THESIS

GENETIC ANALYSIS REVEALS BIDIRECTIONAL FISH MOVEMENT ACROSS THE CONTINENTAL DIVIDE VIA AN INTERBASIN WATER TRANSFER

Submitted by

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ABSTRACT

GENETIC ANALYSIS REVEALS BIDIRECTIONAL FISH MOVEMENT ACROSS THE CONTINENTAL DIVIDE VIA AN INTERBASIN WATER TRANSFER

Interbasin water transfers are becoming an increasingly common tool to satisfy municipal and agricultural water demand, but their impacts on the movement and gene flow of aquatic organisms are poorly understood. The Grand Ditch is an interbasin water transfer that diverts water from tributaries of the upper Colorado River on the west side of the Continental Divide to the upper Cache la Poudre River on the east side of the Continental Divide. I used single nucleotide polymorphisms to characterize population genetic structure in cutthroat trout (*Oncorhynchus clarkii*) and determine if fish utilize the Grand Ditch as a movement corridor. Samples were collected from two sites on the west side and three sites on the east side of the Continental Divide. I identified two genetic clusters, but they did not align with the west and east sides of the Continental Divide. Spatial distributions of admixed individuals indicated that the Grand Ditch facilitated bidirectional fish movement across the Continental Divide, a major biogeographic barrier. Many others have demonstrated the ecological impacts of interbasin water transfers, but this study is one of the first to utilize genetics to understand how interbasin water transfers affect connectivity between previously isolated watersheds. I also discuss implications on native trout management and the need for balancing water demand and biodiversity conservation.

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CHAPTER 1

GENETIC ANALYSIS REVEALS BIDIRECTIONAL FISH MOVEMENT ACROSS THE CONTINENTAL DIVIDE VIA AN INTERBASIN WATER TRANSFER

Introduction

Much of the world currently faces water stress and insecurity (Vörösmarty et al. 2010), and climate change is predicted to exacerbate water demand (IPCC 2014). Issues surrounding water supply are particularly evident in urban centers and high-intensity agricultural areas in arid and semi-arid regions, where water demand already outpaces water supply (Gupta and van der Zaag 2008; Vörösmarty et al. 2010). One solution to satisfy the gap between local water supply and demand is interbasin water transfer (also known as transbasin diversion), in which water from a donor basin is diverted to a recipient basin via artificial structures (Davies et al. 1992; Gupta and van der Zaag 2008). In the United States alone, there are 2,161 interbasin water transfers (Dickson and Dzombak 2017). Worldwide, 34 large-scale water transfer megaprojects already exist, with 76 additional megaprojects to be completed by 2050 in both developed and developing countries (Shumilova et al. 2018). Interbasin water transfers distribute water to areas with the greatest demand, but they can also result in changes to water quality (Fornarelli and Antenucci 2011; Jin et al. 2015), decreased aquatic biodiversity (Campbell Grant et al. 2012; Lin et al. 2017), and increased spread of aquatic invasive species (Bunn and Arthington 2002; Gallardo and Aldridge 2018). As water demand increases and necessitates construction of larger, more complicated water infrastructure, the ecological impacts of interbasin water transfers become a pressing global issue.

Watershed boundaries often serve as biogeographic barriers that prevent movement and gene flow in aquatic organisms (Wishart and Davies 2003). Thus, interbasin water transfers can have considerable effects on genetic population structure (Davies et al. 1992; Snaddon et al. 1998; Wishart and Davies 2002). Interbasin water transfers constructed between historically isolated watersheds connect previously allopatric populations of aquatic species, which could result in unintended hybridization and introgression (Allendorf and Leary 1988; Echelle 1991; Scribner et al. 2001). Despite these potential consequences on intraspecific diversity, there are very few empirical data regarding the effects of interbasin water transfers on genetic population structure of aquatic organisms. In Chile, lack of spatial genetic structure in an endangered catfish was attributed to migration between two basins connected by an interbasin water transfer (Muñoz-Ramírez et al. 2014; Muñoz-Ramírez et al. 2015). Wilson and Haxton (2021) found that an interbasin water transfer scheme greatly affected genetic structure and nearly homogenized two genetically distinct groups of walleye (*Sander vitreus*) in Canada.

Colorado (USA) is an ideal location in which to study the effects of interbasin water transfer on population genetic structure. Colorado is located in the headwaters of four major river basins in an arid region, and there are 44 interbasin water transfer schemes in Colorado, 25 of which move water across the Continental Divide (Water Education Colorado 2014). The Continental Divide is a major biogeographic barrier that has expedited speciation for millennia (Rahel 2007), particularly in cutthroat trout (*Oncorhynchus clarkii*). Cutthroat trout are the most widely distributed salmonid in western North America (Budy et al. 2019), and six distinct lineages of cutthroat trout are native to Colorado, though not all are extant (Metcalf et al. 2012; Bestgen et al. 2019). The Continental Divide serves as a primary feature explaining the historical

diversity of cutthroat trout in Colorado (Metcalf et al. 2012) and is where ranges of distinct cutthroat trout lineages abut.

Modern approaches for native cutthroat trout conservation center on isolating populations in headwater streams to mitigate risk of invasion and displacement by non-native salmonids (Fausch et al. 2009). Managers typically construct barriers at the downstream boundary of an area to protect an existing native trout population or reintroduce a new population. However, this approach does not consider the potential effects of interbasin water transfers, which may be located upstream of the area intended for native trout conservation and connected artificially to another basin. Little is understood about whether interbasin water transfers serve as fish movement corridors and thus undermine physical isolation of headwater areas. In our study, we investigate genetic structure of cutthroat trout populations connected by the Grand Ditch, an interbasin water transfer that moves water across the Continental Divide in Colorado. The downstream portion of our study area is part of an ongoing large-scale reclamation effort to restore a metapopulation of greenback cutthroat trout (*O. c. stomias*), which is federally listed as threatened. Thus, understanding the role interbasin water transfers play in shaping population genetic structure is crucial to evaluating whether an isolated greenback cutthroat trout metapopulation could be established in the project area and if genetic integrity of the reintroduced metapopulation could be maintained.

Methods

Study Area

Our study took place in a high-elevation, snowmelt-driven stream habitat within Rocky Mountain National Park and Arapaho and Roosevelt National Forests (Figure 1a). The Grand

Ditch is a 24-km interbasin water transfer which moves water from headwater tributaries of the Colorado River on the west side of the Continental Divide to the upper Cache la Poudre River on the east side of the Continental Divide (Figure 1b). Construction of the Grand Ditch began in the late 1890s, making it one of Colorado's oldest water diversions. The majority of the Grand Ditch is an open water channel with an earthen levee, but also consists of tunnels and buried conduits. Channel substrate is primarily a coarse gravel-cobble mixture embedded in a fine gravel and sand matrix (unpublished data, U.S. Forest Service). Channel-conveyance capacity at the terminal end of the Grand Ditch is approximately 400 cubic feet per second, and overall, channel slope is approximately 0.3% (unpublished data, U.S. Forest Service). Long Draw Reservoir was built on La Poudre Pass Creek in 1930 to manage water supply more efficiently. Water is typically released from Long Draw Reservoir between mid-May and mid-September, and the lower section of La Poudre Pass Creek experiences periodic intermittency mediated by water releases from Long Draw Reservoir.

Historically, a distinct lineage of Colorado River cutthroat trout (*O. c. pleuriticus*) was native to the upper Colorado River, and the greenback cutthroat trout was native to the Cache la Poudre River, a tributary to the South Platte basin (Metcalf et al. 2012). However, widespread stocking of non-native lineages has greatly modified the modern distribution of cutthroat trout in Colorado (Metcalf et al. 2012; Love Stowell et al. 2015). On the west side of the Continental Divide, cutthroat trout residing in Baker Gulch (Figure 1a) are considered a separate lineage of Colorado River cutthroat trout not native to the area (unpublished data, Colorado Parks and Wildlife), and stocking last occurred in 1932 (unpublished data, U.S. Fish and Wildlife Service). A blended hatchery strain of Colorado River cutthroat trout and Yellowstone cutthroat trout (*O. c. bouvieri*) have historically been stocked in Long Draw Reservoir (Figure 1a) on the east side

of the Continental Divide, though stocking ceased in 2017 (personal communication, Kevin Rogers, Colorado Parks and Wildlife). No fish have been stocked in Grand Ditch (Figure 1a), but cutthroat trout have been observed in the diversion, suggesting that fish move into Grand Ditch from either Baker Gulch or Long Draw Reservoir, or a combination of both (personal observation, Matt Fairchild).

Sample Collection

Cutthroat trout tissue samples were collected in summer and fall of 2019 via backpack electrofishing surveys. We measured each fish for total length and collected an anal or caudal fin clip for genetic analysis before releasing fish alive. Fin clips were dried on Whatman chromatography paper and stored individually. We genotyped a total of 229 fish collected from Baker Gulch (BG), Grand Ditch (GD), La Poudre Pass Creek Above Long Draw Reservoir (LPPA), Neota Creek (NE), and La Poudre Pass Creek Below Long Draw Reservoir (LPPB; Table 1). Both Baker Gulch and Grand Ditch sites contain samples from multiple stream segments that were pooled due to low sample size (Figure 1a).

Laboratory Analysis

We extracted genomic DNA using the Qiagen DNeasy Blood and Tissue Kit (Thermofisher Scientific) according to the manufacturer's protocol. Individuals were genotyped at a panel of 125 single nucleotide polymorphisms (SNPs) developed to differentiate cutthroat trout subspecies (Houston et al. 2012). Using the KASPar primer sequences from Houston et al. (2012), we designed 125 SNP Type™ assays and genotyped individuals using 96.96 Dynamic Array™ integrated fluidic circuits (IFC) on a Fluidigm EP1 system (Fluidigm Corporation). To ensure adequate DNA concentration for genotyping, we performed a specific target amplification

(STA) step and diluted STA products 1:100 in DNA suspension buffer prior to genotyping. We ran each IFC with 10 no template controls and duplicate samples to check for repeatability of genotype calls. We did not detect any genotyping error in duplicate samples. Genotypes were automatically called using the Fluidigm SNP Genotyping Analysis software (version 4.5.1) with an 80% confidence threshold and checked by eye. Full details of STA and genotyping protocols can be found in the Fluidigm SNP Genotyping User Guide (Fluidigm PN 68000098 O1).

Data filtering, genetic diversity, and genetic differentiation

Because these SNPs were developed for all cutthroat trout subspecies (Houston et al. 2012), we expected that not all markers would be informative for our study. Before analysis, we removed monomorphic markers, markers with > 20% missing data, and individuals with > 20% missing data using poppr (Kamvar et al. 2014) and adegenet (Jombart 2008) packages in R (R Core Team 2021). After removing uninformative markers and individuals, our data set contained 104 SNPs and 225 individuals. We then examined Hardy-Weinberg equilibrium (HWE) at each site with 1,000 Monte Carlo permutations using the R package pegas (Paradis 2010) and applied a Bonferroni correction for multiple comparisons across 520 tests. We excluded loci that deviated from HWE in a majority of sites (≥ 3) from further analysis, leaving 38 SNPs in our final data set. The R package hierfstat (Goudet 2005) was used to calculate overall within-population gene diversity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), and rarefied allelic richness (A_R) for each site. We also calculated pairwise F_{ST} following Weir and Cockerham (1984) using hierfstat with 1,000 bootstrap replicates for 95% confidence intervals. Estimates of pairwise F_{ST} were considered significant if 95% confidence intervals did not overlap zero. To minimize bias in cluster analysis arising from uneven sample size (Puechmaille 2016), we randomly subsampled 29 individuals, the minimum sample size, from each site. We repeated

this procedure five times to check for consistency across subsampled data sets. Summary statistics (H_E, H_O, F_{IS}, and A_R) and pairwise F_{ST} were calculated for each subsampled dataset.

Genetic clustering

To understand how Grand Ditch affects spatial population structure of cutthroat trout, we used two clustering methods. The first method, STRUCTURE version 2.3.4 (Pritchard et al. 2000), is a model-based Bayesian clustering method for multilocus genetic data. All STRUCTURE runs were performed with the admixture model, correlated allele frequencies, and no location prior. Each STRUCTURE run consisted of 20,000 burn-in iterations, 100,000 subsequent iterations, and five replicates of each K. We tested K = 1 – 5 and determined the number of clusters likely present in the data using the likelihood of K (Pritchard et al. 2000) and ΔK (Evanno et. al 2005). Within every STRUCTURE run, we merged replicates of each K and visualized results using the R package pophelper (Francis 2017). To further understand spatial structure, we calculated mean cluster assignment probabilities (Q-scores) for each site and plotted resulting pie charts on a map of the study area. The second clustering method, discriminant analysis of principal components (DAPC; Jombart et al. 2010), transforms genetic data using a principal components analysis before applying discriminant functions to maximize between-group variance and minimize within-group variance. We visualized genetic relationships between sites by defining clusters *a priori* as sampling sites. Because the results of DAPC are sensitive to the number of principle components retained, we used the *optim.a.score* function from adegenet (Jombart 2008) to determine the optimum number of principal components to retain for DAPC. All discriminant components were retained, and clusters were plotted in an ordination plot along axes of the first and second discriminant functions.

Results

Genetic Diversity and Differentiation

Results were highly consistent across five subsampled datasets. Thus, we present results from a single subsampled dataset consisting of 145 individuals genotyped at 38 SNPs (dataset 5). Additional results from other subsampled datasets are available as supplementary materials (Table A1; Figures. A2 – A5). Across sites, measures of diversity were consistently lowest in BG (H_E = 0.146; H_O = 0.233, F_{IS} = -0.522; A_R = 1.342), and genetic diversity tended to be highest in LPPB (Table 1; H_E = 0.338, H_O = 0.458, A_R = 1.911). Pairwise F_{ST} ranged from 0.016 (LPPB-NE) to 0.537 (BG-LPPA), and all pairwise F_{ST} values were significantly different from zero (Figure 2; Table A2). Overall, BG was most differentiated genetically, with values from all pairwise F_{ST} comparisons > 0.4 (Figure 2).

Genetic Clustering

Our STRUCTURE analysis showed evidence of two genetic clusters (K = 2), with concordant results from both likelihood of K and ΔK estimators (Figure A1). Cluster 1 was primarily composed of BG individuals, and individuals from LPPA, NE, and LPPB largely assigned to cluster 2 (Figure 3). However, we detected signs of admixture in GD, as evidenced by intermediate *Q*-scores (> 0.25 for cluster 1 and < 0.75 for cluster 2) in a majority of individuals from GD (Figure 3), suggesting that cutthroat trout move westward across the Continental Divide via Grand Ditch. In addition, two individuals from LPPA had intermediate *Q*-scores, indicative of eastward movement across the Continental Divide (Figure 3a). We also performed two hierarchical STRUCTURE analyses—one including GD, LPPA, LPPB, and NE,

and one including LPPA, LPPB, and NE. We did not find evidence of hierarchical structure in either case.

The *optim.a.score* function indicated the optimum number of PCAs to retain for DAPC was 7, which conserved 75.8% of the observed variance. The majority of variance in the discriminant analysis was explained by discriminant functions 1 and 2 (Figure 4 inset). Generally, results from DAPC were consistent with those of STRUCTURE, with individuals from BG tightly clustered and separated from GD, LPPA, NE, and LPPB (Figure 4). Discriminant function 1 (x-axis; eigenvalue = 424.4) and discriminant function 2 (y-axis; eigenvalue = 153.5) separated sites into three identifiable groups—one cluster containing only individuals from BG, a second cluster composed of GD and LPPA, and a third cluster of individuals from NE and LPPB (Figure 4). Though DAPC provided some evidence of separation between GD-LPPA and LPPB-NE, we also observed three LPPB individuals within the GD-LPPA clusters (Figure 4), indicating that movement likely occurs between these sites. More importantly, the high degree of overlap between GD and LPPA suggests that extensive movement occurs between these sites, which are separated by the Continental Divide.

Discussion

Our study characterized fish movement via an interbasin water transfer from the upper Colorado River basin across the Continental Divide and into the upper Cache la Poudre River basin. In the absence of fish movement via the interbasin water transfer, we would have expected to identify two isolated clusters corresponding to the western (BG and GD) and eastern (LPPA, NE, and LPPB) sides of the Continental Divide. However, the two genetic clusters we observed

did not align with this expectation, indicating that the Grand Ditch functions as a fish movement corridor across the Continental Divide. Importantly, we found evidence of bidirectional movement across the Continental Divide, with admixed individuals present in both GD (west side) and LPPA (east side). Bidirectional movement within an interbasin water transfer has not been previously documented, though Wilson and Haxton (2021) found evidence of unidirectional downstream movement of walleye in an interbasin water transfer in Northern Ontario. Our STRUCTURE results indicated that most individuals in GD are admixed, a result of extensive hybridization between individuals from BG and LPPA. Varying degrees of admixture among individuals in GD suggest that movement across the Continental Divide and subsequent hybridization have been occurring over multiple generations. This pattern is also demonstrated in our DAPC results, with a high degree of overlap between GD and LPPA.

Despite evidence of westward movement across the Continental Divide, we found this connectivity did not extend to BG. Results from both STRUCTURE and DAPC indicate that BG is isolated and that upstream movement from GD to BG likely does not occur. This conclusion is reinforced by low levels of genetic diversity in BG and high pairwise F_{ST} values for comparisons with BG. The isolation of BG indicates the presence of barriers downstream of the site, including natural velocity and gradient barriers on Baker Gulch and tunnels and buried conduits on the Grand Ditch (personal observation, Matt Fairchild). However, despite the lack of upstream movement into BG, our analysis provided support for downstream movement into the Grand Ditch, where immigrants subsequently interact with individuals from the eastern side of the Continental Divide.

Implications for greenback cutthroat conservation

To our knowledge, our study is the first to consider the ecological effects of interbasin water transfer in a headwater stream network, and our findings have direct management implications for the planned establishment of a greenback cutthroat trout metapopulation in the headwaters of the Cache la Poudre River. Genetic integrity is critical for the recovery of greenback cutthroat trout, which have been a focus of aggressive conservation efforts since the 1980s. However, researchers recently discovered that the subspecies persisted in a single, genetically pure population and that previously reintroduced and conserved populations were of admixed origin (Metcalf et al. 2012). Furthermore, the artificial origin of the sole remaining population, combined with ongoing isolation and small population size have resulted in extremely low levels of genetic diversity for the subspecies (Greenback Cutthroat Trout Recovery Team 2019). Greenback cutthroat trout reared in hatcheries have high rates of deformity and mortality (Love Stowell 2016), indicating high genetic load and a possible inbreeding depression. In experimental outcrossings of greenback and Colorado River cutthroat trout in a hatchery setting, F1 hybrids had significantly higher survival than pure greenback cutthroat trout offspring (Love Stowell 2016). If hybridization confers a similar fitness advantage (i.e., hybrid vigor) within reintroduced populations of greenback cutthroat trout, hybrid swarms resulting from introgressive hybridization may pose a profound risk to the genetic integrity of reintroduction projects (Rhymer and Simberloff 1996; Crispo et al. 2011; Bohling 2016). This scenario provides additional challenges for the conservation of greenback cutthroat trout because (1) managers view the greenback cutthroat trout as an irreplaceable evolutionary lineage and thus, prioritize maintaining existing genetic purity despite high genetic load and possible inbreeding depression and (2) hybrids are not protected under the U.S. Endangered Species Act and their conservation is controversial (Allendorf et al. 2001; Jackiw et al. 2015; Wayne and Shaffer

2016). Maintaining existing genetic purity is a high priority for managers seeking to conserve greenback cutthroat trout, but our study suggests that if reintroduction proceeds without considering the impacts of interbasin water transfers, the genetic integrity of the reintroduction is likely to be severely undermined by hybridization with cutthroat trout from Baker Gulch.

Interbasin water transfers as fish habitat

Previous studies have focused on water infrastructure as a sink habitat for fishes, where entrained fish experience high levels of mortality (Vinyard 1996; Gale et al. 2008; Roberts and Rahel 2008). Low densities of cutthroat trout in Grand Ditch align with these observations. However, our genetic analyses show the highest degree of admixture in GD fish, which indicates Grand Ditch serves as a spawning habitat. Uncertainties remain as to whether fish spawn in the small, high-gradient tributaries intercepted by Grand Ditch or in Grand Ditch itself (Figure 1A). Cutthroat trout density in the intercepted tributaries is equally low and decreases to zero approximately 100-500 m above their confluences with the Grand Ditch (unpublished data, U.S. Forest Service). Despite low fish abundance, our study suggests that interbasin water transfers may function as both fish movement corridors and habitat for important life history events, such as spawning and rearing. Evidence of water infrastructure providing habitat for self-sustaining populations is relatively rare, though instances have been documented in water diversions (Hooley-Underwood et al. 2018) and irrigation ponds (Woodford et al. 2013). Our findings, combined with previous studies, suggest that water infrastructure serving as fish habitat warrants further investigation.

Invasion via interbasin water transfer

Though many others have documented the role of interbasin water transfers in facilitating the spread of invasive species (Bunn and Arthington 2002; Gallardo and Aldridge 2018), our study provides important lessons for managers implementing aquatic conservation projects to intentionally isolate native fish from invasion using barriers. Managers often balance trade-offs between invasion and isolation when conserving native salmonids (Fausch et al. 2009), and the dominant strategy in the inland western United States is utilizing downstream barriers to isolate native salmonids from invasion and subsequent displacement by non-native salmonids. However, our research demonstrates that interbasin water transfers function as movement corridors for aquatic species and may provide a previously overlooked route of invasion for conservation projects that depend on physical isolation. In addition, the prevailing paradigm of invasion versus isolation typically considers only heterospecific invaders, but our study suggests that intraspecific hybridization can also pose a significant threat to isolation if interbasin water transfers connect populations of previously allopatric subspecies. The cryptic nature of many hybrids may allow them to go undetected, thus representing a more subtle invasion front (Haynes et al. 2012; Morais and Reichard 2018; Quilodrán et al. 2018). In our specific case, distinguishing cutthroat trout subspecies and their hybrids is exceptionally difficult without the aid of morphometric and meristic characters (Bestgen et al. 2019), making correct field identification nearly impossible. Ultimately, in areas where physical isolation is a management goal, interbasin water transfers are likely to compromise the physical isolation needed to prevent invasion, regardless of whether invaders are conspecifics or heterospecifics.

Interbasin water transfers facilitate biotic homogenization

Biotic homogenization occurs when the taxonomic, genetic, or functional similarity among previously distinct biotas increases through invasions and extirpations (Rahel 2002; Olden et al.

2004; Olden 2006). Interbasin water transfers contribute to biotic homogenization by increasing both taxonomic and genetic similarity between donor and recipient basins. The role interbasin water transfer plays in taxonomic homogenization is well-documented across multiple systems, with many examples of interbasin water transfers facilitating the spread of invasive species (Bunn and Arthington 2002; Gallardo and Aldridge 2018) and decreasing aquatic biodiversity (Campbell Grant et al. 2012; Lin et al. 2017). However, genetic homogenization resulting from interbasin water transfer is far less studied. Watershed boundaries often function as barriers to gene flow (Wishart and Davies 2003), and researchers have hypothesized that interbasin water transfers influence population genetic structure (Davies et al. 1992; Snaddon et al. 1998; Wishart and Davies 2002). But few empirical studies have confirmed this hypothesis. Our findings indicate that interbasin water transfers have measurable effects on genetic population structure and can contribute to genetic homogenization (i.e., loss of β diversity), in concordance with a few similar studies (Muñoz-Ramírez et al. 2014; Muñoz-Ramírez et al. 2015; Wilson and Haxton 2021).

Over the next century, climate change is likely to intensify gaps between water supply and demand (IPCC 2014) leading to the construction of additional water infrastructure (Shumilova et al. 2018), such as interbasin water transfers. Unintended ecological consequences of interbasin water transfers can be mitigated by: (1) considering impacts of existing interbasin water transfers during conservation planning, (2) investigating potential ecological outcomes of proposed interbasin water transfers before construction begins, and (3) establishing genetic monitoring programs to understand species distributions and genetic population structure before and after construction of interbasin water transfers.

Table 1. Descriptive summary of genetic diversity. For each site, number of cutthroat trout individuals genotyped in the original dataset after removing uninformative loci and individuals ($N_{original}$) and subsampled datasets ($N_{subsample}$), body size range in millimeters (mm), within-population gene diversity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), and rarefied allelic richness (A_R). Sites are ordered from west to east. BG = Baker Gulch; GD = Grand Ditch; LPPA = La Poudre Pass Creek Above Long Draw Reservoir; NE = Neota Creek; LPPB = La Poudre Pass Creek Below Long Draw Reservoir

Site	$\mathbf{N_{original}}$	$\mathbf{N_{subsample}}$	**Size Range (mm)**	H_E	H_O	F_{IS}	A_R
BG	44	29	97 – 215	0.146	0.233	-0.522	1.342
GD	49	29	58 – 407	0.297	0.328	-0.080	1.839
LPPA	53	29	65 – 438	0.243	0.248	-0.021	1.779
NE	29	29	67 – 239	0.309	0.445	-0.346	1.842
LPPB	50	29	71 – 182	0.338	0.458	-0.278	1.911

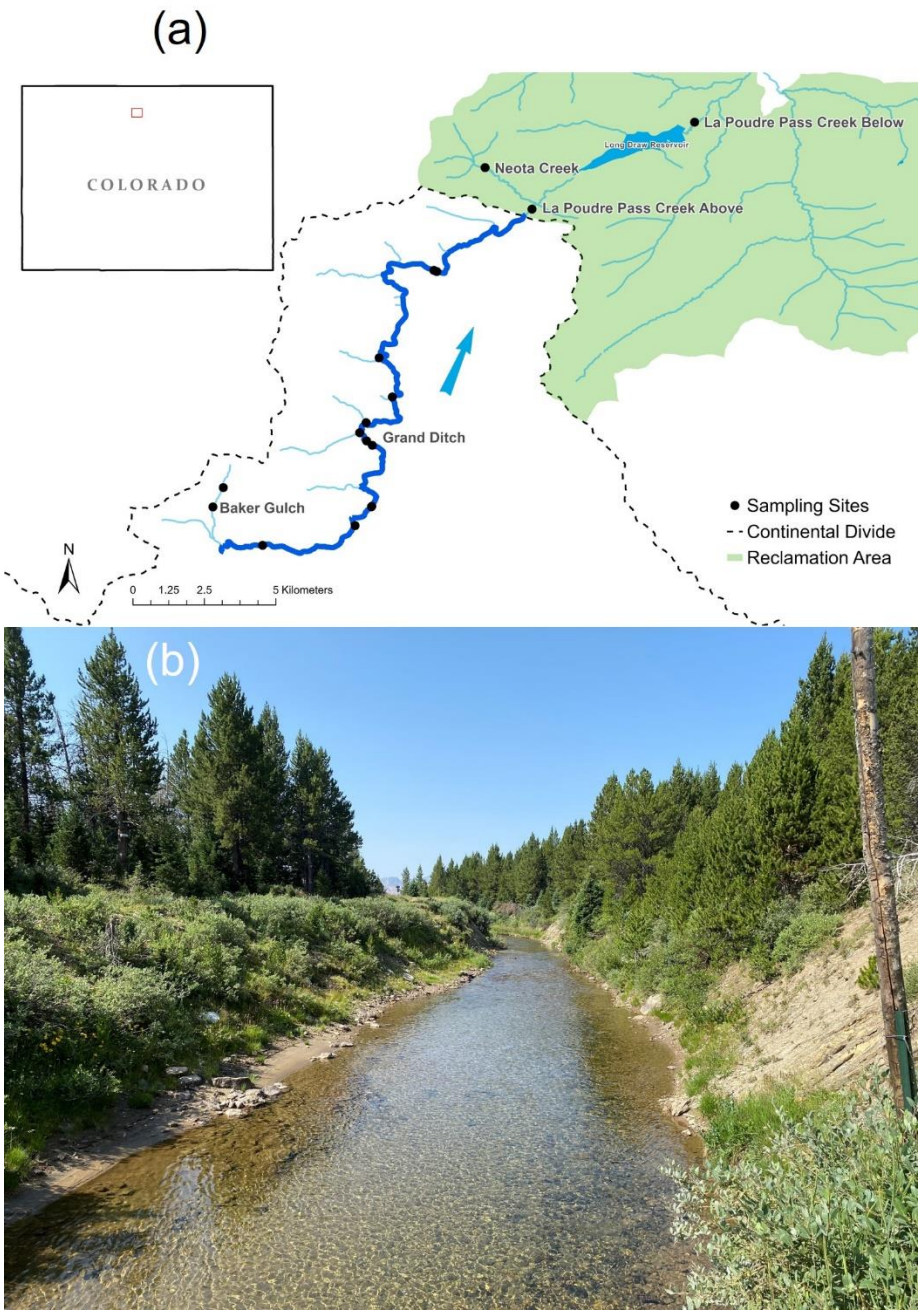

Figure 1. (a) Map of the study area showing sites (black dots) where cutthroat trout were sampled in Arapaho and Roosevelt National Forest and Rocky Mountain National Park in northern Colorado, USA. Streams are shown as light blue lines, and the Grand Ditch is indicated by the dark blue line. The Continental Divide, a major biogeographic barrier, is indicated by the dashed black line, and the greenback cutthroat trout reclamation area is shaded in green. Direction of water flow is indicated by the light blue arrow. Due to low sample size, multiple stream segments were pooled for sites on Grand Ditch and Baker Gulch. (b) Photo of the Grand Ditch, an interbasin water transfer, near the Continental Divide. The majority of the Grand Ditch is a low gradient, open water channel bound by an earthen levee

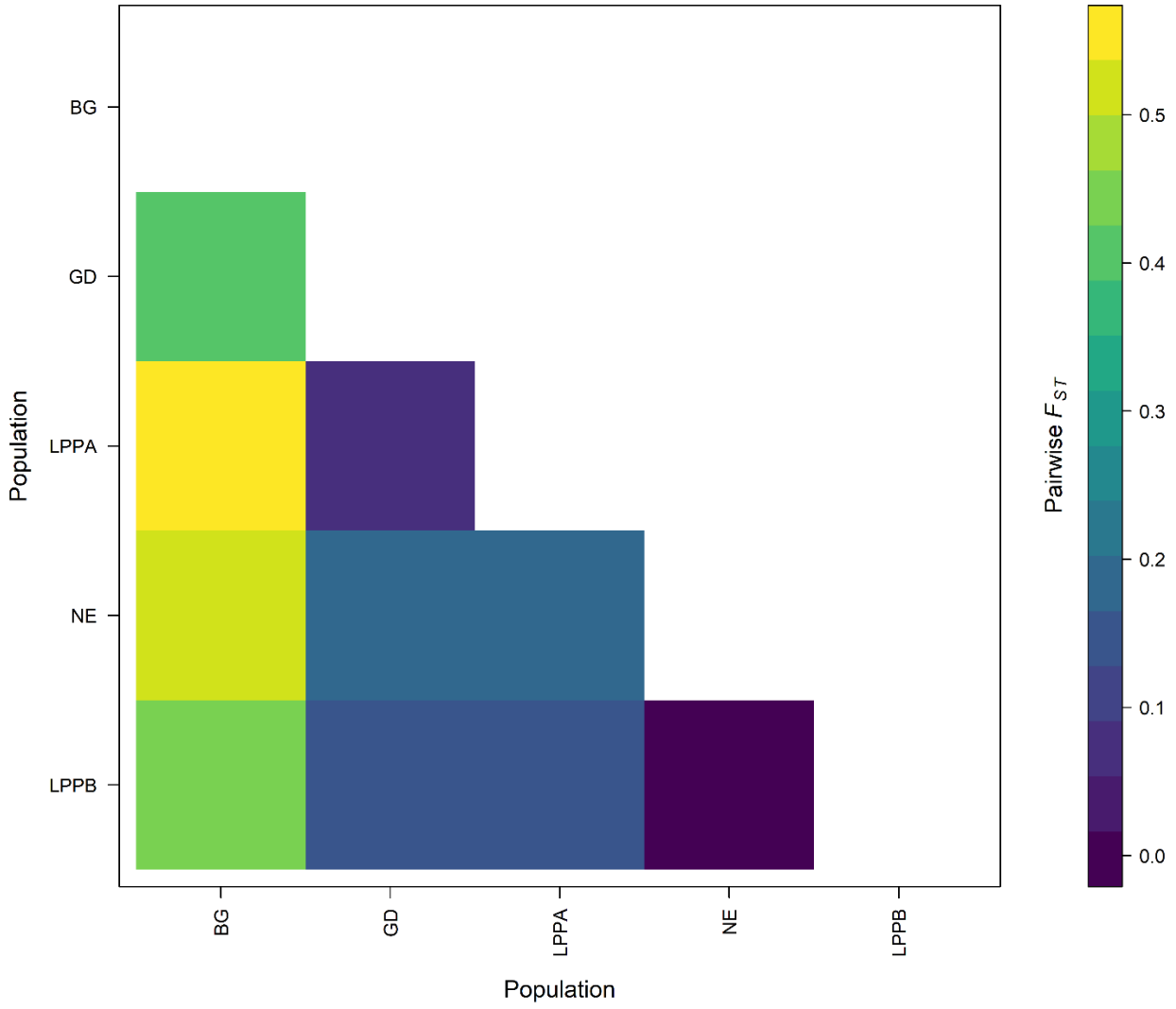

Figure 2. Matrix of pairwise F_{ST} comparisons for all pairs of sites. Values range from 0.016 (purple) to 0.537 (yellow)

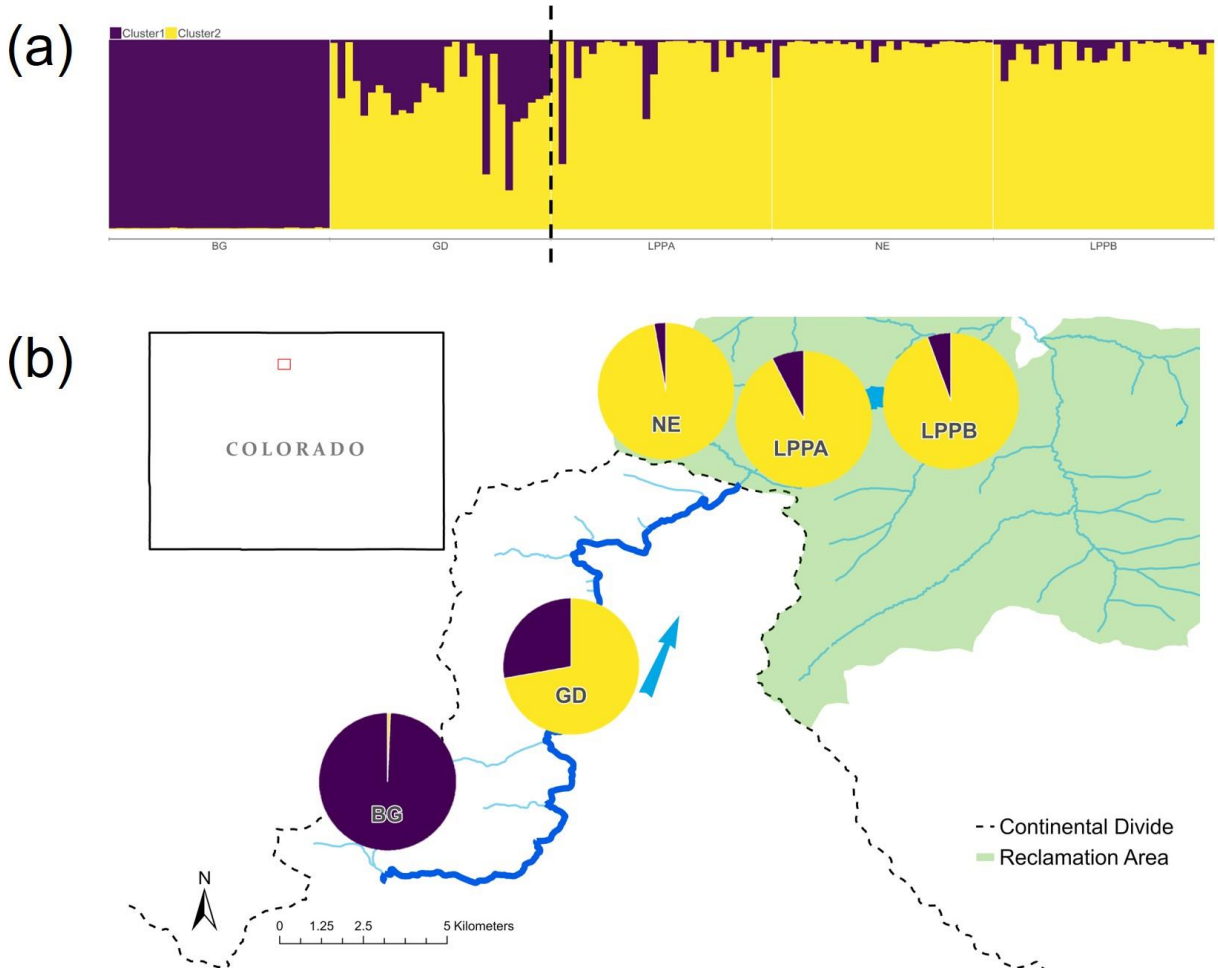

Figure 3. (a) STRUCTURE analysis (K = 2) of 145 cutthroat trout from Baker Gulch (BG), Grand Ditch (GD), La Poudre Pass Creek Above Long Draw Reservoir (LPPA), Neota Creek (NE), and La Poudre Pass Creek Below Long Draw Reservoir (LPPB). Each individual is represented as a vertical bar whose colors correspond to the probability of assignment to different clusters. Sites and individuals within Grand Ditch are ordered from west to east, and location of the Continental Divide is indicated by the dashed black line. (b) Map of the study area showing mean cluster assignment probabilities for each site. The Continental Divide, a major biogeographic barrier, is indicated by the dashed black line, and the greenback cutthroat trout reclamation area is shaded in green. Direction of water flow is indicated by the light blue arrow

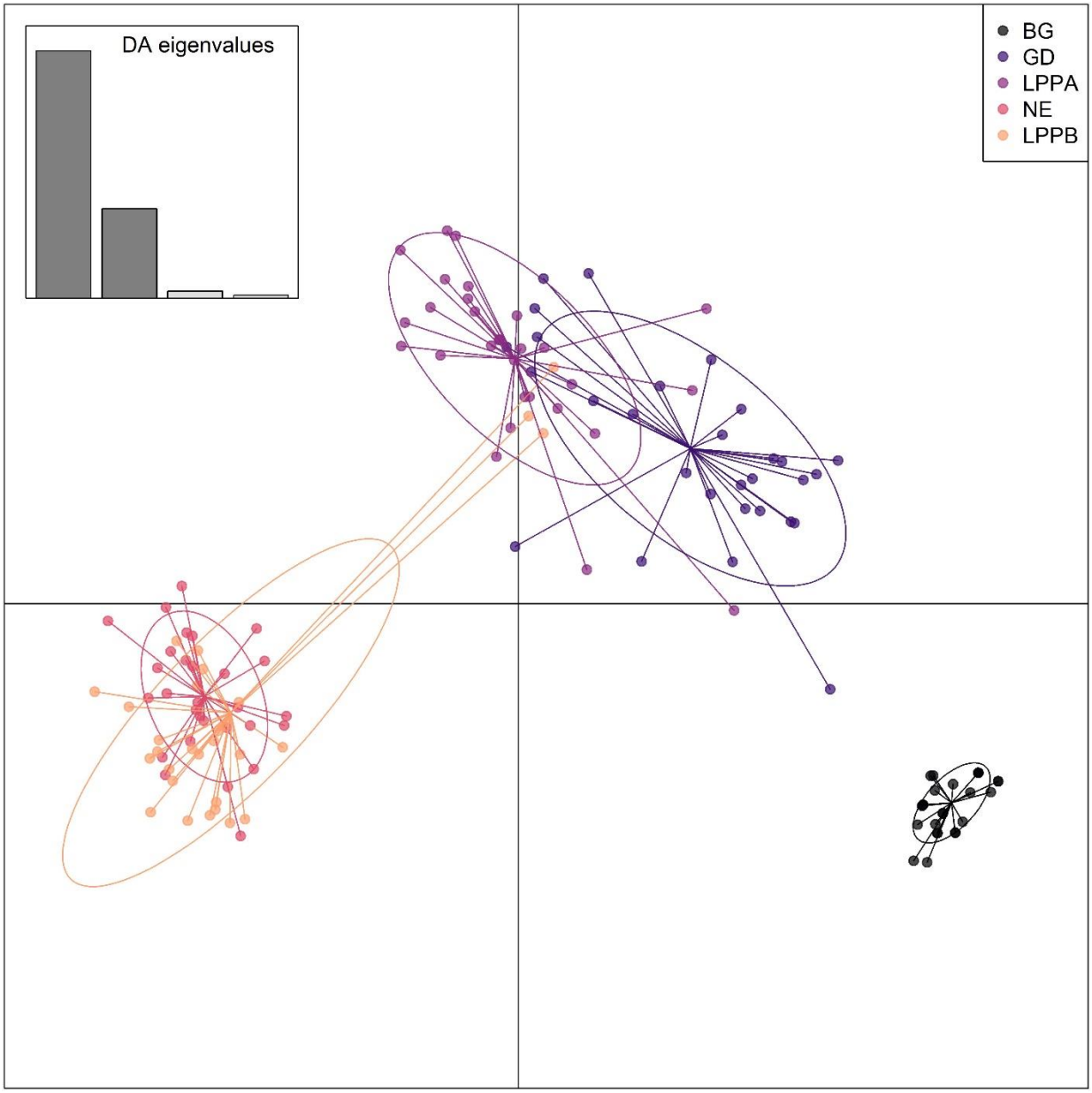

Figure 4. DAPC analysis of 145 cutthroat trout from Baker Gulch (BG), Grand Ditch (GD), La Poudre Pass Creek Above Long Draw Reservoir (LPPA), Neota Creek (NE), and La Poudre Pass Creek Below Long Draw Reservoir (LPPB). Each individual is represented by a point plotted along discriminant function 1 (x-axis; eigenvalue = 424.4) and discriminant function 2 (y-axis; eigenvalue = 153.5). Colors of individual points and inertia ellipses correspond to sampling sites

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APPENDIX 1

Table A1. Descriptive summary of genetic diversity for all subsampled datasets. For each numbered subsampled dataset, within-population gene diversity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), and rarefied allelic richness (A_R) at each sampling site. Sites are ordered from west to east. BG = Baker Gulch; GD = Grand Ditch; LPPA = La Poudre Pass Creek Above Long Draw Reservoir; NE = Neota Creek; LPPB = La Poudre Pass Creek Below Long Draw Reservoir. Subsampled dataset 5 was presented as the primary analysis in the manuscript

	H_E					H_O					F_{IS}					A_R				
Site	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
BG	0.145	0.147	0.145	0.146	0.146	0.229	0.232	0.233	0.235	0.233	-0.503	-0.514	-0.529	-0.536	-0.522	1.341	1.341	1.341	1.342	1.342
GD	0.293	0.282	0.288	0.286	0.297	0.335	0.313	0.318	0.328	0.328	-0.117	-0.07	-0.074	-0.072	-0.08	1.834	1.842	1.828	1.85	1.839
LPPA	0.231	0.237	0.255	0.233	0.243	0.255	0.255	0.268	0.254	0.248	-0.09	-0.058	-0.047	-0.073	-0.021	1.751	1.789	1.801	1.774	1.779
LPPB	0.332	0.337	0.341	0.334	0.338	0.46	0.468	0.454	0.456	0.458	-0.311	-0.319	-0.272	-0.289	-0.278	1.885	1.887	1.912	1.909	1.911
NE	0.309	0.309	0.309	0.309	0.309	0.445	0.445	0.445	0.445	0.445	-0.346	-0.346	-0.346	-0.346	-0.346	1.842	1.841	1.842	1.842	1.842

Table A2. For subsampled dataset 5, lower and upper bounds of 95% confidence intervals for each pairwise F_{ST} comparison. Sites are ordered from west to east. BG = Baker Gulch; GD = Grand Ditch; LPPA = La Poudre Pass Creek Above Long Draw Reservoir; NE = Neota Creek; LPPB = La Poudre Pass Creek Below Long Draw Reservoir

Site	BG	GD	LPPA	NE	LPPB
BG	*				
GD	0.2898 - 0.4991	*			
LPPA	0.4391 - 0.6128	0.0308 - 0.1084	*		
NE	0.4298 - 0.580	0.0980 - 0.2380	0.0945 - 0.2462	*	
LPPB	0.3725 - 0.5083	0.0815 - 0.2016	0.0953 - 0.2236	0.002 - 0.0356	*

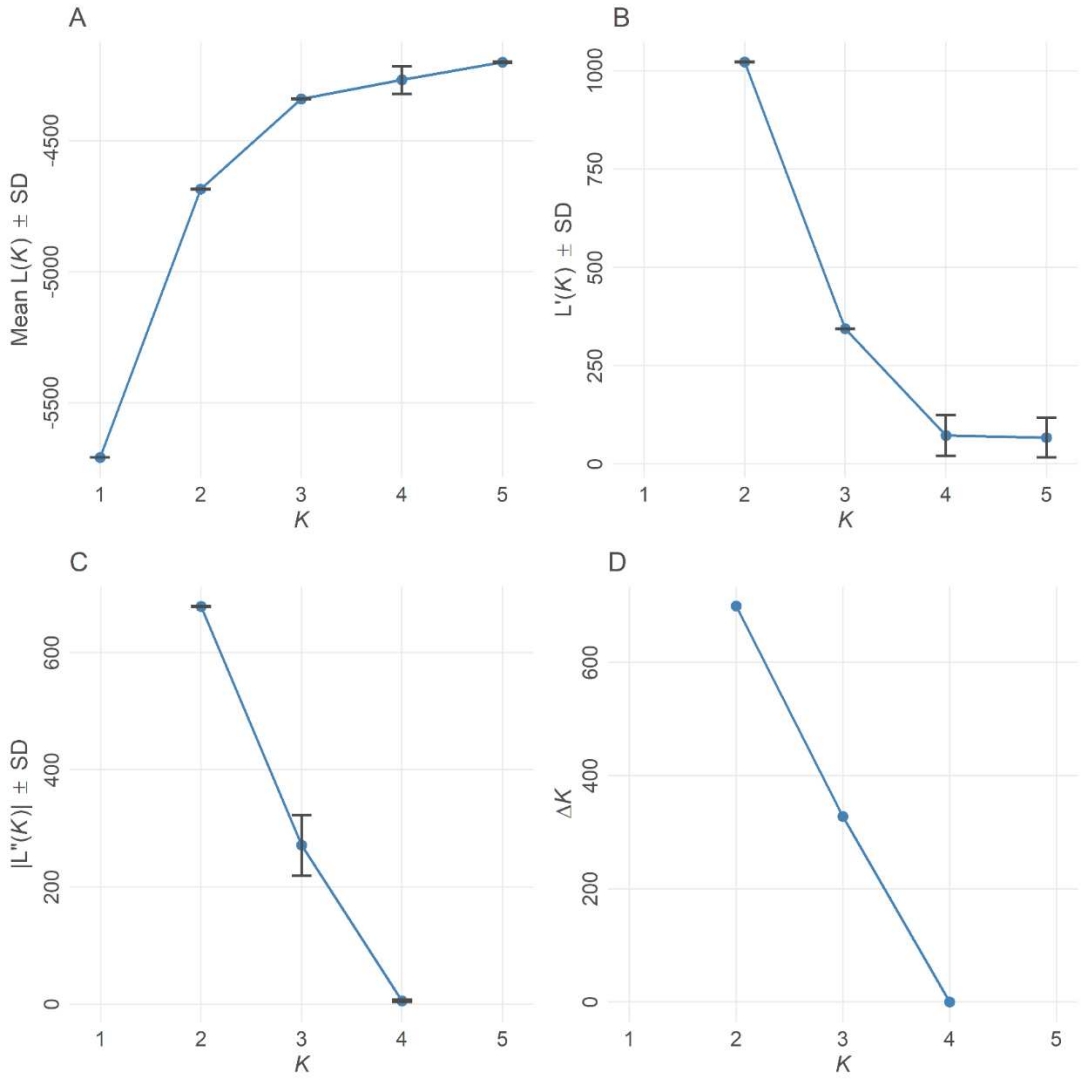

Figure A1. For subsampled dataset 5, STRUCTURE results from analysis of 145 cutthroat trout genotyped at 38 SNPs for K = 1 – 5. (a) Mean estimated log likelihood for each K. Bars represent standard deviation. (b) Rate of change of the likelihood distribution for each K. Bars represent minimum and maximum values across iterations of each K. (c) Absolute values of the second order rate of change of the likelihood distribution for each K. Bars represent minimum and maximum values across iterations of each K. (d) ΔK for each K

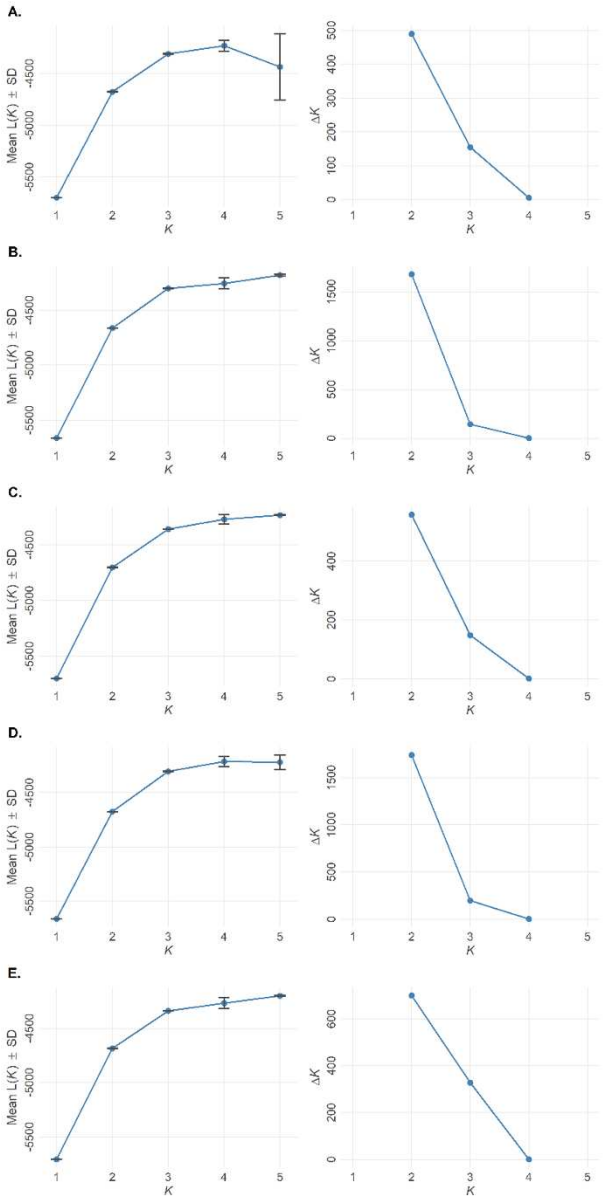

Figure A2. STRUCTURE results from analysis of 145 cutthroat trout genotyped at 38 SNPs for K = 1 – 5. Mean estimated log likelihood (left) and ΔK (right) for levels of K within each subsampled dataset. (a) Subsampled dataset 1. (b) Subsampled dataset 2. (c). Subsampled dataset 3. (d) Subsampled dataset 4. (e) Subsampled dataset 5. Subsampled dataset 5 was presented as the primary analysis in the manuscript

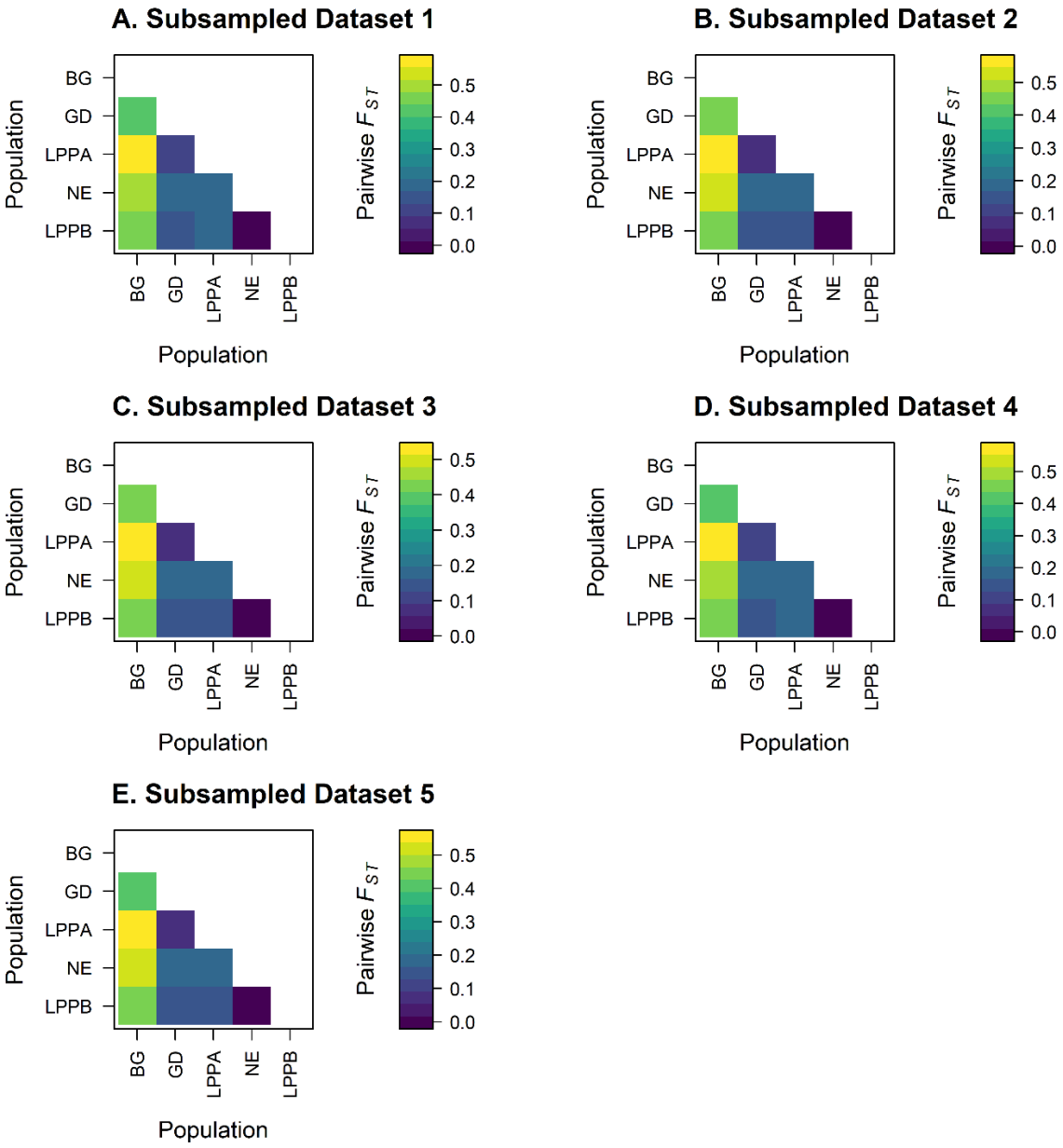

Figure A3. Matrix of pairwise F_{ST} comparisons for all pairs of sites within each subsampled data set. Subsampled dataset 5 was presented as the primary analysis in the manuscript

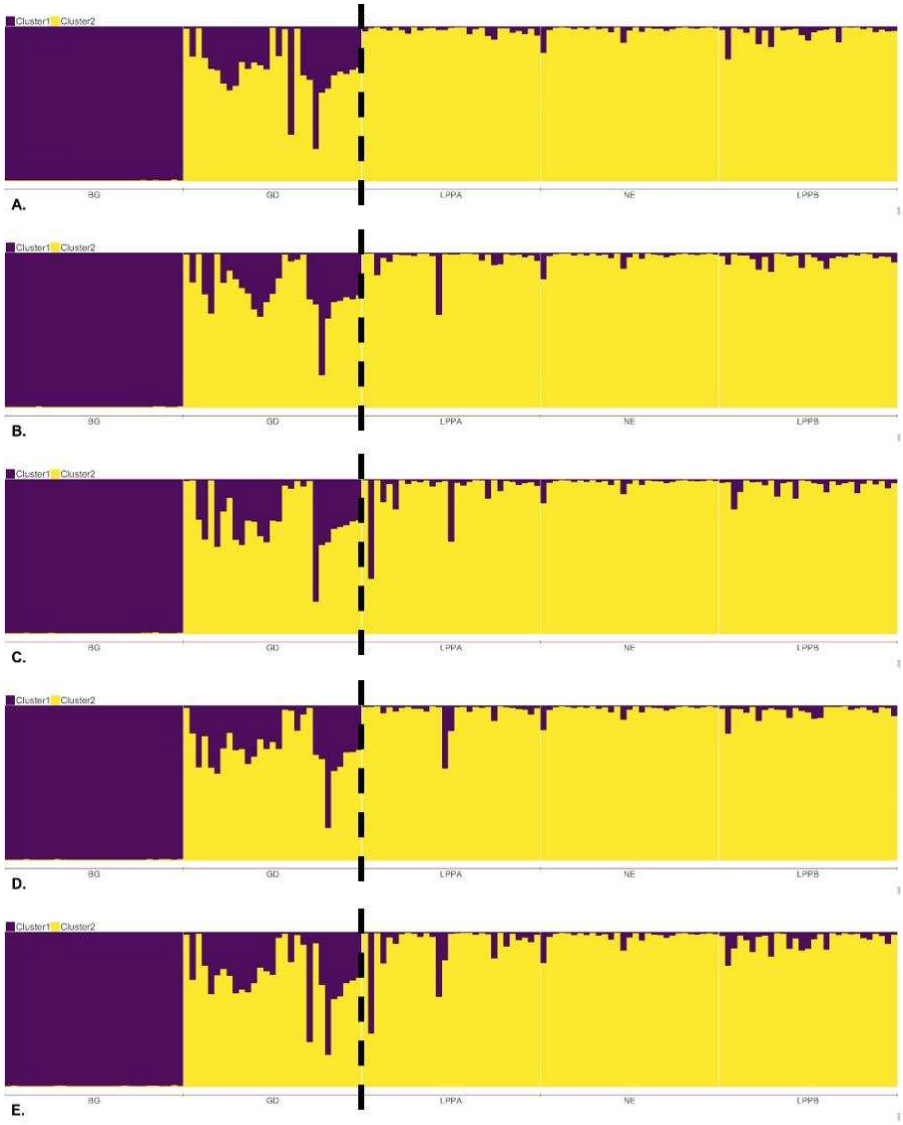

Figure A4. STRUCTURE analysis (K = 2) of 145 cutthroat trout from Baker Gulch (BG), Grand Ditch (GD), La Poudre Pass Creek Above Long Draw Reservoir (LPPA), Neota Creek (NE), and La Poudre Pass Creek Below Long Draw Reservoir (LPPB). Each individual is represented as a vertical bar whose colors correspond to the probability of assignment to different clusters. Sites and individuals within Grand Ditch are ordered from west to east, and location of the Continental Divide is indicated by the dashed black line. (a) Subsampled dataset 1. (b) Subsampled dataset 2. (c) Subsampled dataset 3. (d) Subsampled dataset 4. (e) Subsampled dataset 5. Subsampled dataset 5 was presented as the primary analysis in the manuscript

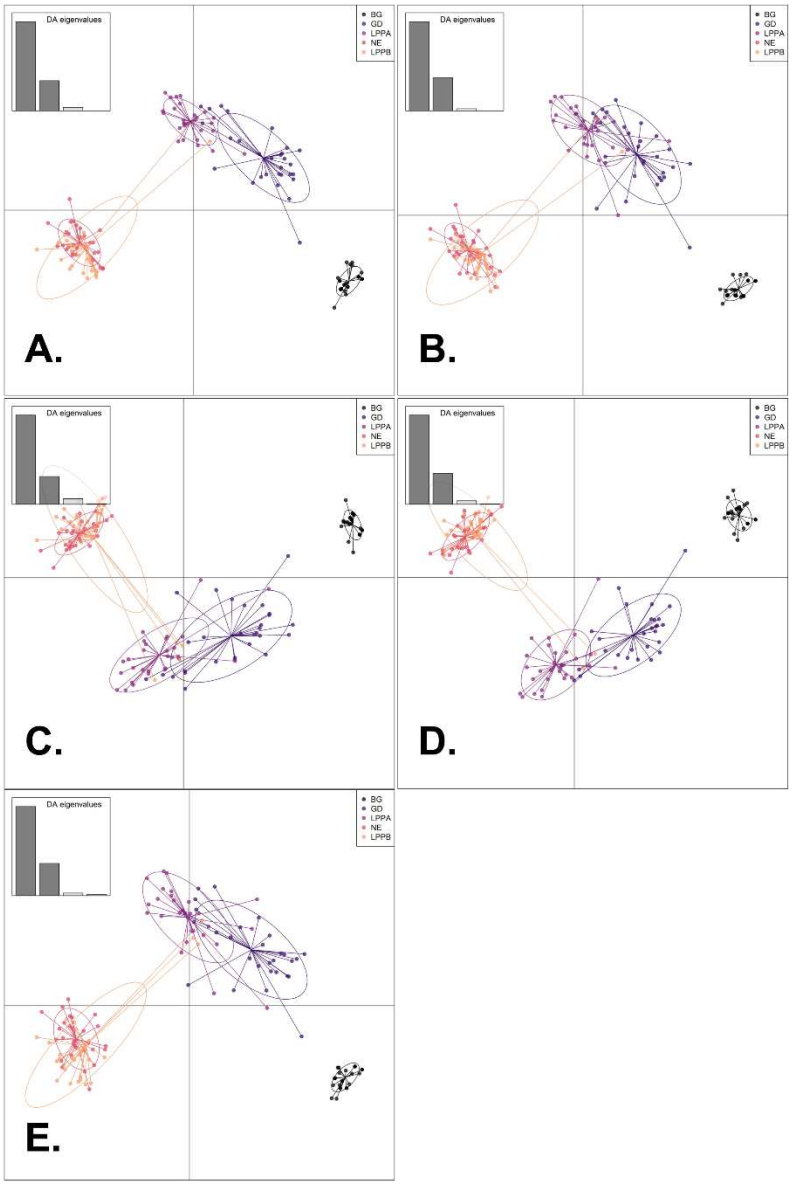

Figure A5. DAPC analysis of 145 cutthroat trout from Baker Gulch (BG), Grand Ditch (GD), La Poudre Pass Creek Above Long Draw Reservoir (LPPA), Neota Creek (NE), and La Poudre Pass Creek Below Long Draw Reservoir (LPPB). Each individual is represented by a point plotted along discriminant function 1 (x-axis) and discriminant function 2 (y-axis). Colors of individual points and inertia ellipses correspond to sampling sites. (a) Subsampled dataset 1. (b) Subsampled dataset 2. (c) Subsampled dataset 3. (d) Subsampled dataset 4. (e) Subsampled dataset 5. Subsampled dataset 5 was presented as the primary analysis in the manuscript