate simple linear regression lines were fit to each group for visualization, and separate Mantel tests were conducted on the split dataset, which verified statistical significance of IBR and IBD relationships within these two groups (Figure S6).

DISCUSSION

This study revealed that riverscape features, waterway distance, and asymmetric streamflow play significant roles in shaping the spatial genetic structure of a nonnative salmonid occupying a future native trout reintroduction area. Brook Trout exhibited spatial population structure, with genetic clusters corresponding to four major tributary drainages (Figure 3). This observation aligns with previous research showing that Brook Trout populations often segregate into genetically distinct tributaries (Kelson et al., 2015; Pilgrim et al., 2012; White et al., 2020). Brook Trout typically spawn in low-order streams near the upstream ends of network branches (Witzel & MacCrimmon, 1983), which can contribute to genetic isolation of neighboring tributaries even in the absence of obvious barriers or resistance features (Kazyak et al., 2016; Beer et al., 2019). Such spatial genetic structure is also driven by the tendency of most Brook Trout individuals to exhibit short dispersal distances (<1 km annually) (Hutchings & Gerber, 2002; Peterson & Fausch, 2003; White, Keagy, et al., 2023). However, individual variation in movement plays a crucial role in maintaining genetic connectivity. While most Brook Trout are relatively sedentary, occasional long-distance dispersal events, including movements exceeding 10 km within a season (Shetter, 1968), have been documented (Gowan & Fausch, 1996; Petty et al., 2012). Despite genetic segregation of tributary groups in our study, pairwise genetic distances among sites (Figure 2) suggest that connectivity across the stream network is facilitated by rare long-distance movements and sequential short-distance dispersals occurring in a stepping-stone manner (Saura et al. 2014). In the native range of Brook Trout, connectivity restoration is underway to reconnect habitat patches fragmented by culverts and land-use changes (Poplar-Jeffers et al., 2009; Wood et al., 2018). Our results reveal that, free of fragmentation, Brook Trout dispersal facilitates genetic connectivity at the watershed scale, highlighting the utility of connectivity restoration as a tool for promoting genetic exchange and improving the robustness of native Brook Trout metapopulations.

Our riverscape genetics models uncovered the roles of specific riverscape features in mediating population connectivity. Variables including stream gradient, barriers, flow direction, and the interaction between flow direction and barriers consistently appeared in all of our top models, suggesting that these are key factors influencing trout movement in this stream network (Table 2). The negative effect of barriers on gene flow was expected (Table 1) and aligns with many other studies that have found genetic evidence of restricted fish movement due to vertical barriers (Deiner et al., 2007; Kelson et al., 2015; Neville et al., 2006; Timm et al., 2016; Torterotot et al., 2014; White et al., 2020; Wofford et al., 2005). We also found that steeper stream gradients negatively affected gene flow, which has been similarly detected in some riverscape genetics studies (Kanno et al., 2011; Nakajima et al., 2023 ; Narum et al., 2008) but not others (White et al., 2020). Ascending steep slopes can be energetically costly for various taxa (Funk et al., 2005), and high-gradient stream segments often contain vertical drops that could hinder fish movement by acting as partial barriers. In a mark–recapture study, Adams et al. (2000) observed Brook Trout ascending streams with gradients as high as 22% (maximum gradient in our study area = 25.7%), but upstream movements were longer and more common in lower-gradient

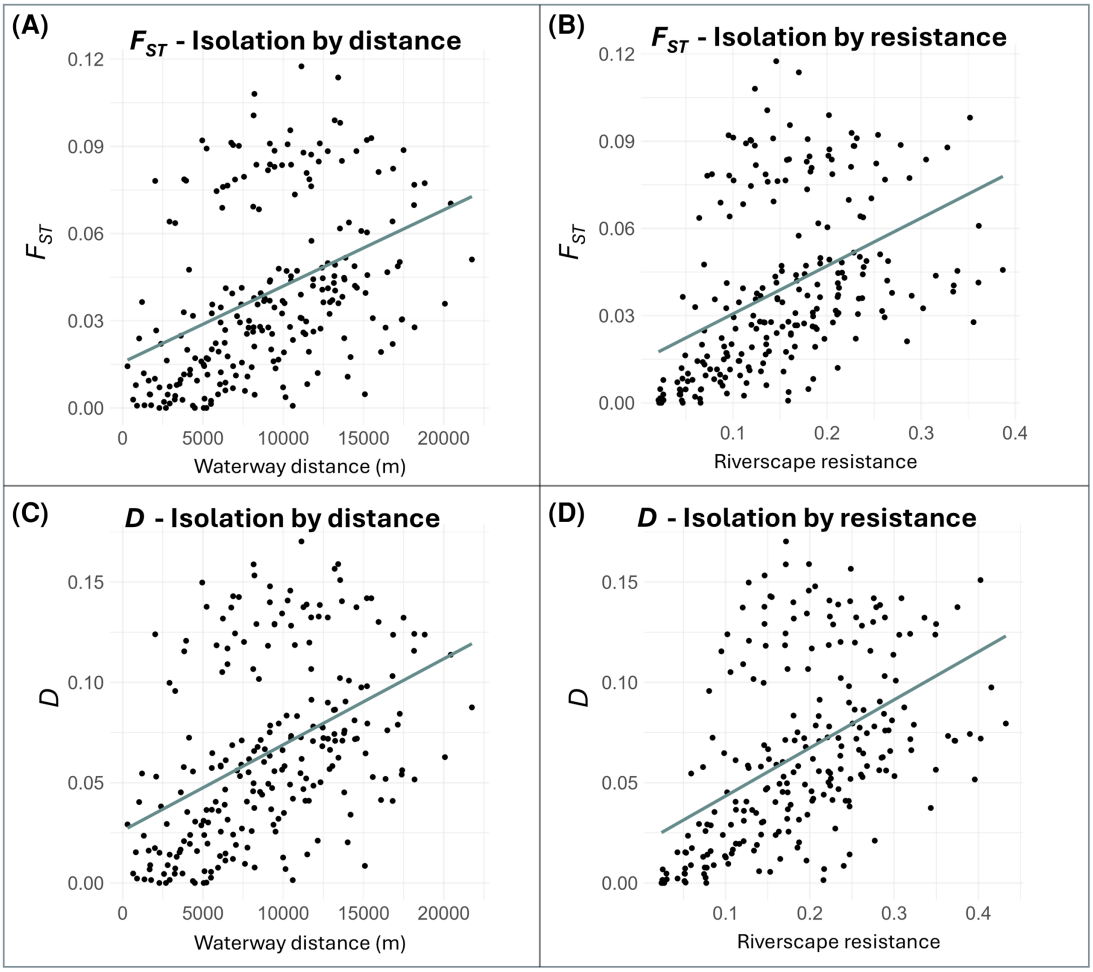

Figure 4. Scatterplots showing isolation by distance and isolation by resistance between all site pairs with simple linear regression lines: (A) pairwise F_{ST} plotted against pairwise waterway distance, (B) pairwise F_{ST} plotted against cumulative riverscape resistance (from bidirectional gene flow in riverscapes model results), (C) pairwise Jost's *D* plotted against pairwise waterway distance and (D) pairwise Jost's *D* plotted against cumulative riverscape resistance.

segments. This aligns with our findings and suggests that steeper gradients hinder trout movement, but upstream passage can still occur when impassable falls are not present.

The ability of the BGR framework to model asymmetric gene flow provided unique insights into trout movement patterns in this proposed reintroduction area. We found a positive effect of downstream direction on gene flow, consistent with most other studies on lotic fishes that have reported downstream-biased gene flow (Lamphere & Blum, 2012; Morrissey & Ferguson, 2011; White et al., 2020). Despite this common trend, several studies have observed upstream-biased movement among adult Brook Trout (Adams et al., 2000; Gutowsky et al., 2023; Hansbarger et al., 2010; Peterson & Fausch, 2003), which seems contrary to our results. However, observations of this upstream movement preference are limited to adult Brook Trout, and a population-level pattern of downstream-biased gene flow can result from passive processes, such as the downstream drift of recently emerged fry and the displacement of smaller-bodied individuals during high-flow events (Morrissey & Ferguson, 2011). We also found significant interactions between flow direction and other riverscape attributes, specifically barriers (using both F_{ST} and Jost's *D*) and stream gradient (using Jost's *D*). These findings support our hypotheses (Table 1) that barriers and steeper stream gradients disproportionately impede trout movement in the upstream direction. This is consistent with the known tendency of waterfalls to block upstream passage while allowing downstream movement (Nathan et al., 2019) and the fact that steep slopes are more energetically costly to ascend than to descend. The BGR framework's ability to formally model the effects of flow direction and its interactions with other riverscape attributes enabled us to uncover unique insights about asymmetric gene flow within this stream network.

Unexpectedly, our BGR model results did not detect a restrictive effect of dam regulation on trout gene flow in our study area. Instead, results suggested that dam-regulated sites exhibited high levels of genetic connectivity with nearby habitats. This lack of a restrictive effect on gene flow was unexpected given that the dam-regulated stream segment in our study area does not support year-round trout occupancy due to seasonal dewatering, and previous research has indicated that habitats with intermittent occupancy typically isolate stream fish populations (Labbe & Fausch, 2000; White et al., 2020). Moreover, the genetically distinct Corral Creek and

Willow Creek tributaries are separated from each other and the rest of the study area by the dam-regulated stream segment (Figure 3A), and Corral Creek is the most genetically isolated drainage (Figure 2), which suggests that the altered hydrology might hinder trout movement between tributaries. Estimated genetic distances and STRUCTURE results for sites located on the dam-regulated segment (sites ULPP and LLPP; Figure 3A) revealed that these sites were genetically similar to nearby locations, particularly those in the adjacent Willow Creek tributary (Figures 2 and 3). Furthermore, trout size data showed that dam-regulated sites were dominated by adult trout, with a notable absence of age-0 fish (the minimum total length among Brook Trout measured at dam-regulated sites was 70 mm, while the average total length of age-0 fish across the study area was 35 mm based on length frequency distributions). Thus, it appears that the dam-regulated segment is predominantly occupied by transient migrants from nearby tributaries during seasonal windows of suitable habitat, causing our BGR models to identify dam-regulation as facilitating genetic connectivity. Importantly, this result does not necessarily indicate that dam-regulated streams facilitate gene flow on the riverscape scale. It is well known that dams restrict longitudinal connectivity along river corridors (Nilsson et al., 2005; Zarri et al., 2022), and fish responses to anthropogenic flow regulation are complex, often depending on location-specific discharge patterns (Kelly et al., 2017; Korman & Campana, 2009; Oliveira et al., 2020). Based on the relatively strong genetic isolation of Corral Creek and Willow Creek, it remains plausible that the dam-altered hydrology in our study area inhibits migration between tributaries, but the genetic similarity of dam-regulated sites to nearby areas may have masked this effect and thus prevented our models from detecting it.

It is important to note that stocking history can have a persistent influence on spatial patterns in fish (Perrier et al., 2013), particularly in scenarios where genetically distinct strains of fish were introduced at different locations or times. Our inferences rest on the assumption that contemporary patterns of genetic structure in this watershed are shaped primarily by mechanisms of Brook Trout gene flow and genetic drift rather than founding conditions involving different hatchery strains. In our study area, available historical records indicate that Brook Trout were stocked between 1892 and 1955 (Colorado Parks and Wildlife, unpublished data; U.S. Fish and Wildlife Service, unpublished data), with a majority of stocking concentrated in the central main stem of the watershed (Cache la Poudre River; Figure 1) and only one single recorded stocking event in the Hague Creek tributary. Available records lack information about genetic strains used, and we cannot dismiss the possibility of additional undocumented introductions or angler translocations. Nonetheless, several lines of evidence support the notion that postestablishment Brook Trout dispersal is the primary driver of the observed patterns of genetic connectivity in this stream network. First, the cessation of stocking more than six decades prior to our sampling provided ample time for gene flow and drift to shape current spatial genetic patterns. Second, the concentration of stocking events in the central main stem of the study area suggests that tributaries were largely colonized from a common central source, with no evidence of tributaries being independently seeded with genetically distinct strains. Third, the observed patterns of IBD and IBR are consistent with dispersal-mediated gene flow across a heterogeneous landscape, whereas differentiation resulting from founding conditions would be expected to be unrelated to spatial or environmental gradients. For example, Eldridge et al. (2009) reported a loss of historical IBD patterns among populations Coho Salmon *Oncorhynchus kisutch* following extensive hatchery transfers, observing that genetic distance in modern populations was better explained by stocking history than geographic proximity. Moreover, Kazyak et al. (2022) found that Brook Trout from 17 hatcheries across the eastern United States were genetically distinct from wild populations but comparatively homogenous among themselves, forming a single genetic cluster in their analyses. This suggests that even if fish from multiple hatcheries were introduced to our study area, they may not have differed substantially in their genetic composition. Collectively, these lines of available information support our assumption that initial differences among introduced stocks, if any, have been largely attenuated by subsequent ecological and evolutionary processes.

In addition to providing insights about Brook Trout population connectivity at the watershed scale, our observations of spatial genetic structure and the effects of riverscape covariates on trout gene flow can inform expectations of future population connectivity of reintroduced Greenback Cutthroat Trout in this stream network and perhaps other proposed reintroduction habitats. However, it is important to consider that differences in reproductive timing between Brook Trout and Rocky Mountain Cutthroat Trout might influence annual periodicity in fish movement and the effects of certain riverscape features on gene flow. Brook Trout spawn during autumn and cutthroat trout spawn during spring, and because salmonid movement is heightened during the prespawning period (Hilderbrand & Kershner, 2000), annual phases of increased mobility in these two species coincide with different levels of stream discharge due to the timing of snowmelt runoff (i.e., prespawn movements of Rocky Mountain Cutthroat Trout occur when flows are higher compared with those of Brook Trout). Elevated discharge can enhance fish mobility (Taylor & Cooke, 2012) and may improve passage around features like waterfalls by filling intermittent side channels or deepening plunge pools, suggesting that the timing of upstream spawning movements in Rocky Mountain Cutthroat Trout could result in greater mobility compared to Brook Trout. On the other hand, Peterson and Fausch (2003) observed that adult Brook Trout have a stronger tendency towards upstream movement than Rocky Mountain Cutthroat Trout, particularly in high-gradient streams. This behavioral difference implies that steep stream gradients could exert a stronger isolating effect on reintroduced Greenback Cutthroat Trout. Despite these nuances, both species exhibit broad similarities in swimming speed and performance (Blank et al., 2020; Castro-Santos et al., 2013; Young, 2011), and available information on the ecology and movement of these two species does not provide strong evidence to suspect that they differ substantially in their movement patterns and consequent metapopulation structure. Future reintroduction of Greenback Cutthroat Trout in this stream network could provide an opportunity to directly test

possible differences, but with the current lack of Greenback Cutthroat Trout populations available for study, we believe that resident Brook Trout serve as a useful surrogate for predicting patterns of connectivity in reintroduced native trout.

Our results suggest that the study stream network provides a large, connected habitat capable of supporting a native trout metapopulation. For reintroduced Greenback Cutthroat Trout, this connectivity could promote resilience to disturbance (Christie & Knowles, 2015) and facilitate allelic exchange to buffer against genetic drift (Allendorf et al., 2022), which is crucial in the face of changing and uncertain environmental conditions. Connectivity across the reintroduction area is impeded by vertical barriers and areas of high stream gradient. However, downstream movement appears less affected by these features, suggesting that stocking Greenback Cutthroat Trout at sites farther upstream may enable more expedient colonization of the reintroduction area. Insights into salmonid gene flow in this system can also help managers identify future reintroduction habitats with high potential for connectivity. Our results suggest that the prioritization of reclamation areas containing fewer waterfalls and more gradual stream gradients could promote population connectivity. However, the presence of these features does not entirely preclude salmonid gene flow, and even infrequent migration (<10 migrants per generation) has been shown to provide functional genetic connectivity in trout (Nathan et al., 2017). Overall, our work adds to a growing body of information about the factors influencing genetic connectivity in salmonids. This knowledge is crucial for managing genetic diversity and promoting metapopulation resilience in sensitive fish species amid increasing habitat fragmentation and environmental change.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Transactions of the American Fisheries Society* online.

DATA AVAILABILITY

The data and sample R code for conducting the riverscape genetic analysis presented are available on Dryad (Stack et al., 2025).

ETHICS STATEMENT

Fish capture and handling were conducted following a protocol approved by the Colorado State University Institutional Animal Care and Use Committee (IACUC Protocol Number 1505), and collections were permitted under the State of Colorado Department of Natural Resources aquatic scientific collection license number 2407861825.

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CONFLICTS OF INTEREST

None declared.

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REFERENCES

Adams, S. B., Frissell, C. A., & Rieman, B. E. (2000). Movements of nonnative Brook Trout in relation to stream channel slope. *Transactions of the American Fisheries Society, 129*, 623–638. https://doi.org/10.1577/1548-8659(2000)129<0623:MONBTI>2.3.CO;2

Allendorf, F. W., Funk, W. C., Aitken, S. N., Byrne, M., Luikart, G., & Antunes, A. (2022). *Conservation and the genomics of populations*. Oxford University Press.

Anderson, D. R. (2008). *Model based inference in the life sciences: A primer on evidence*. Springer.

Balkenhol, N., Cushman, S. A., Storfer, A., & Waits, L. P. (2015). *Landscape genetics: Concepts, methods, applications*. John Wiley & Sons.

Beer, S. D., Bartron, M. L., Argent, D. G., & Kimmel, W. G. (2019). Genetic assessment reveals population fragmentation and inbreeding in populations of Brook Trout in the Laurel Hill of Pennsylvania. *Transactions of the American Fisheries Society, 148*, 620–635. https://doi.org/10.1002/tafs.10153

Bell, D. A., Kovach, R. P., & Whiteley, A. R. (2024). Inbreeding avoidance and cost in a small, isolated trout population. *Proceedings of the Royal Society of London B: Biological Sciences, 291*, Article 20241164. https://doi.org/10.1098/rspb.2024.1164

Biermann, C., & Havlick, D. G. (2021). Genetics and the question of purity in cutthroat trout restoration. *Restoration Ecology, 29*, Article e13516. https://doi.org/10.1111/rec.13516

Blank, M. D., Kappenman, K. M., Plymesser, K., Banner, K., & Cahoon, J. (2020). Swimming performance of Rainbow Trout and Westslope Cutthroat Trout in an open-channel flume. *Journal of Fish and Wildlife Management, 11*, 217–225. https://doi.org/10.3996/052019-JFWM-040

Cain, M. K., & Zhang, Z. (2019). Fit for a Bayesian: An evaluation of PPP and DIC for structural equation modeling. *Structural Equation Modeling: A Multidisciplinary Journal, 26*, 39–50. https://doi.org/10.1080/10705511.2018.1490648

Castro-Santos, T., Sanz-Ronda, F. J., & Ruiz-Legazpi, J. (2013). Breaking the speed limit—Comparative sprinting performance of Brook Trout (*Salvelinus fontinalis*) and Brown Trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences: Journal Canadien Des Sciences Halieutiques Et Aquatiques, 70*, 280–293. https://doi.org/10.1139/cjfas-2012-0186

Che-Castaldo, J. P., & Neel, M. C. (2012). Testing surrogacy assumptions: Can threatened and endangered plants be grouped by biological similarity and abundances? *PLoS One, 7*, Article e51659. https://doi.org/10.1371/journal.pone.0051659

Christie, M. R., & Knowles, L. L. (2015). Habitat corridors facilitate genetic resilience irrespective of species dispersal abilities or population sizes. *Evolutionary Applications, 8*, 454–463. https://doi.org/10.1111/eva.12255

Correa Ayram, C. A., Mendoza, M. E., Etter, A., & Salicrup, D. R. P. (2016). Habitat connectivity in biodiversity conservation: A review of recent studies and applications. *Progress in Physical Geography: Earth and Environment, 40*, 7–37. https://doi.org/10.1177/0309133315598713

Cullingham, C. I., Miller, J. M., Peery, R. M., Dupuis, J. R., Malenfant, R. M., Gorrell, J. C., & Janes, J. K. (2020). Confidently identifying the

correct K value using the ΔK method: When does $K = 2$? *Molecular Ecology*, *29*, 862–869. https://doi.org/10.1111/mec.15374
Davis, C. D., Epps, C. W., Flitcroft, R. L., & Banks, M. A. (2018). Refining and defining riverscape genetics: How rivers influence population genetic structure. *WIREs: Water*, *5*, Article e1269. https://doi.org/10.1002/wat2.1269
Deiner, K., Garza, J. C., Coey, R., & Girman, D. J. (2007). Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and man-made barriers in the Russian River, California. *Conservation Genetics*, *8*, 437–454. https://doi.org/10.1007/s10592-006-9183-0
Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science: Official Organ of the International Association for Vegetation Science*, *14*, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x
Dunham, J. B., Taylor, E. B., & Allendorf, F. W. (2014). *Bull Trout in the Boundary System: Managing connectivity and the feasibility of a reintroduction in the lower Pend Oreille River, northeastern Washington* (Report No. 2014–1229). U.S. Geological Survey. https://doi.org/10.3133/ofr20141229
Eldridge, W. H., Myers, J. M., & Naish, K. A. (2009). Long-term changes in the fine-scale population structure of Coho Salmon (*Oncorhynchus kisutch*) populations subject to extensive supportive breeding. *Heredity*, *103*, 299–309. https://doi.org/10.1038/hdy.2009.69
Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x
Fausch, K. D., Rieman, B. E., Dunham, J. B., Young, M. K., & Peterson, D. P. (2009). Invasion versus isolation: Trade-offs in managing native salmonids with barriers to upstream movement. *Conservation Biology*, *23*, 859–870. https://doi.org/10.1111/j.1523-1739.2008.01159.x
Francis, R. M. (2017). Pophelper: An R package and web app to analyze and visualize population structure. *Molecular Ecology Resources*, *17*, 27–32. https://doi.org/10.1111/1755-0998.12509
Funk, W. C., Blouin, M. S., Corn, P. S., Maxell, B. A., Pilliod, D. S., Amish, S., & Allendorf, F. W. (2005). Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, *14*, 483–496. https://doi.org/10.1111/j.1365-294X.2005.02426.x
Gomez-Uchida, D., Knight, T. W., & Ruzzante, D. E. (2009). Interaction of landscape and life history attributes on genetic diversity, neutral divergence and gene flow in a pristine community of salmonids. *Molecular Ecology*, *18*, 4854–4869. https://doi.org/10.1111/j.1365-294X.2009.04409.x
Goudet, J. (2005). Hierfstat, a package for R to compute and test hierarchical *F*-statistics. *Molecular Ecology Notes*, *5*, 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x
Gowan, C., & Fausch, K. D. (1996). Mobile Brook Trout in two high-elevation Colorado streams: Reevaluating the concept of restricted movement. *Canadian Journal of Fisheries and Aquatic Sciences: Journal Canadien Des Sciences Halieutiques Et Aquatiques*, *53*, 1370–1381. https://doi.org/10.1139/f96-058
Greenback Cutthroat Trout Recovery Team. (2019). *Recovery outline for the Greenback Cutthroat Trout* (Oncorhynchus clarkii stomias). U.S. Fish and Wildlife Service.
Gresswell, R. E. (2011). Biology, status, and management of the Yellowstone Cutthroat Trout. *North American Journal of Fisheries Management*, *31*, 782–812. https://doi.org/10.1080/02755947.2011.608980
Gutowsky, L. F. G., Blair, S. G., Cooke, S. J., & Fox, M. G. (2023). Summer and autumn movement ecology of native Brook Trout (*Salvelinus fontinalis*) in urban headwater streams of eastern North America. *Hydrobiologia*, *850*, 3481–3495. https://doi.org/10.1007/s10750-023-05169-8
Hanks, E. M. (2017). Modeling spatial covariance using the limiting distribution of spatio-temporal random walks. *Journal of the American Statistical Association*, *112*, 497–507. https://doi.org/10.1080/01621459.2016.1224714
Hanks, E. M. (2025). *rwc: Random walk covariance models* (Version 1.12). https://cran.r-project.org/web/packages/rwc/index.html
Hansbarger, J. L., Petty, J. T., & Mazik, P. M. (2010). Brook Trout movement within a high-elevation watershed: Consequences for watershed restoration. In J. S. Rentch & T. M. Schuler (Eds.), *Proceedings from the conference on the ecology and management of high-elevation forests in the central and southern Appalachian Mountains* (General Technical Report NRS-P-64, pp. 74–84). U.S. Forest Service.
Hao, W., & Storey, J. D. (2019). Extending tests of Hardy–Weinberg equilibrium to structured populations. *Genetics*, *213*, 759–770. https://doi.org/10.1534/genetics.119.302370
Harig, A., & Fausch, K. (2002). Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications*, *12*, 535–551. https://doi.org/10.1890/1051-0761(2002)012[0535:MHRFET]2.0.CO;2
Harig, A. L., Fausch, K. D., & Young, M. K. (2000). Factors influencing success of Greenback Cutthroat Trout translocations. *North American Journal of Fisheries Management*, *20*, 994–1004. https://doi.org/10.1577/1548-8675(2000)020<0994:FISOGC>2.0.CO;2
Harris, A. C., Oyler-McCance, S. J., Fike, J. A., Fairchild, M. P., Kennedy, C. M., Crockett, H. J., Winkelman, D. L., & Kanno, Y. (2022). Population genetics reveals bidirectional fish movement across the continental divide via an interbasin water transfer. *Conservation Genetics*, *23*, 839–851. https://doi.org/10.1007/s10592-022-01455-5
Hayes, M., & Banish, N. (2017). Translocation and reintroduction of native fishes: A review of Bull Trout (*Salvelinus confluentus*) with applications for future reintroductions. *Endangered Species Research*, *34*, 191–209. https://doi.org/10.3354/esr00849
Henry, E., Brammer-Robbins, E., Aschehoug, E., & Haddad, N. (2019). Do substitute species help or hinder endangered species management? *Biological Conservation*, *232*, 127–130. https://doi.org/10.1016/j.biocon.2019.01.031
Hilderbrand, R. H., & Kershner, J. L. (2000). Movement patterns of stream-resident Cutthroat Trout in Beaver Creek, Idaho–Utah. *Transactions of the American Fisheries Society*, *129*, 1160–1170. https://doi.org/10.1577/1548-8659(2000)129<1160:MPOSRC>2.0.CO;2
Hodgson, J. A., Moilanen, A., Wintle, B. A., & Thomas, C. D. (2011). Habitat area, quality and connectivity: Striking the balance for efficient conservation. *Journal of Applied Ecology*, *48*, 148–152. https://doi.org/10.1111/j.1365-2664.2010.01919.x
Hudy, M., Thieling, T. M., Gillespie, N., & Smith, E. P. (2008). Distribution, status, and land use characteristics of subwatersheds within the native range of Brook Trout in the eastern United States. *North American Journal of Fisheries Management*, *28*, 1069–1085. https://doi.org/10.1577/M07-017.1
Hutchings, J. A., & Gerber, L. (2002). Sex-biased dispersal in a salmonid fish. *Proceedings of the Royal Society of London B: Biological Sciences*, *269*, 2487–2493. https://doi.org/10.1098/rspb.2002.2176
Janes, J. K., Miller, J. M., Dupuis, J. R., Malenfant, R. M., Gorrell, J. C., Cullingham, C. I., & Andrew, R. L. (2017). The $K = 2$ conundrum. *Molecular Ecology*, *26*, 3594–3602. https://doi.org/10.1111/mec.14187
Jost, L. (2008). G_{ST} and its relatives do not measure differentiation. *Molecular Ecology*, *17*, 4015–4026. https://doi.org/10.1111/j.1365-294X.2008.03887.x
Jost, L., Archer, F., Flanagan, S., Gaggiotti, O., Hoban, S., & Latch, E. (2018). Differentiation measures for conservation genetics. *Evolutionary Applications*, *11*, 1139–1148. https://doi.org/10.1111/eva.12590
Kanno, Y., Vokoun, J. C., & Letcher, B. H. (2011). Fine-scale population structure and riverscape genetics of Brook Trout (*Salvelinus fontinalis*) distributed continuously along headwater channel networks. *Molecular Ecology*, *20*, 3711–3729. https://doi.org/10.1111/j.1365-294X.2011.05210.x
Katopodis, C., & Gervais, R. (2012). Ecohydraulic analysis of fish fatigue data. *River Research and Applications*, *28*, 444–456. https://doi.org/10.1002/rra.1566

Kazyak, D. C., Hilderbrand, R. H., King, T. L., Keller, S. R., & Chhatre, V. E. (2016). Hiding in plain sight: A case for cryptic metapopulations in Brook Trout (*Salvelinus fontinalis*). *PLoS One*, *11*, Article e0146295. https://doi.org/10.1371/journal.pone.0146295

Kazyak, D. C., Lubinski, B. A., Kulp, M. A., Pregler, K. C., Whiteley, A. R., Hallerman, E., Coombs, J. A., Kanno, Y., Rash, J. M., Morgan, R. P., Habera, J., Henegar, J., Weathers, T. C., Sell, M. T., Rabern, A., Rankin, D., & King, T. L. (2022). Population genetics of Brook Trout in the southern Appalachian mountains. *Transactions of the American Fisheries Society*, *151*, 127–149. https://doi.org/10.1002/tafs.10337

Keeley, A. T. H., Basson, G., Cameron, D. R., Heller, N. E., Huber, P. R., Schloss, C. A., Thorne, J. H., & Merenlender, A. M. (2018). Making habitat connectivity a reality. *Conservation Biology*, *32*, 1221–1232. https://doi.org/10.1111/cobi.13158

Kelly, B., Smokorowski, K. E., & Power, M. (2017). Downstream effects of hydroelectric dam operation on thermal habitat use by Brook Trout (*Salvelinus fontinalis*) and Slimy Sculpin (*Cottus cognatus*). *Ecology of Freshwater Fish*, *26*, 552–562. https://doi.org/10.1111/eff.12299

Kelson, S. J., Kapuscinski, A. R., Timmins, D., & Ardren, W. R. (2015). Fine-scale genetic structure of Brook Trout in a dendritic stream network. *Conservation Genetics*, *16*, 31–42. https://doi.org/10.1007/s10592-014-0637-5

King, T. L., Lubinski, B. A., Burnham-Curtis, M. K., Stott, W., & Morgan, R. P. (2012). Tools for the management and conservation of genetic diversity in Brook Trout (*Salvelinus fontinalis*): Tri- and tetranucleotide microsatellite markers for the assessment of genetic diversity, phylogeography, and historical demographics. *Conservation Genetics Resources*, *4*, 539–543. https://doi.org/10.1007/s12686-012-9603-z

Kondratieff, M. C., & Myrick, C. A. (2006). How high can Brook Trout jump? A laboratory evaluation of Brook Trout jumping performance. *Transactions of the American Fisheries Society*, *135*, 361–370. https://doi.org/10.1577/T04-210.1

Korman, J., & Campana, S. E. (2009). Effects of hydropeaking on nearshore habitat use and growth of age-0 Rainbow Trout in a large regulated river. *Transactions of the American Fisheries Society*, *138*, 76–87. https://doi.org/10.1577/T08-026.1

Labbe, T. R., & Fausch, K. D. (2000). Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. *Ecological Applications*, *10*, 1774–1791. https://doi.org/10.1890/1051-0761(2000)010[1774:DOISHR]2.0.CO;2

Lahontan Cutthroat Trout Coordinating Committee. (2019). *Updated goals and objectives for recovery of Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*)*. U.S. Fish and Wildlife Service.

Lamphere, B. A., & Blum, M. J. (2012). Genetic estimates of population structure and dispersal in a benthic stream fish. *Ecology of Freshwater Fish*, *21*, 75–86. https://doi.org/10.1111/j.1600-0633.2011.00525.x

Lindenmayer, D. B., & Likens, G. E. (2011). Direct measurement versus surrogate indicator species for evaluating environmental change and biodiversity loss. *Ecosystems*, *14*, 47–59. https://doi.org/10.1007/s10021-010-9394-6

McCullagh, P. (2009). Marginal likelihood for distance matrices. *Statistica Sinica*, *19*, 631–649.

McRae, B. H. (2006). Isolation by resistance. *Evolution*, *60*, 1551–1561. https://doi.org/10.1111/j.0014-3820.2006.tb00500.x

Metcalf, J. L., Love Stowell, S., Kennedy, C. M., Rogers, K. B., McDonald, D., Epp, J., Keepers, K., Cooper, A., Austin, J. J., & Martin, A. P. (2012). Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. *Molecular Ecology*, *21*, 5194–5207. https://doi.org/10.1111/mec.12028

Morrissey, M. B., & Ferguson, M. M. (2011). Individual variation in movement throughout the life cycle of a stream-dwelling salmonid fish: Brook Charr movement. *Molecular Ecology*, *20*, 235–248. https://doi.org/10.1111/j.1365-294X.2010.04921.x

Myrick, C. A., & Kondratieff, M. C. (2004). *An evaluation of a potential barrier to the upstream movement of Brook Trout in Rocky Mountain National Park, Colorado* (Technical Report NPS/NRWRD/NRTR-2005/337). National Park Service.

Nakajima, S., Suzuki, H., Nakatsugawa, M., Matsuo, A., Hirota, S. K., Suyama, Y., & Nakamura, F. (2023). Inferring future changes in gene flow under climate change in riverscapes: A pilot case study in fluvial sculpin. *Landscape Ecology*, *38*, 1351–1362. https://doi.org/10.1007/s10980-023-01633-x

Narum, S. R., Zendt, J. S., Graves, D., & Sharp, W. R. (2008). Influence of landscape on resident and anadromous life history types of *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences: Journal Canadien Des Sciences Halieutiques Et Aquatiques*, *65*, 1013–1023. https://doi.org/10.1139/F08-025

Nathan, L. R., Kanno, Y., & Vokoun, J. C. (2017). Population demographics influence genetic responses to fragmentation: A demogenetic assessment of the "one migrant per generation" rule of thumb. *Biological Conservation*, *210*, 261–272. https://doi.org/10.1016/j.biocon.2017.02.043

Nathan, L. R., Welsh, A. B., & Vokoun, J. C. (2019). Watershed-level Brook Trout genetic structuring: Evaluation and application of riverscape genetics models. *Freshwater Biology*, *64*, 405–420. https://doi.org/10.1111/fwb.13230

Neville, H. M., Dunham, J. B., & Peacock, M. M. (2006). Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. *Landscape Ecology*, *21*, 901–916. https://doi.org/10.1007/s10980-005-5221-4

Nilsson, C., Reidy, C. A., Dynesius, M., & Revenga, C. (2005). Fragmentation and flow regulation of the world's large river systems. *Science*, *308*, 405–408. https://doi.org/10.1126/science.1107887

Nordberg, B. J., Mandeville, E. G., Walters, A. W., Burckhardt, J. C., & Wagner, C. E. (2021). Historical data provide important context for understanding declines in cutthroat trout. *North American Journal of Fisheries Management*, *41*, 809–819. https://doi.org/10.1002/nafm.10593

Novinger, D. C., & Rahel, F. J. (2003). Isolation management with artificial barriers as a conservation strategy for cutthroat trout in headwater streams. *Conservation Biology*, *17*, 772–781. https://doi.org/10.1046/j.1523-1739.2003.00472.x

Oliveira, I. C., Alexandre, C. M., Quintella, B. R., & Almeida, P. R. (2020). Impact of flow regulation for hydroelectric production in the movement patterns, growth and condition of a potamodromous fish species. *Ecohydrology: Ecosystems, Land and Water Process Interactions, Ecohydrogeomorphology*, *13*, Article e2250. https://doi.org/10.1002/eco.2250

Paradis, E. (2010). Pegas: An R package for population genetics with an integrated–modular approach. *Bioinformatics*, *26*, 419–420. https://doi.org/10.1093/bioinformatics/btp696

Pebesma, E. (2018). Simple features for R: Standardized support for spatial vector data. *The R Journal*, *10*, 439–446. https://doi.org/10.32614/RJ-2018-009

Pennock, C. A., Healy, B. D., Bogaard, M. R., McKinstry, M. C., Gido, K. B., Cathcart, C. N., & Hines, B. (2024). Translocation in a fragmented river provides demographic benefits for imperiled fishes. *Ecosphere*, *15*, Article e4874. https://doi.org/10.1002/ecs2.4874

Perrier, C., Guyomard, R., Bagliniere, J., Nikolic, N., & Evanno, G. (2013). Changes in the genetic structure of Atlantic Salmon populations over four decades reveal substantial impacts of stocking and potential resiliency. *Ecology and Evolution*, *3*, 2334–2349. https://doi.org/10.1002/ece3.629

Peterson, D. P., & Fausch, K. D. (2003). Upstream movement by nonnative Brook Trout (*Salvelinus fontinalis*) promotes invasion of native Cutthroat Trout (*Oncorhynchus clarkii*) habitat. *Canadian Journal of Fisheries and Aquatic Sciences: Journal Canadien Des Sciences Halieutiques Et Aquatiques*, *60*, 1502–1516. https://doi.org/10.1139/f03-128

Peterson, E. E., Hanks, E. M., Hooten, M. B., Ver Hoef, J. M., & Fortin, M. (2019). Spatially structured statistical network models for landscape genetics. *Ecological Monographs*, *89*, Article e01355. https://doi.org/10.1002/ecm.1355

Petty, J. T., Hansbarger, J. L., Huntsman, B. M., & Mazik, P. M. (2012). Brook Trout movement in response to temperature, flow, and thermal refugia within a complex Appalachian riverscape. *Transactions of the American Fisheries Society*, *141*, 1060–1073. https://doi.org/10.1080/00028487.2012.681102

Pilgrim, B. L., Perry, R. C., Keefe, D. G., Perry, E. A., & Marshall, H. D. (2012). Microsatellite variation and genetic structure of Brook Trout (*Salvelinus fontinalis*) populations in Labrador and neighboring Atlantic Canada: Evidence for ongoing gene flow and dual routes of post-Wisconsinan colonization. *Ecology and Evolution*, *2*, 885–898. https://doi.org/10.1002/ece3.200

Poplar-Jeffers, I. O., Petty, J. T., Anderson, J. T., Kite, S. J., Strager, M. P., & Fortney, R. H. (2009). Culvert replacement and stream habitat restoration: Implications from Brook Trout management in an Appalachian watershed, USA. *Restoration Ecology*, *17*, 404–413. https://doi.org/10.1111/j.1526-100X.2008.00396.x

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959. https://doi.org/10.1093/genetics/155.2.945

R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Reemeyer, J. E., Rumball, D., Mandrak, N. E., & Chapman, L. J. (2024). Seasonal variation in thermal tolerance and hypoxia tolerance of a threatened minnow and a non-imperilled congener: A cautionary tale for surrogate species in conservation. *Conservation Physiology*, *12*, Article coae071. https://doi.org/10.1093/conphys/coae071

Roberts, J. J., Fausch, K. D., Hooten, M. B., & Peterson, D. P. (2017). Nonnative trout invasions combined with climate change threaten persistence of isolated cutthroat trout populations in the southern Rocky Mountains. *North American Journal of Fisheries Management*, *37*, 314–325. https://doi.org/10.1080/02755947.2016.1264507

Rogers, K., Anderson, J. R., Brinkman, S. F., & Martin, A. P. (2022). Inbreeding depression reduces fitness in Colorado's last remaining Greenback Cutthroat Trout: Consequences for management. In J. Gregory (Ed.), *Wild Trout XIII: Reducing the gap between science and public opinion* (pp. 185–194). Wild Trout Symposium.

Saura, S., Bodin, Ö, & Fortin, M. (2014). Stepping stones are crucial for species' long-distance dispersal and range expansion through habitat networks. *Journal of Applied Ecology*, *51*, 171–182. https://doi.org/10.1111/1365-2664.12179

Shao, X., Fang, Y., Jawitz, J. W., Yan, J., & Cui, B. (2019). River network connectivity and fish diversity. *The Science of the Total Environment*, *689*, 21–30. https://doi.org/10.1016/j.scitotenv.2019.06.340

Shetter, D. S. (1968). Observations on movements of wild trout in two Michigan stream drainages. *Transactions of the American Fisheries Society*, *97*, 472–480. https://doi.org/10.1577/1548-8659(1968)97[472:OOMOWT]2.0.CO;2

Shreve, R. L. (1966). Statistical law of stream numbers. *The Journal of Geology*, *74*, 17–37. https://doi.org/10.1086/627137

Stack, T., Harris, A., Fairchild, M., Oyler-McCance, S., Fike, J., Winkelman, D., & Kanno, Y. (2025). *Riverscape genetics of non-native Brook Trout to inform native cutthroat trout conservation* [Data set]. Dryad. https://doi.org/10.5061/dryad.nk98sf84b

Strahler, A. (1957). Quantitative analysis of watershed geomorphology. *Eos, Transactions American Geophysical Union*, *38*, 913–920. https://doi.org/10.1029/TR038i006p00913

Taylor, M. K., & Cooke, S. J. (2012). Meta-analyses of the effects of river flow on fish movement and activity. *Environmental Reviews*, *20*, 211–219. https://doi.org/10.1139/a2012-009

Thomaz, A. T., Christie, M. R., & Knowles, L. L. (2016). The architecture of river networks can drive the evolutionary dynamics of aquatic populations. *Evolution; International Journal of Organic Evolution*, *70*, 731–739. https://doi.org/10.1111/evo.12883

Timm, A., Hallerman, E., Dolloff, C. A., Hudy, M., & Kolka, R. (2016). Identification of a barrier height threshold where Brook Trout population genetic diversity, differentiation, and relatedness are affected. *Environmental Biology of Fishes*, *99*, 195–208. https://doi.org/10.1007/s10641-015-0467-4

Torterotot, J., Perrier, C., Bergeron, N. E., & Bernatchez, L. (2014). Influence of forest road culverts and waterfalls on the fine-scale distribution of Brook Trout genetic diversity in a boreal watershed. *Transactions of the American Fisheries Society*, *143*, 1577–1591. https://doi.org/10.1080/00028487.2014.952449

U.S. Fish and Wildlife Service. (2009). *Endangered and threatened wildlife and plants; Apache Trout* (Oncorhynchus apache) *recovery plan, second revision*. Federal Register 74:170(3 September 2009):45649–45650.

U.S. Geological Survey. (2022). *National Hydrography Dataset*. Accessed August, 2023. https://www.usgs.gov/national-hydrography/access-national-hydrography-products

U.S. Geological Survey. (2023). *3D Elevation Program*. Accessed August, 2023. https://www.usgs.gov/the-national-map-data-delivery

Wagenmakers, E.-J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin & Review*, *11*, 192–196. https://doi.org/10.3758/BF03206482

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Wenger, S. J. (2008). Use of surrogates to predict the stressor response of imperiled species. *Conservation Biology*, *22*, 1564–1571. https://doi.org/10.1111/j.1523-1739.2008.01013.x

White, S. L., Hanks, E. M., & Wagner, T. (2020). A novel quantitative framework for riverscape genetics. *Ecological Applications*, *30*, Article e02147. https://doi.org/10.1002/eap.2147

White, S. L., Keagy, J., Batchelor, S., Langlois, J., Thomas, N., & Wagner, T. (2023). Movement beyond the mean: Decoupling sources of individual variation in Brook Trout movement across seasons. *Environmental Biology of Fishes*, *106*, 2205–2218. https://doi.org/10.1007/s10641-023-01501-2

White, S. L., Rash, J. M., & Kazyak, D. C. (2023). Is now the time? Review of genetic rescue as a conservation tool for Brook Trout. *Ecology and Evolution*, *13*, Article e10142. https://doi.org/10.1002/ece3.10142

Whiteley, A. R., Coombs, J. A., Hudy, M., Robinson, Z., Colton, A. R., Nislow, K. H., & Letcher, B. H. (2013). Fragmentation and patch size shape genetic structure of Brook Trout populations. *Canadian Journal of Fisheries and Aquatic Sciences: Journal Canadien Des Sciences Halieutiques Et Aquatiques*, *70*, 678–688. https://doi.org/10.1139/cjfas-2012-0493

Whiteley, A. R., Coombs, J. A., Hudy, M., Robinson, Z., Nislow, K. H., & Letcher, B. H. (2012). Sampling strategies for estimating Brook Trout effective population size. *Conservation Genetics*, *13*, 625–637. https://doi.org/10.1007/s10592-011-0313-y

Wickham, H. (2016). *Ggplot2: Elegant graphics for data analysis*. Springer.

Winter, D. J. (2012). MMOD: An R library for the calculation of population differentiation statistics. *Molecular Ecology Resources*, *12*, 1158–1160. https://doi.org/10.1111/j.1755-0998.2012.03174.x

Witzel, L. D., & MacCrimmon, H. R. (1983). Redd-site selection by Brook Trout and Brown Trout in southwestern Ontario streams. *Transactions of the American Fisheries Society*, *112*, 760–771. https://doi.org/10.1577/1548-8659(1983)112<760:RSBBTA>2.0.CO;2

Wofford, J. E. B., Gresswell, R. E., & Banks, M. A. (2005). Influence of barriers to movement on within-watershed genetic variation of Coastal Cutthroat Trout. *Ecological Applications*, *15*, 628–637. https://doi.org/10.1890/04-0095

Wood, D. M., Welsh, A. B., & Petty, J. T. (2018). Genetic assignment of Brook Trout reveals rapid success of culvert restoration in headwater streams. *North American Journal of Fisheries Management*, *38*, 991–1003. https://doi.org/10.1002/nafm.10185

Wright, S. (1943). Isolation by distance. *Genetics*, *28*, 114–138. https://doi.org/10.1093/genetics/28.2.114

Young, M. K. (2008). *Colorado River Cutthroat Trout: A technical conservation assessment* (General Technical Report RMRS-GTR-207-WWW). U.S. Forest Service, Rocky Mountain Research Station.

Young, M. K. (2011). Generation-scale movement patterns of Cutthroat Trout (*Oncorhynchus clarkii pleuriticus*) in a stream network. *Canadian Journal of Fisheries and Aquatic Sciences: Journal Canadien Des Sciences Halieutiques Et Aquatiques*, *68*, 941–951. https://doi.org/10.1139/f2011-023

Young, M. K., & Harig, A. L. (2001). A critique of the recovery of Greenback Cutthroat Trout. *Conservation Biology*, *15*, 1575–1584. https://doi.org/10.1046/j.1523-1739.2001.00291.x

Zarri, L. J., Palkovacs, E. P., Post, D. M., Therkildsen, N. O., & Flecker, A. S. (2022). The evolutionary consequences of dams and other barriers for riverine fishes. *BioScience*, *72*, 431–448. https://doi.org/10.1093/biosci/biac004