THESIS

RIVERSCAPE FEATURES AND ISOLATION-BY-DISTANCE SHAPE SPATIAL GENETIC STRUCTURE OF BROOK TROUT IN A COLORADO HEADWATER STREAM NETWORK

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ABSTRACT

RIVERSCAPE FEATURES AND ISOLATION-BY-DISTANCE SHAPE SPATIAL GENETIC STRUCTURE OF BROOK TROUT IN A COLORADO HEADWATER STREAM NETWORK

Understanding the influences of riverscape characteristics on gene flow in stream networks is crucial for managing population connectivity in freshwater species. In this study, we examined the fine-scale genetic structure of non-native Brook Trout (*Salvelinus fontinalis*) in a headwater stream network proposed for future reintroduction of native trout. Using 12 microsatellite loci, we genotyped 757 individual Brook Trout from 22 sampling sites throughout the dendritic stream network and modelled the effects of physical riverscape features on gene flow. Genetic clustering analysis identified four distinct tributary groups, indicating fine-scale population structure, while pairwise genetic differentiation estimates (mean $F_{ST} = 0.04$; mean Jost's $D = 0.06$) revealed some genetic connectivity across the network. Riverscape genetics models identified vertical barriers and steep stream gradients as key factors impeding gene flow, whereas higher order mainstem streams were more conducive to trout movement. Gene flow was stronger in the downstream direction, and models with interaction terms revealed that asymmetries between upstream and downstream gene flow were more pronounced in stream reaches with barriers and steep gradients. Mantel tests confirmed that both waterway distance between sites and riverscape resistance significantly influence genetic connectivity across the network. Overall, this study demonstrates that spatial genetic patterns in stream networks are shaped by a combination of isolation-by-distance, riverscape resistance, and asymmetric stream flow. Our findings suggest that this reintroduction area provides sufficient genetic connectivity to support a metapopulation of native trout.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

RIVERSCAPE FEATURES AND ISOLATION-BY-DISTANCE SHAPE SPATIAL GENETIC STRUCTURE OF BROOK TROUT IN A COLORADO HEADWATER STREAM NETWORK

Introduction

Genetic population structure in freshwater systems is shaped by numerous factors, including habitat connectivity, species life history, and the presence of natural or anthropogenic barriers to movement (Davis et al. 2018). In riverine environments, gene flow is further influenced by the linear structures of stream networks, where dendritic architectures and asymmetric stream flow create patterns of isolation and connectivity based on network positioning. For example, sites near the upstream ends of network branches are often more isolated, while downstream sites near network confluences experience greater connectivity (Thomaz et al. 2016, White et al. 2020). The evolving 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. 2016, Davis et al. 2018). This knowledge is crucial for managing genetic connectivity in stream networks to promote population resilience and adaptive potential (Greene et al. 2010, Schindler et al. 2015), particularly in the face of ongoing habitat fragmentation and environmental change.

Unlike in terrestrial systems, where organisms can take multiple paths across a landscape, movement paths for obligatory aquatic species in riverscapes are clearly defined along linear stream channels. This confinement allows for precise quantification of riverscape variables that might facilitate or impede movement along these paths. Population connectivity in riverscapes is often influenced by both isolation-by-distance (IBD; Wright 1943), where geographic separation leads to genetic differentiation, and isolation-by-resistance (IBR; McRae 2006), where landscape features mediate gene flow by influencing species movement. Isolation-by-distance is a commonly observed genetic pattern in stream fishes (Hanfling & Wheetman 2006, Kanno et al. 2011, Nakajima et al. 2023) and is typically assessed by relating genetic distances between populations to the waterway distances separating them. Isolation-by-resistance approaches aim to provide additional information by estimating "resistance distances" based on specific landscape variables and their effects on gene flow (McRae 2006, Zeller et al. 2012, White et al. 2020). Some riverscape studies have applied IBR approaches by defining resistance surfaces and linking genetic distance to cumulative resistance between populations (Inoue & Berg 2017, Escalante et al. 2018), but these surfaces are often parametrized a priori based on expert opinion or empirical estimates (Zeller et al. 2012, Spear et al. 2010). Directly modelling resistance to gene flow from genetic data can provide a more accurate understanding of the factors influencing connectivity (Sartor et al. 2022). However, attempts to do so using common statistical techniques in landscape genetics have been criticized for failing account for important riverscape characteristics such as linear network architectures and asymmetric stream flow (Ver Hoef & Peterson 2010, White et al. 2020, Chafin et al. 2021), making them poorly suited for riverscape analyses. Recent applications of network models have led to the development of more rigorous techniques for modelling connectivity in river systems (Murphy et al. 2016, Hanks et al. 2017, Peterson et al. 2019, White et al. 2020). Using spatially explicit graph-theoretic frameworks, these methods account for the spatial autocorrelation arising from the dendritic structures of stream habitats and directional asymmetries in geneflow caused by differences between upstream and downstream movement (Hanks and Hooten 2013, Hanks et al. 2017, White et al 2020).

Riverscape genetics may be particularly relevant for informing conservation strategies for native inland salmonid species, which have become increasingly confined to small headwater habitats 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 translocation of native fish into headwater habitats devoid of nonnative competitors, but these reintroductions may be less successful when recovery habitats are small and fragmented (Harig et al. 2000, Hayes & Banish 2017).

Additionally, low genetic diversity in available broodstocks of some native species could limit the success of reintroduction efforts due to inbreeding depression (Biermann & Havlick 2021, Rogers et al. 2022). Therefore, identifying reintroduction habitats with sufficient patch size and connectivity could help buffer against genetic drift and improve the success of native fish recovery efforts (Dunham et al. 2014, Pennock et al. 2024). Managing the trade-off between isolating sensitive populations from invasive species while promoting connectivity within recovery areas requires a comprehensive understanding of factors influencing population connectivity and genetic isolation in headwater trout populations (Fausch et al. 2009).

In this study, we examined the spatial genetic structure of a metapopulation of nonnative Brook Trout (*Salvelinus fontinalis*) occupying a 60-km headwater stream network proposed for future reintroduction of federally threatened Greenback Cutthroat Trout (*Oncorhynchus virginalis stomias*), a species of conservation concern. 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 is unknown. Using Brook Trout as surrogate species to characterize salmonid gene flow, we applied a recently developed statistical framework for riverscape genetics (White et al. 2020) to quantify the effects of specific riverscape features on gene flow and estimate levels of migration throughout the network. This knowledge is critical for predicting future patterns of genetic connectivity among reintroduced Greenback Cutthroat Trout and assessing the feasibility of establishing a robust metapopulation in the reintroduction area. This work may also provide useful insights for the conservation of Brook Trout within their native range, where genetic connectivity is a key management consideration (White et al. 2023).

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, USA. The network is located in Rocky Mountain National Park and Arapaho and Roosevelt National Forests and encompasses 60 km of headwater stream habitat.

Major tributary streams in the network include Corral Creek, Willow Creek, the upper Cache la Poudre River, and Hague Creek (Figure 1). Long Draw Reservoir was constructed for water supply in 1930, and the dam at the terminal end of the reservoir blocks upstream fish passage. Water release from the reservoir typically occurs between mid-May and mid-September and ceases during winter, causing the section of stream below the reservoir to experience an annual cycle of dewatering and flow intermittency. Our study streams are occupied primarily by Brook Trout, with occasional occurrence of hybridized non-native cutthroat trout originating from stocked reservoirs and tributaries. According to historical stocking records, Brook Trout stocking occurred within and around the study area beginning in 1892, with no records of stocking after 1955 (Colorado Parks and Wildlife, unpublished data). The watershed making up our study area is a proposed reintroduction habitat for federally-threatened Greenback Cutthroat Trout.

Field sampling and genotyping

During the summer and fall of 2018 and 2019, we collected Brook Trout tissue samples from 22 sites by backpack electrofishing (Figure 1). Sampling sites were stream reaches approximately 100 meters in length. Multiple sites were sampled along each major tributary drainage to capture 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 with total lengths ranging from 56–260 mm were genotyped.

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 selected 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). Genomic DNA was extracted from Brook Trout fin clip specimens 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 genomic DNA, 5 µl Qiagen Multiplex PCR Mastermix, 0.04- 0.1 µl of each forward and reverse PCR primer (10 µM), and 2.18-2.2 µl nuclease free water. The thermal profile for PCR amplification consisted of denaturing at 95°C for 15 minutes, 35 cycles of denaturation at 95°C for 45 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 2 minutes, followed by a final extension of 60°C for 30 minutes. 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

Using microsatellite genotype data, we assessed potential 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 and Cockerham 1984) and D (Jost 2008) and corresponding 95% confidence intervals were calculated using the R packages “hierfstat” for F_{ST} and “diversity” (Keenan et al. 2013) for D.

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 within different regions of the study area, we performed additional STRUCTURE runs with subsets of study sites separated into the four major 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. Within each STRUCTURE run, we merged replicates of each K using the R package "pophelper" (Francis 2017) and visualized individual cluster assignment probabilities using "ggplot" (Wickam 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 plotted resulting pie charts on a map of the study area.

Riverscape genetic analysis

We applied the Bidirectional Geneflow in Riverscapes (BGR) model (White et al. 2020) to examine the influences of six riverscape variables (Table 1) on Brook Trout gene flow. This method models directional 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 delineating the study streams into a spatially structured ecological network (SSEN), a graph made up of nodes (points on the stream network) and edges (stream segments connecting nodes). The network consists of both observed nodes (sampling locations) and unobserved nodes which are positioned to minimize heterogeneity of riverscape characteristics within edges (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 et al. 2017, Petersen et al. 2019, White et al. 2020). Thus, the BGR model infers the effects of measured riverscape covariates on gene flow using observed genetic distance matrices as input data.

To construct our SSEN, observed nodes were placed at all sampling locations, and additional unobserved nodes were placed at every tributary confluence, following White et al. (2020). Riverscape covariates (Table 1) 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). 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 and 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 which 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 upstream of the confluence point between the Long Draw Reservoir outflow and the Cache la Poudre River (see Figure 1) 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, non-binary 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. All models were run in R using a Markov Chain Monte Carlo (MCMC) sampler to estimate posterior distributions of the regression coefficients for each covariate (i.e., their effects on trout gene flow). For model selection, we fit models with all possible combinations of covariates and models were ranked using deviance information criteria (DIC). To address potential collinearity among covariates, we calculated Pearson correlations between all covariates and excluded covariate pairs with a correlation magnitude > 0.4 from being included in the same model. Models with fewer than 4 variables were run for 50,000 MCMC iterations with 25,000 burn-in, and models with 4 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 the effects of riverscape covariates on trout gene flow from the best models, we considered models within 4 DIC values of the model with the lowest DIC as competing models (Anderson 2008, Cain and Zhang 2019), and averaged covariate effects across competing models using DIC model weights. Following Wagenmakers and Farrell (2004), model weights were calculated as

$$m_i(DIC) = \frac{\exp\left(-\frac{1}{2}\Delta_i(DIC)\right)}{\sum_{k=1}^{K}\exp\left(-\frac{1}{2}\Delta_k(DIC)\right)}$$

where m_i(DIC) is the weight for model i so that $\sum m_i$(DIC) = 1. Separately for F_{ST} and Jost's D, final 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 one.

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

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

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_{ijk} 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 calculation of edge weights. Edge weights are directed, such that w_{ij} need not equal w_{ji}, accommodating potential asymmetries in migration rates based on flow direction and its interaction terms. The estimated resistance to geneflow for each edge was calculated as 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) 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) was also evaluated using Mantel tests to assess correlations between pairwise genetic distances and waterway distances between sampling sites. Mantel tests were performed using the R package "vegan" (Dixon 2003) with 9999 permutations.

Results

Genetic diversity and differentiation

Across 22 sites, a total of 757 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 A2) which did not warrant the exclusion of any markers. Site-level observed heterozygosity (H_O) ranged from 0.51– 0.69 with a mean of 0.61, expected heterozygosity (H_E) ranged from 0.53– 0.69 with a mean of 0.62, and rarefied allelic richness (A) ranged from 3.9 – 6.9 with a mean of 5.7 (Table A1). Measures of genetic diversity were lowest at sites in the Corral Creek drainage compared to 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 A3), 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 is K = 4 (Figure A2). Although the largest value for ΔK occurred at K = 2, we concluded that K = 4 provides the best explanation because 1) the ΔK method has been shown to frequently identify 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 an additional modal value for ΔK at K = 4 (figure A2). The four genetic clusters identified by STRUCTURE correspond 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 A3). However, some moderate genetic structuring was detected in the Hague Creek drainage (Figure A5).

Effects of riverscape covariates on Brook Trout gene flow

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: flow direction, stream gradient, barriers, dam regulation, and 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, statistically significant negative effects on Brook Trout gene flow were observed for barriers (posterior mean = -4.96, 95% CRI [-7.39, -3.12]) and stream gradient (posterior mean = -1.97, 95% CRI [-4.21, -0.71]), while significantly positive effects were observed for downstream flow direction (posterior mean = 0.39, 95% CRI [0.16, 0.57]), Shreve's stream order (posterior mean = 0.56, 95% CRI [0.00, 1.18]), and unexpectedly, dam regulation (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 on 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, 0.89]) or the interaction between stream gradient and flow direction (posterior mean = 0.87, 95% CRI [-0.57, 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, -3.15]) and stream gradient (posterior mean = -3.25, 95% CRI [-5.35, -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, 0.62]) and Strahler's stream order (posterior mean = -0.26, 95% CRI [-0.87, 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, 3.06]), indicating that differences between rates of upstream and downstream movement were more pronounced on steeper gradient streams.

Relative migration rates

Edge weights consistently indicated higher relative migration rates in the downstream direction compared to upstream (Figure A4). When averaging percent differences between upstream and downstream edge weights across all SSEN edges, downstream migration rates were 51.5% higher (using F_{ST}) and 57.8% higher (using Jost's *D*) than upstream rates. Due to the significant interaction between barriers and flow direction, the discrepancy between upstream and downstream migration rates was amplified by the presence of barriers; upstream migration rates on SSEN edges with barriers were, on average, 198.4% lower (F_{ST}) and 197.9% lower (Jost's D) than downstream rates. Additionally, edge weights calculated using Jost's D model results showed a mediating effect of stream gradient on directional asymmetries in movement. Upstream migration rates on high-gradient edges (75th percentile) were, on average, 120.7% lower than downstream rates, while upstream migration on low-gradient edges (25th percentile) averaged only 49.8% lower compared to downstream. 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, estimated migration rates on dam-regulated edges were 56.4% higher than those on free-flowing edges.

Isolation-by-distance and isolation-by-resistance

Mantel tests revealed statistically significant patterns of isolation-by-distance (IBD) and isolation-by-resistance (IBR) in this stream network (Figure 4). 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 ($r = 0.48$, $p = 0.0001$). Mantel statistics indicated that the strengths of the IBD and IBR correlations were similar.

Discussion

This study revealed that both isolation-by-distance and riverscape resistance play significant roles in shaping the spatial genetic structure of a headwater trout metapopulation. Fine-scale population structure was evident, 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 (Pilgrim et al. 2012, Kelson et al. 2015, White et al. 2020). Brook Trout typically spawn in low-order streams near the upstream ends of network branches (Witzel and 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 fine-scale genetic structure is common in Brook Trout populations (Hudy et al. 2010, Kanno et al. 2011), and reflects that most individuals exhibit relatively short dispersal distances (< 1 km annually) (Hutchings and Gerber 2002, White et al. 2023). Nonetheless, genetic connectivity has been detected between Brook Trout populations at larger spatial scales (Aunins 2015, Kelson et al. 2015) and mark-recapture studies have shown that individual Brook Trout occasionally disperse over longer distances (Gowan and Fausch 1996, Petty et al. 2012), sometimes moving more than 10 km within a season (Shetter 1968). Despite the genetic segregation of tributary groups in our study, pairwise genetic distances among sites indicate that genetic connectivity persists across the stream network (Figure 2) which could be facilitated by a combination of infrequent long-distance dispersal events and sequential short-distance dispersals occurring in a stepping-stone manner (Saura 2013).

Our riverscape genetic analysis indicated that genetic connectivity throughout this stream network is significantly influenced by riverscape features that facilitate or impede trout movement. Variables such as 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 patterns 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 movement due to vertical barriers (Wofford et al. 2005, Neville et al. 2006, Deiner et al. 2007, Torterotot et al. 2014, Kelson et al. 2015, Timm et al. 2015, White et al. 2020). We also found that steeper stream gradients negatively affected geneflow, which has been similarly detected in some riverscape genetics studies (Narum et al. 2008, Kanno et al. 2011, Nakajima et al. 2023), 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 step falls which 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 at lower gradient segments. This aligns with our findings and suggests that steeper gradients hinder Brook Trout movement, but upstream passage can still occur when impassable falls are not present.

Our results highlight the BGR framework's ability to model asymmetries in gene flow, providing a key advantage over other statistical methods in riverscape genetics. We found a positive effect of downstream direction on gene flow, consistent with most other studies on lotic fishes which have reported downstream-biased gene flow (Morrissey and Ferguson 2011, Lamphere and Blum 2012, White et al. 2020). Despite this common trend, several studies have observed upstream-biased movement among adult Brook Trout (Adams et al. 2000, Peterson and Fausch 2003, Hansbarger et al. 2010, Gutowsky et al. 2023) 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 alevins and the displacement of smaller-bodied individuals during high flow events (Morissey and 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 vertical waterfalls to block upstream passage while allowing downstream movement (Nathan et al. 2019). The significant interaction between gradient and flow direction also coheres because steep slopes are more energetically costly to ascend than to descend, and high-gradient stream segments often contain vertical step falls which could be disproportionately restrictive to upstream movement. The BGR framework's ability to formally model the effects of flow direction and its interactions with other riverscape attributes enabled us to uncover novel insights into 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 surprising 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 and 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 are genetically similar to nearby locations, particularly those in the adjacent Willow Creek tributary (Figures 2 and 3). Furthermore, our trout sampling 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 (Korman and Campana 2009, Kelly et al. 2017, 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.

The estimated relative migration rates on SSEN edges provide a useful visualization of the mosaic of riverscape resistance contributing to the observed genetic differentiation between, and connectivity within, the major tributaries in the network (Figure 3a). Each of the four tributary groups are separated by regions of relatively high riverscape resistance, primarily due to steep stream gradients and barriers. Within each tributary, genetic homogenization appears to be facilitated by lower riverscape resistance, with the exception of Hague Creek which contains a barrier and the steepest stream gradients in the network (Figure A1). Some weak hierarchical genetic structure was observed among sites within the Hague Creek drainage (Figure A5), but the degree of genetic differentiation within this tributary is considerably lower than the variation between tributaries. Considering the significance of isolation-by-distance in this network, it is likely that the close spatial proximity of sites within the Hague Creek drainage contributes to their genetic similarity despite high riverscape resistance. Furthermore, downstream movement, which we found to be less affected by steep gradients and barriers, may also play a role in maintaining connectivity among Hague Creek sites.

Our results add to a growing body of information about the factors influencing population connectivity in aquatic systems. There is a broad consensus among conservation biologists that landscape connectivity promotes population viability in many species (Hodgson et al. 2010, Correa Ayram et al. 2016, Keely et al. 2018, Allendorf et al. 2022). Although reproductive isolation of groups across diverse environmental conditions can facilitate local adaptation (Kawecki and Ebert 2004), connectivity is important for maintaining the genetic diversity of small populations that are vulnerable to genetic drift and increased extinction risk (Allendorf et al. 2022). Stream fish populations are uniquely susceptible to isolation because movement of individuals is restricted to connected waterways (Davis et al. 2017, Shao et al. 2019). Thus, in native fish conservation, selecting reintroduction habitats with ample connectivity might improve the success of recovery efforts. Although we studied nonnative Brook Trout, our study area is a proposed reintroduction habitat for native Greenback Cutthroat Trout. Insights about salmonid geneflow in this system can help predict future patterns of connectivity among reintroduced trout and could be used to optimize release strategies to promote connectivity. Our results show that while trout segregate into genetically distinct tributaries, a level of connectivity persists across the study area, which could provide resilience to disturbance (Christie and Knowles 2015) and facilitate genetic exchange to buffer against genetic drift (Allendorf et al. 2022). Connectivity in this region is impeded by vertical barriers and areas of high stream gradient. However, downstream movement appears to be less affected by these features, suggesting that stocking native fish at farther upstream sites may enable more expedient colonization of the recovery area. For future translocation efforts, our results suggest that reintroduction areas with gradual stream gradients and few vertical waterfalls might be more conducive to connectivity. However, the presence of these features does not entirely preclude connectivity among salmonid populations, and even infrequent migration (<10 migrants per generation) has been shown to provide functional genetic connectivity in trout (Nathan et al. 2017). Our results may also be informative for the management of Brook Trout in their native range, where they are in decline (White et al. 2023), as well as other lotic fish species with similar dispersal capabilities.

Table 1. Descriptions of riverscape variables included in this study. All listed covariates were tested for their effects on Brook Trout geneflow using the Bidirectional Geneflow in Riverscapes model (White et al. 2020).

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 *ij*.	Binary	The presence of complete or partial barriers to trout movement will reduce gene flow. Vertical drops block the upstream movement of brook trout, restricting gene flow and increasing genetic differentiation (Kondratieff and Myrick 2006, Gomez-Uchida and Ruzzante 2009, White et. al 2020).
Mean stream gradient	The mean stream gradient of edge *ij,* calculated as the ratio of elevation change (meters) to distance (meters).	0.003 – 0.257	Higher stream gradients will reduce gene flow. Steep stream gradients create resistance to upstream movement of fish, leading to genetic differentiation (Davis et al. 2019, Nakajima et al. 2023)
Dam regulation	Whether edge *ij* 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 and 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 greater in the downstream direction. Trout dispersal is often biased in the downstream direction (Morrissey and Ferguson 2011, Lamphere and Blum 2012, White et al. 2020)
Shreve's stream order	Link magnitude (i.e. the number of tributary confluences upstream) of the stream segment containing edge *ij*.	1-8	Gene flow will be greater on higher order streams. High order mainstem 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 of the stream segment containing edge *ij*.	1-3	Gene flow will be greater on higher order streams. See hypothesis for Shreve's stream order (above).
Barriers × direction	Interaction between barriers and flow direction.	Binary	Barriers will have a greater influence on upstream movement compared to downstream. 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	Stream gradient will have a greater influence on upstream movement compared to downstream. Steeper stream gradients might be more restrictive to upstream movement due to the presence of step falls and a greater energy expenditure required to gain vs. lose elevation (Adams et al. 2000).

Table 2. Deviance Information Criteria (DIC) values of the top 5 riverscape genetics models 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) separated by ×. Competing models (those within 4 DIC points of the model with lowest DIC) are shown in italic font and were averaged based on model weights to obtain final parameter estimates.

F_{ST}

Variables	**DIC**	**Model weight**
Direction + Gradient + Barriers + Dam Reg. + Shreve + (Bar.×Dir.)	*-1388.3*	*0.35*
Direction + Gradient + Barriers + Dam Reg. + (Bar.×Dir.) + (Grad.×Dir.)	*-1387.9*	*0.27*
Direction + Gradient + Barriers + Dam Reg. + Shreve + (Bar.×Dir.) + (Grad.×Dir.)	*-1387.8*	*0.23*
Direction + Gradient + Barriers + Dam Reg. + Strahler + (Bar.×Dir.)	*-1386.5*	*0.15*
Direction + Gradient + Barriers + Shreve + (Bar.×Dir.) + (Grad.×Dir.)	-1378.4	NA

Jost's *D*

Variables	**DIC**	**Model weight**
Direction + Gradient + Barriers + Dam Reg. + (Bar.×Dir.) + (Grad.×Dir.)	*-1249.6*	*0.44*
Direction + Gradient + Barriers + Dam Reg. + Strahler + (Bar.×Dir.) + (Grad.×Dir.)	*-1249.1*	*0.31*
Direction + Gradient + Barriers + Dam Reg. + Shreve + (Bar.×Dir.) + (Grad.×Dir.)	*-1247.0*	*0.16*
Direction + Gradient + Barriers + Dam Reg. + (Bar.×Dir.)	*-1246.4*	*0.09*
Direction + Gradient + Barriers + (Bar.×Dir.) + (Grad.×Dir.)	-1245.4	NA

Table 3. Estimated β values (posterior mean) and 95% credible intervals (CRI) from top-ranked BGR 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 shown in italics.

F_{ST}

Variable	***β***	**95% CRI**
Intercept	3.67	(3.24, 4.11)
Flow direction	0.39	(0.16, 0.57)
Shreve's stream order	0.56	(0.00, 1.18)
Strahler's stream order	*0.36*	*(-0.19, 0.89)*
Dam regulation	0.49	(0.18, 0.78)
Barriers	-4.96	(-7.39, -3.12)
Stream gradient	-1.97	(-4.21, -0.71)
Gradient × direction	*0.87*	*(-0.57, 2.46)*
Barriers × direction	4.86	(2.95,7.34)

Jost's *D*

Variable	***β***	**95% CRI**
Intercept	3.73	(3.34, 4.26)
Flow direction	0.25	(0.04, 0.46)
Shreve's stream order	*0.05*	*(-0.49, 0.62)*
Strahler's stream order	*-0.26*	*(-0.87, 0.34)*
Dam regulation	0.39	(0.07, 0.71)
Barriers	-5.00	(-7.41, -3.15)
Stream gradient	-3.25	(-5.35, -1.30)
Gradient × direction	1.50	(0.09, 3.06)
Barriers × direction	4.95	(3.06, 7.39)

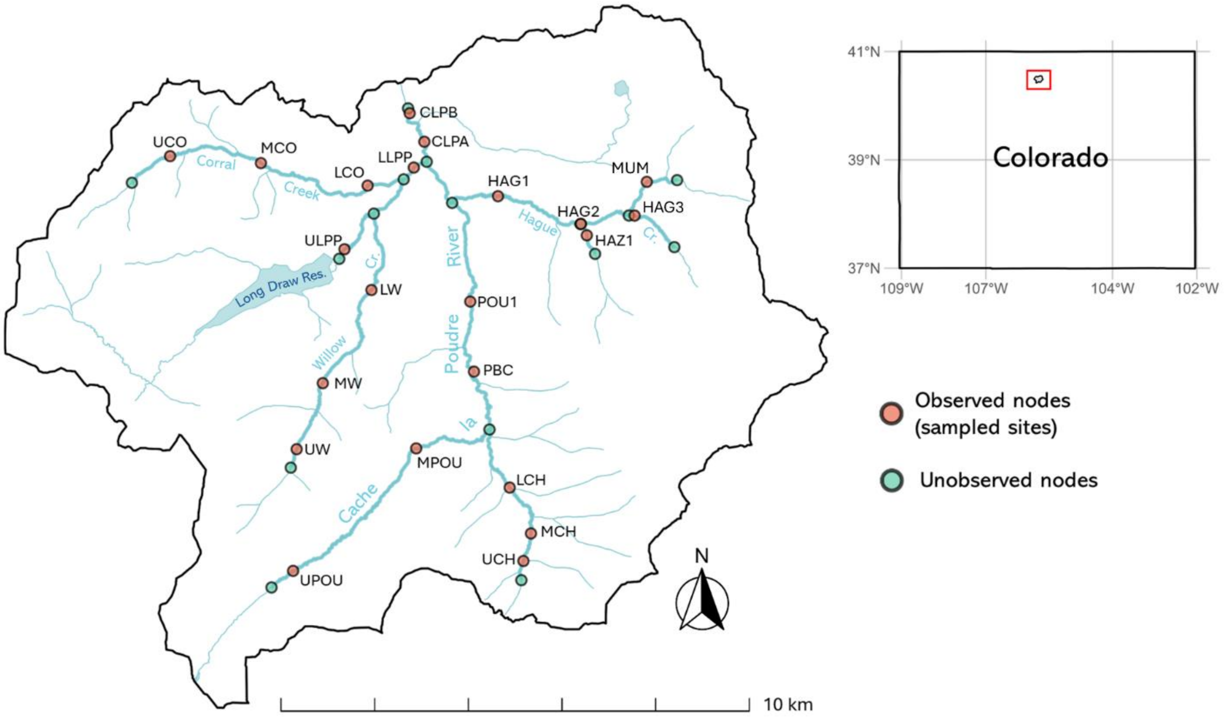

Figure 1. A map of the upper Cache la Poudre River watershed (flow direction: North) showing locations of nodes in the spatially structured ecological network. Observed nodes represent the locations of 22 Brook Trout sampling sites.

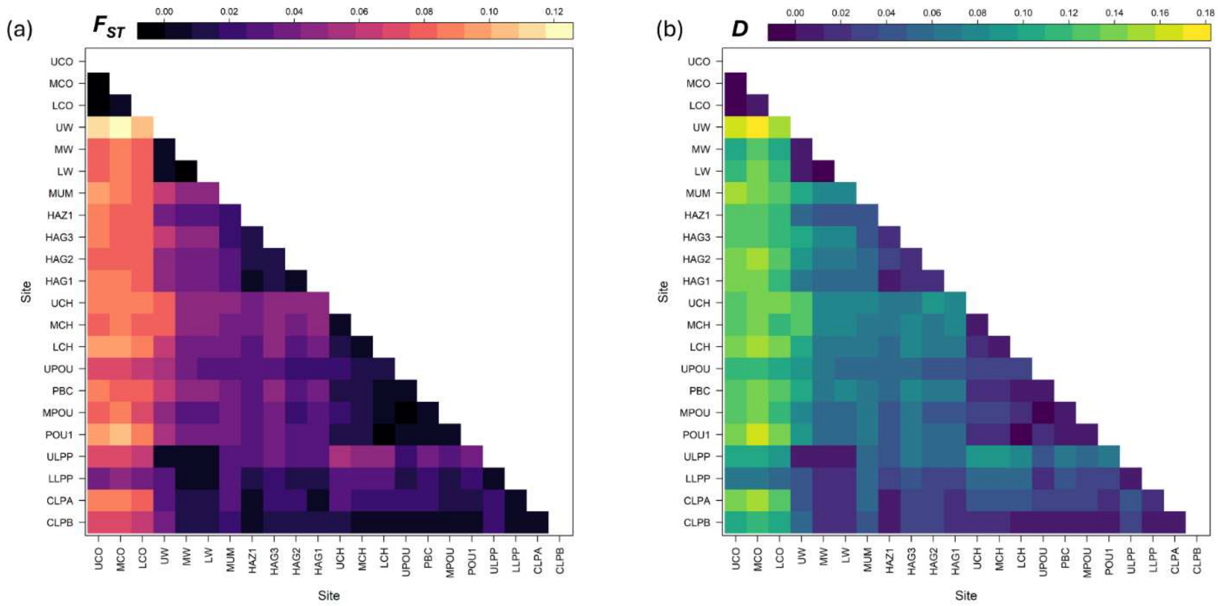

Figure 2. Heatmaps of pairwise genetic distance between 22 Brook Trout sampling sites in the upper Cache la Poudre River watershed. (a) Pairwise F_{ST} (Weir and Cockerham 1984) and (b) pairwise D (Jost 2008). Refer to Figure 1 for site locations.

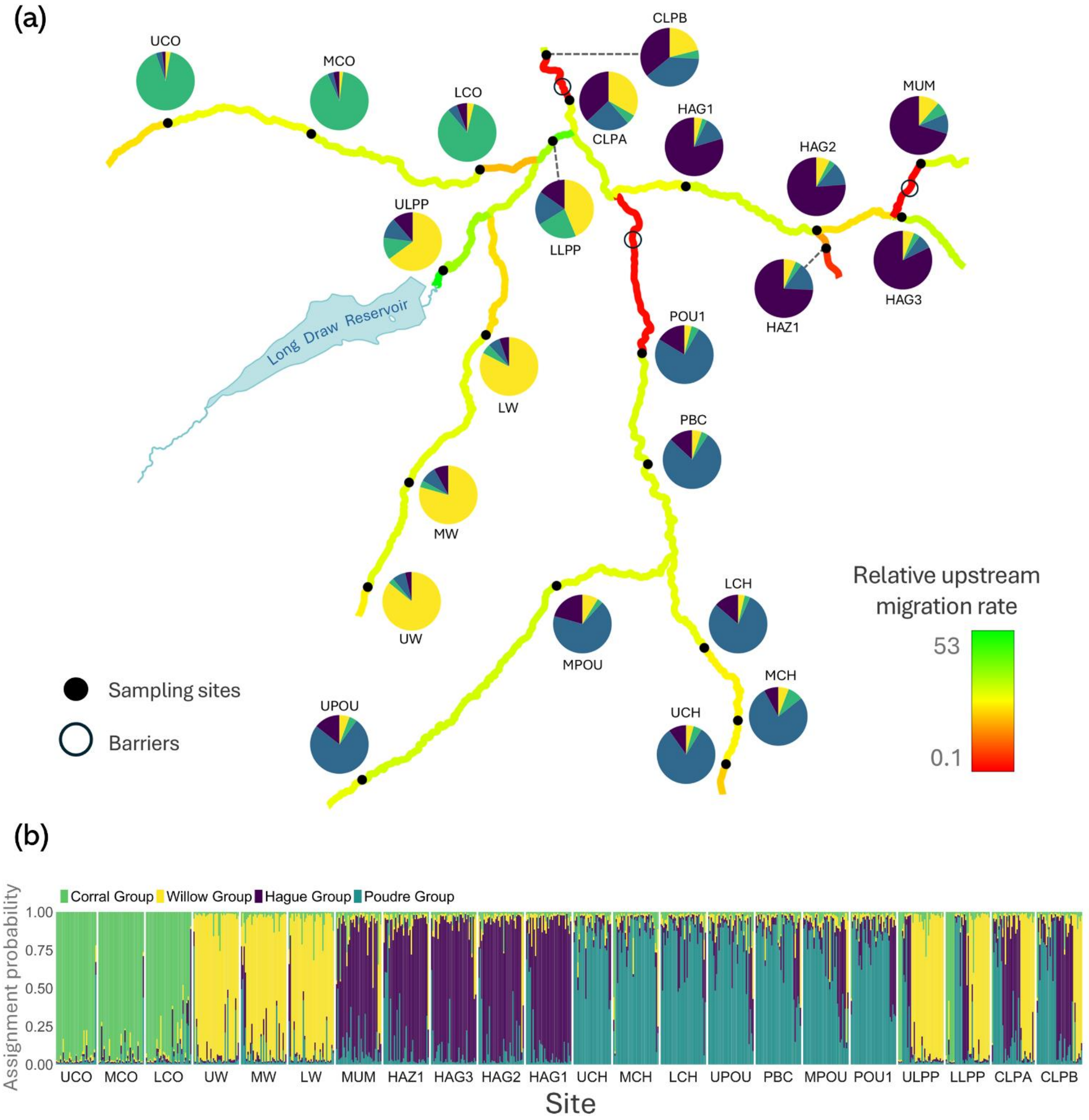

Figure 3. (a) Estimated relative upstream migration rates on SSEN edges, quantified from the estimated effects of riverscape covariates on Brook Trout geneflow from top-ranked BGR models (using pairwise Jost's *D* as the response genetic distance matrix). Pie charts show mean site-level assignment proportions to 4 genetic clusters from the program STRUCTURE. (b) Individual STRUCTURE assignment probabilities to 4 genetic clusters across 22 Brook Trout sampling sites.

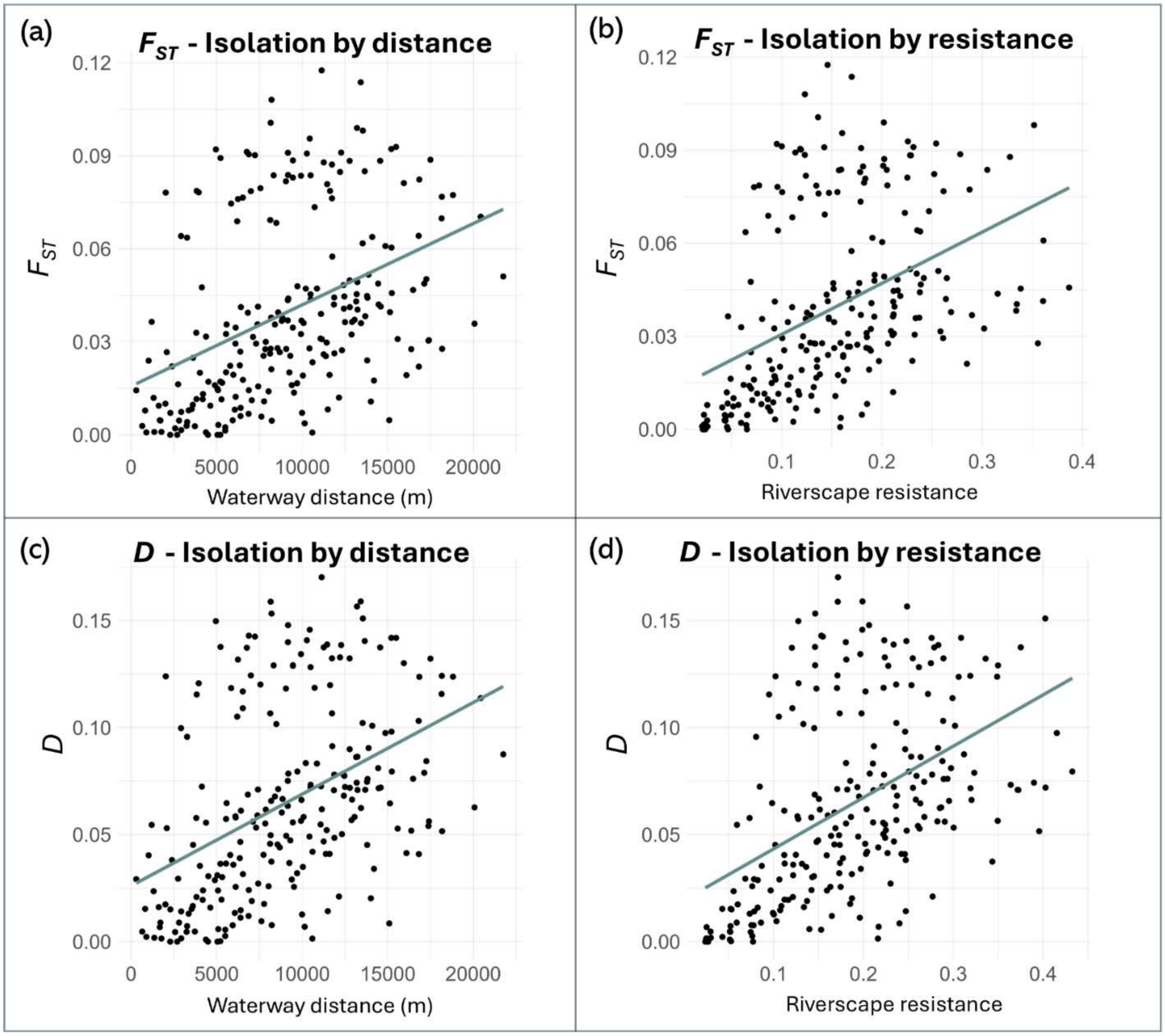

Figure 4. Scatter plots showing isolation-by-distance and isolation-by-resistance between all site pairs. (a) Pairwise F_{ST} plotted against pairwise waterway distance. (b) Pairwise F_{ST} plotted against cumulative riverscape resistance (from BGR model results). (c) Pairwise Jost's D plotted against pairwise waterway distance. (d) Pairwise Jost's D plotted against cumulative riverscape resistance.

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APPENDIX 1

Table A1. Numbers of individuals sampled (N) and genetic summary statistics for Brook Trout collected from 22 sampling sites in the Long Draw region of Colorado. Numeric values for genetic summary statistics are the means across 12 microsatellite loci of observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (*A*), and inbreeding coefficient (F_{IS}).

Site	**N**	H_O	H_E	***A***	F_{IS}
UCO	31	0.511	0.531	3.888	0.039
MCO	35	0.571	0.550	4.142	-0.046
LCO	35	0.543	0.544	4.224	0.003
UW	35	0.555	0.573	4.894	0.026
MW	35	0.600	0.593	5.071	-0.015
LW	35	0.636	0.608	4.874	-0.041
MUM	35	0.643	0.628	5.560	-0.023
HAZ1	35	0.595	0.625	6.366	0.033
HAG3	35	0.621	0.620	5.746	0.001
HAG2	35	0.636	0.685	6.577	0.063
HAG1	35	0.610	0.630	6.504	0.024
UCH	29	0.615	0.621	5.500	0.015
MCH	35	0.631	0.625	5.632	-0.023
LCH	35	0.605	0.626	6.316	0.028
UPOU	35	0.693	0.667	5.950	-0.038
PBC	35	0.664	0.644	5.928	-0.043
MPOU	35	0.657	0.655	6.316	-0.008
POU1	35	0.612	0.624	6.007	0.015
ULPP	35	0.600	0.624	5.712	0.042
LLPP	34	0.603	0.634	6.389	0.041
CLPA	33	0.609	0.644	6.789	0.035
CLPB	35	0.636	0.641	6.916	-0.001

Table A2: *P*-values for deviations from Hardy Weinberg equilibrium (HWE) for 22 Brook Trout sampling sites across 12 loci using 1000 Monte Carlo permutations. Rows are identifiers for Brook Trout sampling sites and columns are the names of 12 microsatellite loci. P-values with asterisks (*) indicate statistical significance of deviation from HWE after Bonferroni correction for multiple comparisons across 264 tests.

	Sfo-C113	**Sfo-C115**	**Sfo-C129**	**Sfo-C38**	**Sfo-C88**	**Sfo-D91**	**Sfo-B52**	**Sfo-C24**	**Sfo-C28**	**Sfo-C79**	**Sfo-C86**	**Sfo-D75**
UCO	0.895	0.516	0.271	0.341	0.637	0.026	0.889	1.000	0.089	1.000	0.815	0.295
MCO	0.364	0.871	0.800	1.000	0.398	0.688	0.423	0.699	0.384	1.000	0.315	0.891
LCO	0.480	0.102	0.289	0.157	0.086	0.541	0.731	0.490	0.684	1.000	0.241	0.180
UW	0.791	0.638	0.008	1.000	0.557	0.112	0.873	0.334	0.230	1.000	0.630	0.543
MW	0.524	0.682	0.604	0.580	0.232	0.993	0.388	0.367	0.042	1.000	0.325	0.834
LW	0.647	0.868	0.464	0.912	0.795	0.634	0.916	0.457	0.154	1.000	0.522	0.142
MUM	0.922	0.402	0.129	0.007	0.196	0.297	0.454	0.928	0.653	1.000	0.976	0.152
HAZ1	0.081	0.318	0.046	0.625	0.588	0.327	0.259	0.675	0.895	1.000	0.466	0.253
HAG3	0.729	0.703	0.612	0.575	0.649	0.618	0.120	0.207	0.908	1.000	0.033	0.049
HAG2	0.271	0.771	0.384	0.200	0.952	0.000*	0.170	0.232	0.820	1.000	0.792	0.264
HAG1	0.697	0.924	0.529	0.192	0.910	0.556	0.088	0.762	0.428	1.000	0.096	0.133
UCH	0.409	0.933	0.879	0.101	0.723	0.004	0.308	0.814	0.300	1.000	0.809	0.343
MCH	0.869	0.308	0.769	1.000	0.227	0.453	0.261	0.046	0.675	1.000	0.367	0.041
LCH	0.127	0.552	0.467	0.205	0.815	0.014	0.110	1.000	0.498	1.000	0.906	0.557
UPOU	0.885	0.130	0.983	0.180	0.664	0.976	0.529	0.860	0.250	1.000	0.034	0.853
PBC	0.238	0.594	0.600	0.547	0.030	0.552	0.030	0.838	0.428	0.582	0.846	0.343
MPOU	0.753	0.449	0.682	0.740	0.371	0.428	0.395	0.386	0.657	1.000	0.381	0.259
POU1	0.019	0.485	0.558	0.133	0.874	0.260	0.044	0.904	0.251	1.000	0.217	0.880
ULPP	0.679	0.089	0.230	0.784	0.006	0.890	0.317	0.552	0.478	1.000	0.777	0.273
LLPP	0.480	0.076	0.053	0.054	0.132	0.602	0.245	0.793	0.152	1.000	0.493	0.817
CLPA	0.599	0.306	0.432	0.905	0.047	0.288	0.030	0.937	0.617	1.000	0.490	0.847
CLPB	0.070	0.880	0.327	0.532	0.080	0.275	0.440	0.550	0.606	1.000	0.906	0.265

Table A3. Matrices of pairwise F_{ST} (bottom triangular) and Jost's D (top triangular) between 22 Brook Trout sampling locations. Row and column headings indicate sampling location identifiers.

	UCO	**MCO**	**LCO**	**UW**	**MW**	**LW**	**MUM**	**HAZ1**	**HAG3**	**HAG2**	**HAG1**	**UCH**	**MCH**	**LCH**	**UPOU**	**PBC**	**MPOU**	**POU1**	**ULPP**	**LLPP**	**CLPA**	**CLPB**
UCO		0.000	0.000	0.159	0.107	0.118	0.151	0.132	0.132	0.139	0.140	0.132	0.124	0.142	0.114	0.133	0.130	0.146	0.102	0.061	0.143	0.107
MCO	0.000		0.002	0.170	0.129	0.137	0.138	0.129	0.128	0.148	0.143	0.142	0.137	0.157	0.116	0.134	0.140	0.159	0.105	0.072	0.150	0.118
LCO	0.000	0.087		0.153	0.109	0.115	0.129	0.117	0.120	0.132	0.121	0.139	0.120	0.141	0.098	0.124	0.119	0.138	0.096	0.055	0.124	0.100
UW	0.002	0.083	0.036		0.007	0.001	0.097	0.058	0.101	0.090	0.072	0.124	0.124	0.103	0.088	0.102	0.084	0.091	0.010	0.041	0.045	0.056
MW	0.114	0.077	0.027	0.049		0.000	0.074	0.041	0.078	0.073	0.060	0.079	0.076	0.065	0.063	0.071	0.053	0.058	0.008	0.013	0.024	0.018
LW	0.118	0.037	0.014	0.046	0.093		0.073	0.044	0.079	0.068	0.058	0.081	0.075	0.068	0.054	0.075	0.057	0.059	0.005	0.013	0.020	0.024
MUM	0.108	0.025	0.012	0.036	0.099	0.036		0.038	0.040	0.053	0.056	0.079	0.072	0.071	0.052	0.066	0.071	0.066	0.056	0.056	0.053	0.037
HAZ1	0.076	0.027	0.091	0.042	0.091	0.03	0.037		0.016	0.029	0.004	0.062	0.072	0.052	0.052	0.050	0.049	0.047	0.046	0.030	0.006	0.011
HAG3	0.089	0.022	0.09	0.043	0.064	0.028	0.028	0.038		0.024	0.017	0.072	0.076	0.077	0.056	0.079	0.066	0.071	0.063	0.039	0.032	0.027
HAG2	0.077	0.088	0.078	0.047	0.040	0.028	0.044	0.026	0.036		0.015	0.086	0.072	0.062	0.041	0.062	0.041	0.059	0.055	0.031	0.036	0.026
HAG1	0.005	0.084	0.045	0.082	0.041	0.031	0.030	0.036	0.036	0.068		0.083	0.083	0.067	0.045	0.065	0.047	0.058	0.057	0.029	0.014	0.021
UCH	0.082	0.080	0.037	0.088	0.040	0.019	0.036	0.019	0.038	0.069	0.049		0.005	0.017	0.035	0.020	0.037	0.019	0.090	0.055	0.047	0.021
MCH	0.091	0.064	0.035	0.079	0.030	0.024	0.011	0.025	0.027	0.064	0.044	0.008		0.002	0.029	0.016	0.029	0.015	0.086	0.049	0.042	0.014
LCH	0.079	0.048	0.032	0.077	0.045	0.019	0.009	0.020	0.041	0.006	0.022	0.032	0.022		0.026	0.007	0.015	0.000	0.078	0.049	0.037	0.007
UPOU	0.001	0.048	0.002	0.047	0.031	0.016	0.004	0.016	0.029	0.005	0.039	0.017	0.014	0.011		0.008	0.000	0.013	0.041	0.020	0.034	0.009
PBC	0.000	0.024	0.010	0.045	0.038	0.014	0.005	0.008	0.033	0.003	0.027	0.022	0.020	0.029	0.020		0.005	0.001	0.071	0.040	0.036	0.012
MPOU	0.098	0.009	0.007	0.041	0.010	0.085	0.081	0.000	0.011	0.033	0.039	0.015	0.005	0.002	0.018	0.069		0.003	0.050	0.026	0.032	0.001
POU1	0.088	0.081	0.089	0.041	0.001	0.084	0.085	0.003	0.009	0.026	0.041	0.016	0.090	0.018	0.019	0.075	0.021		0.069	0.035	0.024	0.006
ULPP	0.084	0.084	0.092	0.044	0.070	0.079	0.073	0.096	0.000	0.037	0.048	0.031	0.092	0.017	0.017	0.064	0.006	0.012		0.009	0.045	0.031
LLPP	0.061	0.076	0.091	0.036	0.070	0.062	0.050	0.101	0.007	0.028	0.036	0.028	0.078	0.007	0.013	0.035	0.016	0.008	0.007		0.015	0.009
CLPA	0.045	0.050	0.077	0.047	0.060	0.042	0.031	0.089	0.001	0.033	0.025	0.028	0.028	0.026	0.025	0.011	0.012	0.004	0.001	0.017		0.002
CLPB	0.044	0.039	0.049	0.003	0.051	0.043	0.032	0.057	0.001	0.052	0.008	0.011	0.014	0.023	0.008	0.014	0.012	0.005	0.003	0.005	0.001	

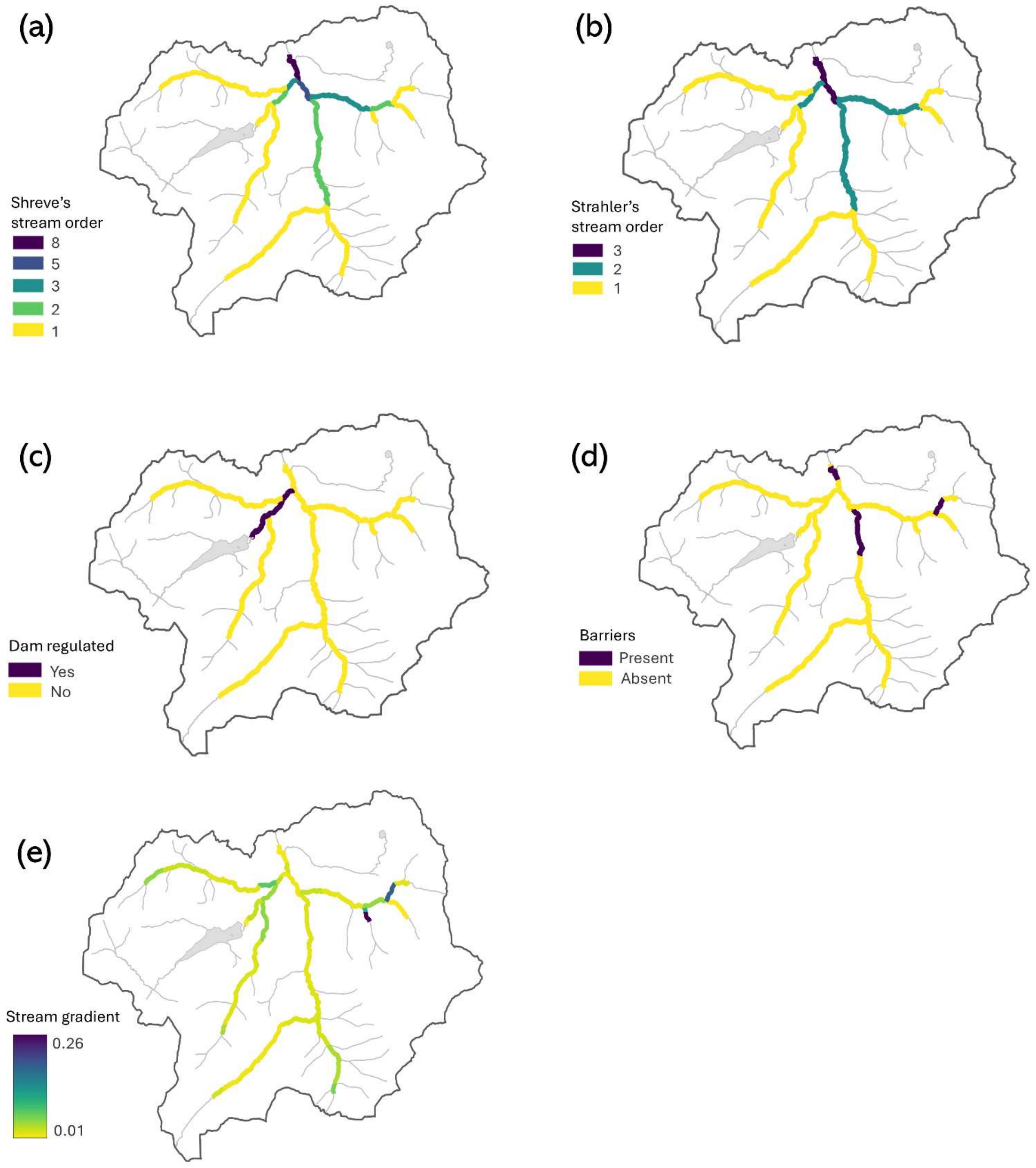

Figure A1: Maps of the upper Cache la Poudre River watershed showing SSEN edges symbolized by the values of riverscape covariates tested for their effects on Brook Trout geneflow. (a) Shreve's stream order. (b) Strahler's stream order. (c) Dam regulation. (d) Movement barriers. (e) Stream gradient.

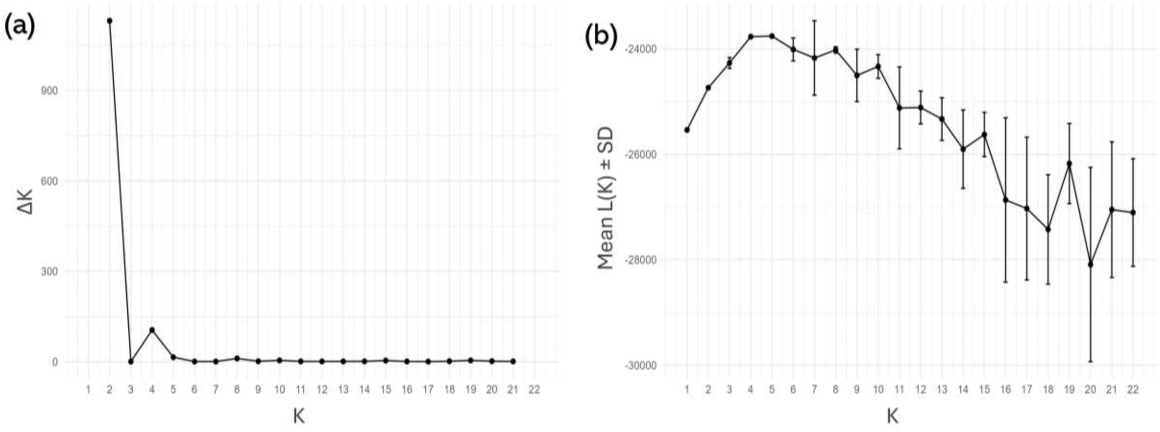

Figure A2: STRUCTURE results for determining the most likely value of K across 22 Brook Trout sampling sites in the upper Cache la Poudre River watershed. (a) ΔK for each of K = 1-22. (b) Mean estimated log likelihood of K for each of K = 1-22. Error bars indicate standard deviation.

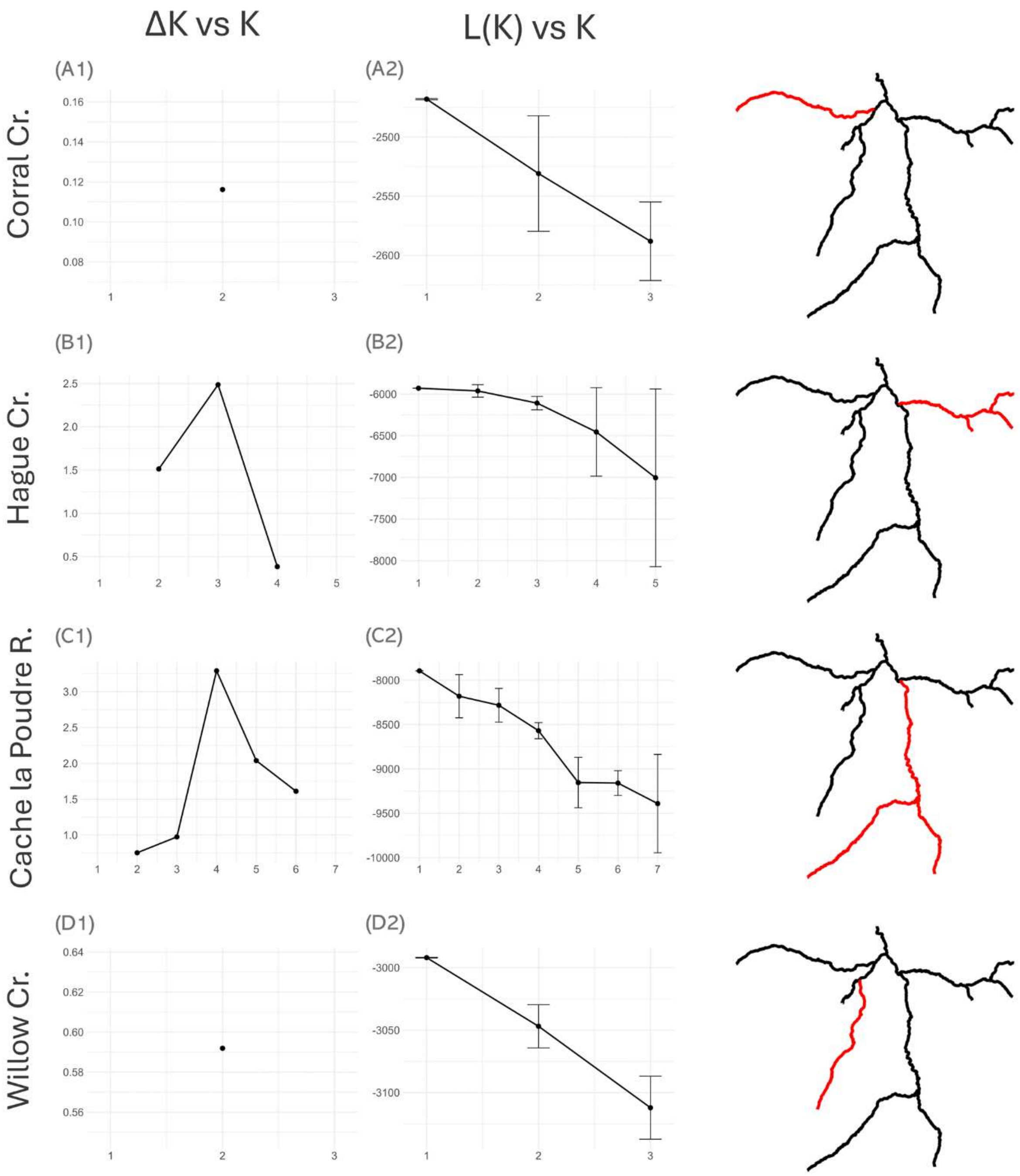

Figure A3: STRUCTURE results for evaluating hierarchical genetic structure within each of 4 major tributary drainages in the upper Cache la Poudre River watershed. Within each tributary, K was evaluated from 1 – *X* where *X* is the number of sampling sites in that tributary. Rows (A-D) represent the 4 major tributary drainages (A: Corral Creek; B: Hague Creek; C: Cache la Poudre River; D: Willow Creek) and columns (1-2) show diagnostic plots from STRUCTURE results (1. ΔK for each K; 2. mean estimated log likelihood of K for each K). The third column shows maps of the stream network with the corresponding tributary for each row highlighted in red.

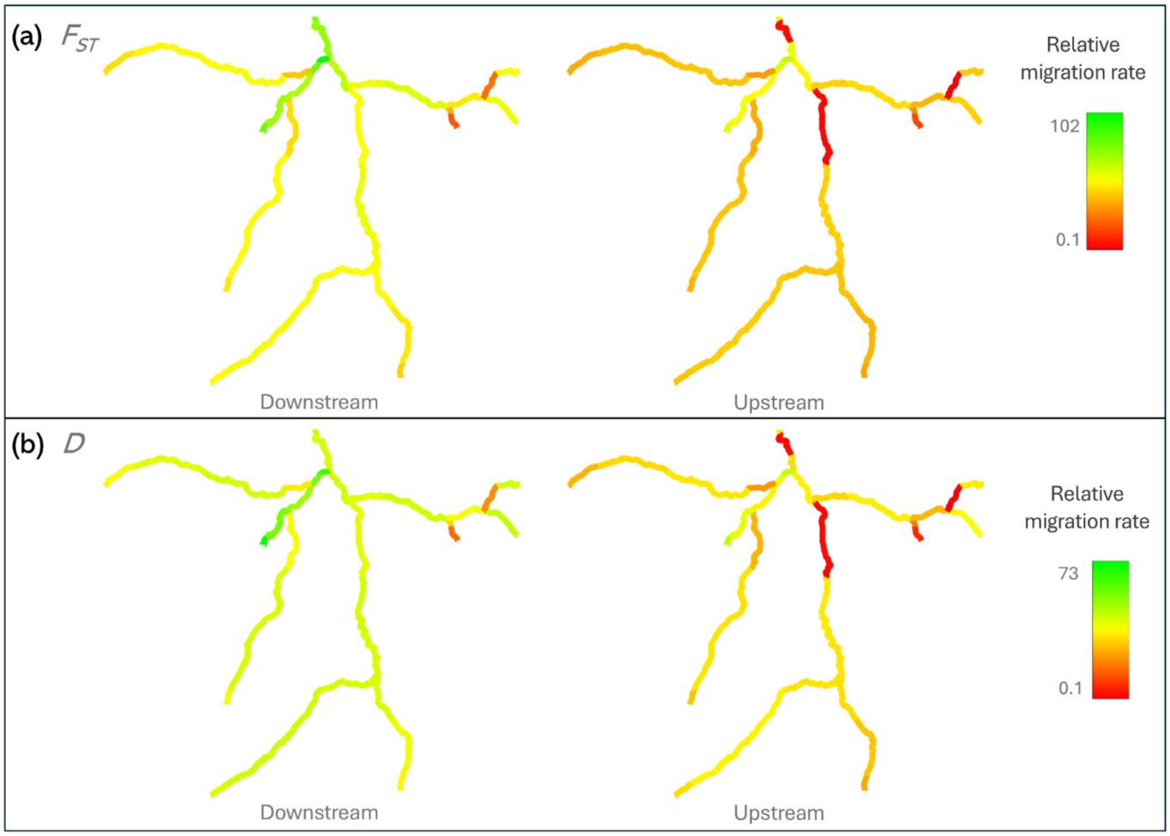

Figure A4. Estimated relative upstream (right) and downstream (left) migration rates on SSEN edges from models using (a) pairwise F_{ST} and (b) Jost's D as response variables.

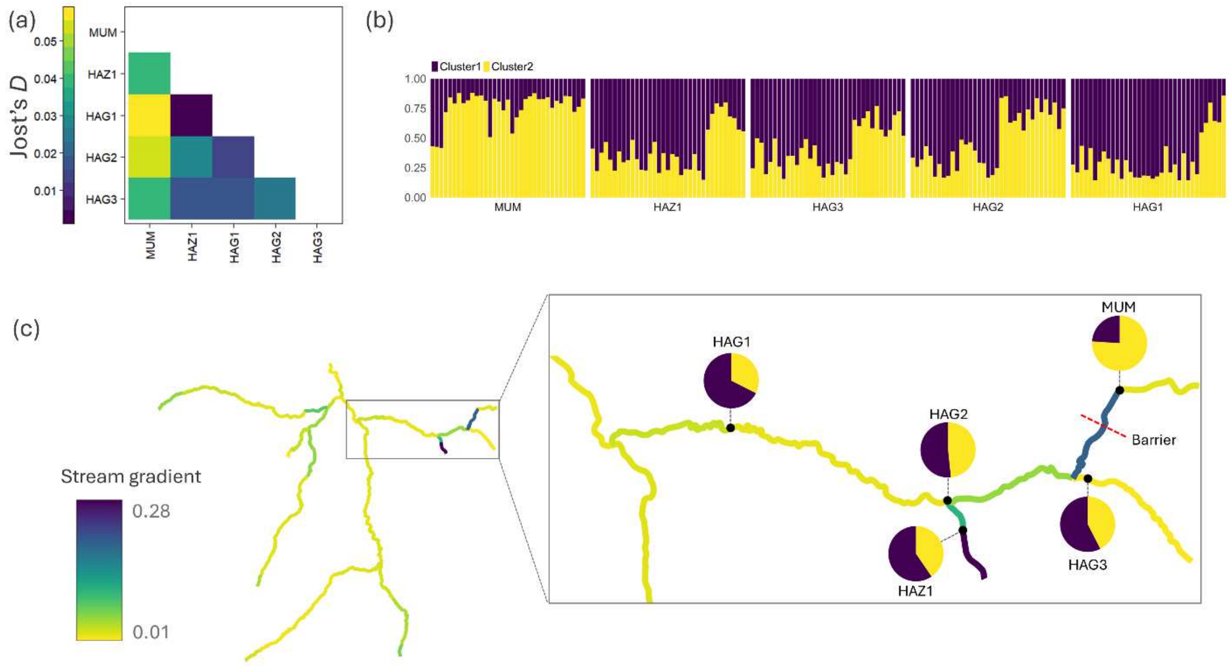

Figure A5: Hierarchical population structure of Brook Trout within the Hague Creek drainage, showing moderate genetic isolation of site MUM due to barriers and high-gradient stream segments. (a) Pairwise Jost's *D* between sites in the Hague Creek drainage. (b) Individual STRUCTURE assignment plots assuming K = 2 for sites in the Hague Creek drainage. (c) Map of SSEN edges colored by stream gradient in the Hague Creek drainage with pie charts indicating mean site-level STRUCTURE assignment proportions to 2 genetic clusters, along with the location of an upstream movement barrier.