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Connections or Containers: Using Genetic Data to Understand How Watershed Evolution and Human Activities May Influence Cutthroat Trout Biogeography

by

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To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Kendra R. Eaton find it satisfactory and recommend that it be accepted.

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Dedication

I dedicate this to all of those who have supported me and guided me over the years to ultimately help me get to this point. I am so blessed to have such incredible encouragement from so many loved ones, especially my family, and I know I would not have been successful without them. I am eternally grateful to my husband for his continued love and support, and for always believing in me, especially in the times when I felt discouraged. He has been my rock throughout this entire process and his unwavering support was truly invaluable to me.

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Table of Contents

List of Figures

List of Tables

Abstract

Species with large geographic distributions often exhibit complex patterns of diversity that can be further complicated by human activities. Cutthroat trout are one of the most widely distributed native freshwater species in western North America that exhibit substantial phenotypic and genetic variability; however, fish stocking practices have translocated populations outside of their native range and may have obscured intraspecific boundaries. This study focuses on cutthroat trout populations representing three distinct evolutionary lineages that are found intermixed within the Bonneville and upper Snake River watersheds. I use mitochondrial and microsatellite genetic data to determine if populations of cutthroat trout in this contact zone are native or introduced by stocking activities. Results of this study reveal that the distribution of cutthroat trout is due to historical connections between the watersheds. This information will help identify where historical connections existed and prioritize populations of conservation concern.

Key Words: conservation genetics, cutthroat trout, native distribution, natural connections

Introduction

Natural geological processes have a critical influence on population structure and gene flow by altering the landscape through volcanism, glaciation, mountain building, and continental drift (Zeisset and Beebee 2008; Sexton et al. 2009). Similarly, habitat variability can lead to ecological specialization through behavioral, morphological, or physiological adaptation. Given sufficient time, natural isolating mechanisms can lead to local adaptive differentiation and speciation, creating a complex mosaic of unique populations organized by geographic and habitat-related features (Sexton et al. 2009; Kim and Conway 2014). Although natural processes can sub-divide populations and promote diversification, human activities can also obscure natural evolutionary patterns (Castley et al. 2001; Riley et al. 2005; Metcalf et al. 2012). The translocation of species outside of their native range is arguably one of the most important human mediated factors that complicates native species distribution patterns (Castley et al. 2001; Kohout et al. 2012; Doornik et al. 2013; Glotzbecker et al. 2016). For species with extensive geographic structuring, disentangling natural and human-mediated factors affecting their distribution can be difficult, but is critical to the development of management plans for protecting species and their role within ecosystems.

In freshwater ecosystems, natural features commonly isolate populations because many aquatic animals cannot move around physical barriers that extend across the land-water interface (Dunham et al. 2002). As a result, the contemporary distribution of aquatic taxa is often a reflection of once widely inter-

connected populations subsequently isolated by natural events such as major changes in climate and hydrological conditions (Smith 1978a; Hershler et al. 1999). In western North America, the Great Basin and adjacent regions include a vast area of deserts and mountains with watersheds that have experienced wetter and cooler periods with high levels of connectivity followed by periods of desiccation (Hubbs and Miller 1948a; Sigler and Sigler 1987; Meffe and Vrijenhoek 1988). During pluvial times, lakes covered large areas of the Great Basin allowing widespread dispersal of aquatic species; however, when the climate became more arid, connections were lost and populations isolated (Smith 1978a; Sigler and Sigler 1987; Reheis 1999). Over time, isolated populations accumulated differences as selection acted on adaptive variation or as small populations became subject to genetic drift, creating genetically distinct endemic taxa (Sexton et al. 2009; Kim and Conway 2014). A variety of aquatic taxa have been identified with localized endemic species in remnant aquatic habitat of arid regions, including amphibians, mollusks, insects, and fish (Hubbs and Miller 1948b; Austin and Murphy 1987; Hershler 1998; Kuchta et al. 2009). The geographic proximity, but phylogenetic distinctiveness of such taxa, often creates a mosaic of adjacent ranges separated by movement barriers across arid landscapes (Smith 1978a; Hershler 1998; Kuchta et al. 2009). Understanding the range and extent of endemic taxa is essential for protecting and conserving native biodiversity. Yet, as human activities continue to expand in ecosystems such as the Great Basin, translocations of closely related species outside of their native range is becoming an increasing concern as it threatens the genetic

integrity of native populations, decreasing their abundance through competition or predation (Hubbs et al. 1974; Mills et al. 1996; Huxel 1999; Alonso and Castro-Díez 2008).

In addition to historical geographic features, contemporary processes have been instrumental in shaping the genetic population structure of fish species through various human activities (Arthington et al. 1983; Riley et al. 2005; Dudgeon et al. 2006). Increasingly, the movement of freshwater fish species to areas outside of their native range has become a common occurrence, often to support the demand for recreational fishing opportunities and to supplement natural populations (Cowx 1998). The cutthroat trout (*Oncorhynchus clarkii*) is one of the most widespread freshwater fish species native to western North America and is also a popular sport fish that has been propagated and translocated from relatively few hatchery stocks (Harig et al. 2000; Harig and Fausch 2002; Young et al. 2005). Cutthroat trout trace their ancestry in North America to between eight and 16 million years BP (Smith et al. 2002; Stearley and Smith 2016), and as such, natural geological events have influenced their distribution and diversification throughout its range (Campbell et al. 2011). In western North America, significant changes in watershed connections and landscape topology have occurred from processes associated with mountain building, volcanism, and altered flow regimes of rivers during multiple periods of climatic cooling and glaciation (Grayson 1993; Kruse et al. 1997; Link et al. 1999; Bunn and Arthington 2002; Pielou 2008; Campbell et al. 2011). As result of these processes, cutthroat trout have diversified into

genetically distinct taxa; largely organized by geographic features such as major watershed boundaries (Behnke 1992; Trotter 2008). Furthermore, the contemporary distribution of cutthroat trout has been complicated by hatchery propagation and translocation to areas outside of their natural range of diversification. While geographic features can largely explain the main axes of cutthroat trout diversification and distribution, overlap in the distribution of some cutthroat trout taxa, and the widespread stocking of hatchery fish have created confusion about whether some cutthroat trout populations are native or have been introduced (Loxterman and Keeley 2012; Metcalf et al. 2012; AMEC Environment & Infrastructure Inc 2014). With ongoing efforts to restore and recover endangered cutthroat trout subspecies, determining the extent and frequency of native populations is of critical importance to developing management plans.

Bonneville cutthroat trout (*O. c. utah*) and Yellowstone cutthroat trout (*O. c. bouvieri*) are two subspecies whose range is defined by a watershed boundary separating the upper Snake River from the adjacent Bonneville Basin within the Great Basin region of the western U.S. (Behnke 1992, Trotter 2008; Fig. 1). However, even early genetic investigations revealed a third evolutionary lineage that was present in the southwestern portions of the Bonneville watershed and was at least as divergent as populations assigned as Bonneville or Yellowstone cutthroat trout (Loudenslager and Gall 1980; Smith et al. 2002). Later genetic studies also documented a distribution of haplotypes thought to be representative of Bonneville cutthroat trout in areas of the upper Snake River (Campbell et al.

2011). More recent geographic studies of cutthroat trout revealed an intermixing of these three evolutionary lineages in a contact zone surrounding the southern portion of the upper Snake River and northern portions of the Bonneville Basin, with the third lineage being more closely related to populations from the Colorado River watershed (Campbell et al. 2011; Loxterman and Keeley 2012). In Loxterman and Keeley, these 3 evolutionary lineages were defined as Clade A and Clade B in the Bonneville-Yellowstone intermixed clade, with the third lineage called the Great Basin clade. Clade A was found primarily in the upper Snake River range, clade B was detected mostly in the Bear River watershed in the Bonneville Basin, and the Great Basin clade was found in the remainder of the Bonneville Basin with an intermixing of all 3 occurring in the contact zone (Loxterman and Keeley 2012). While similarities of native fish fauna between the Bonneville Basin and upper Snake River have long been associated with pluvial events, such as the Bonneville Flood that connected the two watersheds at about 17,400 years ago (McPhail and Lindsey 1986; Janecke and Oaks 2011), translocations of hatchery trout are common and may also explain unexpected distribution patterns of cutthroat trout subspecies (Behnke 1992; Metcalf et al. 2007). In this study, I use population genetic data to determine if there is evidence of natural admixture for cutthroat trout between the upper Snake River and the adjacent Bonneville Basin or whether human translocation of closely related subspecies better explains the current distribution of cutthroat trout within the study area.

Genetic analyses provide a powerful tool to resolve the status of

populations whose taxonomy and biogeography are poorly understood (Teske et al. 2014). Many studies have used genetic approaches to determine source populations of introduced species (DeWalt et al. 2011; Zhao et al. 2013), identify invasive species (Teske et al. 2014; Krzemińska et al. 2016), or simply to uncover genetic differences that occur between known native or introduced populations (Squirrell et al. 2001; Dynes et al. 2001). In some instances, the distribution of populations is such that it is not clear whether specific populations are native or introduced because individuals may be morphologically indistinguishable from each other despite exhibiting genetic differences (Bernatchez et al. 1995). Population genetic data can be used to identify evidence of recent translocations through estimates of genetic differentiation, diversity, and structure (DeWalt et al. 2011; Teske et al. 2014; Signorile et al. 2016; Frantz et al. 2017). Here, I examine the population genetic structure of cutthroat trout along a contact zone, where multiple evolutionary lineages are intermixed, to determine if secondary contact may be due to natural processes or recent human-mediated introductions.

Methods

Study area and sample collection

To describe genetic variation of cutthroat trout, tissue samples were collected from individuals representing 30 populations within the Bonneville Basin and upper Snake River (Fig. 1). I collected samples of cutthroat trout from headwater streams with backpack electro-fishing. Once captured, each individual

was fin-clipped for genetic analysis and then released near the point of capture. Genomic DNA was extracted using the ZR Genomic DNA tissue extraction kit (Zymo Research) following the manufacturer's protocol. All sampling locations were presumed to be native populations of cutthroat trout, except Six Mile Creek in the Raft River watershed. Six Mile Creek was chemically treated to remove the fish population because it was introgressed with non-native rainbow trout (*Oncorhynchus mykiss*) (D. Megaragle, Idaho Department of Fish and Game, Magic Valley Region, personal communication) and subsequently recolonized using cutthroat trout from neighboring Eight Mile Creek. We included Six Mile Creek as a method for comparison describing the genetic population structure of a known translocated population of cutthroat trout.

Mitochondrial DNA data and analyses

I examined the diversity and geographic distribution of cutthroat trout lineages by comparing mtDNA haplotypes from all study populations. Mitochondrial DNA is a maternally inherited haploid molecule that is used in evolutionary studies because it is highly conserved across generations. Mitochondrial DNA was sequenced for 10 individuals from each of the 30 populations (n=300) for the NADH dehydrogenase subunit 2 gene (ND2). Amplification by polymerase chain reaction (PCR) used the sequencing primers NDintF6 and NDVarR (Novak et al. 2005; Campbell et al. 2011). PCR reactions were performed in 25 µl total volumes using 8 µl of 2X ReddyMix PCR Master Mix, 1 µl (10 mM) of each primer, and 2 µl of genomic DNA. The thermal profile

included an initial 94°C denaturation step followed by 35 cycles at 94°C for 30 s, annealing at 58°C for 45 s, and extension at 72°C for 75 s, with a final extension at 72°C for 10 min. PCR products were submitted to the Idaho State University Molecular Research Core Facility for purification and DNA sequencing on an ABI 3130*xl* automated sequencer.

Sequences were edited and aligned to a reference sequence using Sequencer v.4.9 software and Mega v. 6 (Tamura et al. 2013). I estimated haplotype and nucleotide diversity, as well as haplotype frequency, using DnaSp v. 5.0 (Librado and Rozas 2009) and Mega v. 6 software. Evolutionary relationships of cutthroat trout haplotypes in the contact zone will show either historical connections if geographic patterns of haplotypes are identified, or stocking practices if randomized haplotypes of neighboring populations exist. To illustrate evolutionary relationships, I constructed a phylogenetic tree with representatives of each unique ND2 haplotype in addition to sequences from other subspecies of cutthroat trout (Loxterman and Keeley, 2012) and a rainbow trout haplotype was used as an outgroup. Trees were constructed using the Tamura-Nei substitution model with invariant sites based on jModeltest (Posada 2008) results. The final phylogenetic tree was generated with 1000 bootstrap replicates as implemented in the program PhyML (Guindon et al. 2010) and edited in FigTree v.1.4.3 (Rambaut 2016).

Microsatellite DNA data and analyses

Estimates of genetic population structure, organization, and diversity were compared using nuclear microsatellite loci. Microsatellite DNA involves repeat

segments of nuclear DNA in non-coding regions that are highly variable, making them useful for contemporary data and identifying differences between closely related populations. All individuals from the populations sampled were genotyped for 11 polymorphic loci (Och18, Och24, Och27, Och29, Och30, Och35, Ocl1, Ogo4, Omm1036, Omy77, and Ots107) (Olsen et al. 1998; Nelson and Beacham 1999; Rexroad Iii et al. 2002; Robinson et al. 2009). I amplified microsatellite loci in 15 µl PCR reactions using 6 µl of 2X ReddyMix PCR Master Mix (ABgene), 0.5 µl (10 mM) of each labeled primer, and 2 µl of genomic DNA. A PCR temperature profile for Och18, Och27, Och29, Och30, Och35, Omy77, and Ots107 loci included an initial 94°C denaturation step for 180 s, followed by 40 cycles at 94°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 60 s, with a final extension at 72°C for 30 min. To maximize yield of DNA for all remaining loci, I changed the thermal profile to 35 cycles and annealing at 50°C (Ogo4), 57°C (Och24 and Omm1036) and 55°C (Ocl1). All PCR products were submitted to the Idaho State University Molecular Research Core Facility for fragment analysis and genotyping using an ABI3130*xl* automated sequencer. I subsequently used GeneMapper software (ver. 3.7) to genotype every individual at each locus. All peaks were verified manually to ensure accuracy.

Microsatellite diversity and fine scale genetic structure were examined using the number of alleles per loci, average heterozygosity, allelic richness, and pairwise genetic differentiation (Fst) with Microsatellite Analyzer (MSA, ver. 4.05; Dieringer and Schlötterer 2003), FSTAT (ver. 2.9.3; Goudet 2001), and Arlequin software (Excoffier and Lischer 2010). I used MSA to test for population level

differences in the number of alleles per loci and heterozygosity to identify diversity measures that could indicate stocking or natural causes. FSTAT was used to test for significant differences in allelic richness based on 10,000 permutations. Analyses for pairwise genetic differentiation estimates were calculated in Arlequin with 10,000 iterations.

Geographic structuring of genetic data was visualized both at the population level, as well as at the watershed level, using a neighbor-joining tree and population assignment tests. If cutthroat trout populations have a natural distribution history, the neighbor joining tree and clustering should group by watershed and migration should be between neighboring populations. Alternatively, the absence of geographic structure or migration events between watersheds would suggest a history of non-native introductions. For the neighbor-joining tree, I estimated genetic distance using Cavalli-Sforza chord distance (Cavalli-Sforza and Edwards 1967) and constructed the tree using Phylip (ver. 3.695; Felsenstein 1993). I generated a bootstrap tree using 100 bootstrap replicates and visualized it in FigTree. In addition to illustrating geographic structure using genetic distance, patterns of migration and population clustering were examined using GeneClass2 (Piry et al. 2004) and Structure (ver. 2.3.4; Pritchard et al. 2000) software programs. First, I identified migrants between populations through assignment tests that assign each individual to the most likely population of origin using genetic similarity. To assess geographic genetic structure, I estimated the number of populations (K) with Structure using an individual-based Bayesian assignment method, with no prior information of

population origin. For the Structure analysis, five independent runs for each K (2-30) were conducted using the admixture model at 500,000 iterations with a burn-in of 200,000. The most likely number of population clusters (K) was determined by the estimation of ΔK and the likelihood of the posterior probability [L(K)] (Evanno et al. 2005). To visualize the assignment of each population in the resulting clusters, I used the programs Clumpp (ver. 1.1.2; Jakobsson and Rosenberg 2007) and Distruct (ver. 1.1; Rosenberg 2004).

To examine the degree of geographic structuring and isolation among populations, I compared stream distance and genetic distance between population pairs and tested for associations of genetic data. Stream distance was measured between sampling locations as a measure of the geographic distance between populations using ArcMap (version 10.3) and the Spatial Tools for the Analysis of River Systems (STARS) extension (Peterson and ver Hoef 2014). Geographic distance between watersheds was calculated by connecting existing rivers to historical linkages through a GIS representation of Lake Bonneville outflow into the upper Snake River (McCoy et al. 2001). A distance matrix between all sampling locations was obtained using the Spatial Stream Network (SSN) package for R statistical software (ver Hoef et al. 2014). Isolation by distance (IBD) was assessed using 10,000 randomizations with IBD web service (Jensen et al. 2005). If cutthroat trout populations colonized these areas through watershed connections, I would expect a significant pattern of isolation by distance. Conversely, no relationship between genetic and geographic distance would be expected if the populations were translocated. A Mantel test was used

to test for a relationship between genetic distance (Fst) and geographic distance (km). To test for associations of genetic data between and within watersheds, I used a principal component analysis (PCA) to determine if the geographic distribution of microsatellite alleles was primarily organized by watershed boundaries or if they were intermixed across the contact zone. The placement of populations on the principal components axis was based on the similarities across all microsatellite allele sizes. PCA scores were calculated in R statistical software and the average PCA score per population was used to compare population association among locations sampled.

Stocking data and analyses

If stocking activities have been a primary factor influencing the diversity of trout in the study area, then the frequency and extent of stocking should be related to measures of genetic diversity. To test for an association between stocking history and the population genetic structure of cutthroat trout within the contact zone, I compiled all available historical records of cutthroat trout introductions from the Idaho Department of Fish and Game database (https://idfg.idaho.gov/fish/stocking). Stocking records from the Snake, Southeast, and Magic Valley regions were compiled by waterbody location name for all years available in the database (1967-2016) and used to assign the site of translocation within upper Snake River and northern Bonneville watersheds. While most of the study area is covered by the Idaho database, a small portion of the watersheds occur in the state of Utah (Fig. 1). For these populations there

are no electronic records available; however, past reviews of Utah populations indicate no stocking of cutthroat trout in these areas (Thompson 2002). I used a geospatial database to visualize the distribution, frequency, and distance of stocking events to the sampling locations. Each location was standardized to the smallest scale watershed boundary dataset layer (12-digit hydrological unit code, HUC) available from the US Watershed Boundary Dataset (US Geological Survey et al, 2013). I estimated the total number of cutthroat trout stocked at a location by summing the number of fish listed for each stream, river, lake, or reservoir site. I also estimated the frequency of events by counting the number of times stocking occurred at a location for the 49 years of data available. To test for any association between stocking history and genetic diversity, I compared three measures of genetic diversity for each sampling location (allelic richness, number of alleles, and heterozygosity) with the three measures of stocking extent and intensity (distance to nearest stocking location, total number of fish, and the number of events). I used a simple and multiple regression analysis to test for the effect of each variable on genetic diversity of cutthroat trout. Tests of significance for each stocking variable was based on a type III sum of squares, implemented in R statistical software.

Results

For all the cutthroat trout populations sampled, 14 locations were in the Portneuf River watershed, 11 were in the Raft River watershed, and three were in the Malad River watershed (Table 1, Fig. 1). Two additional populations were

used as outgroups, one in a tributary of the South Fork of the Snake River and one from a tributary of the Bear River: Pine Creek and Maple Creek respectively.

Mitochondrial DNA

Mitochondrial sequences were generated at the ND2 gene (1056bp) for 300 cutthroat trout representing 30 populations with 18 unique haplotypes detected. The number of haplotypes per population ranged from one to five and were differentially distributed among populations with two haplotypes shared across a total of 12 populations and eight haplotypes each unique to a single population (Table 1). The most frequent haplotype (H8) occurred in 57 individuals from 12 different populations, while the least frequent haplotypes (H7, H12, H17, and H18) were found in a single individual (Table 2).

Phylogenetic comparisons using 34 unique haplotypes, revealed distinct clades for each subspecies of cutthroat trout with samples from this study consisting of the Bonneville-Yellowstone intermixed clade and the Great Basin clade (following Loxterman and Keeley, 2012) (Fig. 2). Fifteen haplotypes from this study were identical to Genbank sequences. However, three novel haplotypes were detected, one each from One Mile Creek, Second Creek, and Pine Creek respectively (Table 2). In the phylogeny, the Great Basin clade includes individuals from 24 populations and shares an evolutionary history with other Colorado River subspecies (Fig. 2). The remaining haplotypes from 21 populations are nested in the Bonneville-Yellowstone clade and are more closely related to Columbia River subspecies.

Upon closer examination of haplotype distribution, there is further geographic structuring within the contact zone and among watersheds. All populations from the Malad River watershed were associated with the Great Basin clade (Fig. 3). While most of the populations from the southern Portneuf River drainage identified as Great Basin, the northern Portneuf populations were in the Bonneville-Yellowstone clade (Fig. 3). Conversely, populations from the Raft River watershed were found equally in both the Great Basin and the Bonneville-Yellowstone clades. However, populations sampled in the eastern Raft River watershed were predominantly Bonneville-Yellowstone, while western populations were in the Great Basin clade (Fig. 3).

Microsatellite DNA

A total of 718 cutthroat trout (11-25 individuals per stream) were genotyped from the contact zone at 11 microsatellite loci. All loci were polymorphic, with the average number of alleles per locus ranging from 1.82 in Wildcat Creek to 11.55 in Pine Creek. Allelic richness was also lowest in Wildcat Creek (1.76) and highest in Pine Creek (8.27). Overall, average heterozygosity ranged from 0.25 in Grape Creek to 0.70 in First Creek (Table 1). Estimates of pairwise genetic differentiation (Fst) indicate significant differentiation between all 30 population pairs. The lowest genetic differentiation occurred between neighboring populations, Fish Creek and Pebble Creek (Fst=0.045) and was highest between two Raft River populations, Six Mile Creek and Wildcat Creek (Fst=0.67; Supplemental Table 2).

The primary divergence among populations in the neighbor-joining tree separated the upper Portneuf River on the east side of the valley from all other populations (Figs. 3 and 4). Secondary divergence in the tree separated lower and western Portneuf River populations from those in the Malad River of the Bonneville Basin, as well as the two upper Marsh Creek populations of the Portneuf Valley. All Raft River populations clustered together in the tree and tended to be organized primarily by geography. Cassia Creek, the most downstream location, was most divergent from the others in the Raft River, while the remaining populations were closely associated with neighboring sites (Fig. 4).

On average, 92% of individuals assigned to their sampling location (range 55-100%). While some migration was detected, most of these events occurred between neighboring populations (Fig. 5). Not surprisingly, the largest number of misassignments was detected between adjacent populations in the Portneuf River. Most of these streams are flow connected or would have been within the last 50-100 years. No recent migration was detected between upper Snake River and Malad River populations (Fig. 5a). Very little migration was detected in the Raft River populations and five locations had no misassignments (Fig. 5b).

Bayesian cluster analyses of the 30 trout populations suggested the most likely number of clusters, based on the data, was K=5 or K=18 (Fig. 6). Results based on five clusters divided the 30 populations largely based on geographic location within the watersheds. The clusters included upper Portneuf River, lower Portneuf River, Malad River, and two groupings within the Raft River. In all cases, intermixing primarily occurred within watersheds (Fig. 6a). At K=18,

greater levels of intermixing occurred; however, the patterns of geographic structure observed for this level of organization were similar to those for K=5. The dominant proportion of individuals were assigned to a cluster for a single population or for a group of populations from neighboring locations within a watershed (Fig. 6b).

The degree of geographic structuring and isolation among populations is further supported by comparisons of geographic distances and genetic distance between population pairs. Isolation by distance tests reveal that a significant proportion of the genetic variation between populations is explained by geographic distance (Mantel test, r=0.3106, p=0.0016; Fig. 7a). Within watersheds, geographic distance only explains a significant proportion of the variation in genetic distance in the Portneuf watershed (Mantel test, r=0.297, p=0.022). However, I detected a positive correlation between geographic distance and genetic distance in the Raft River (Mantel test, r=0.085, p=0.264) and Malad River populations (Mantel test, r=0.371, p=0.521; Fig. 7b). Principal Component Analyses also revealed population structuring both within and between the three major watersheds. The first four axes of the PCA explained 47.2% of the genetic variation: PC axis 1 16.7%, PC axis 2 12.8%, PC axis 3 9.46%, and PC axis 4 8.28%. The first axis separated the Portneuf River from the Raft River and Malad River watersheds, as well as the eastern and western populations of the Portneuf River watershed (Fig. 8). The second (Fig. 8a) and third axes (Fig. 8b) further organized populations within watersheds and separated Raft River populations from Malad River populations.

Stocking records

Based on stocking records available from the Idaho Department of Fish and Game for the years 1967 to 2016, about 123 million cutthroat trout were stocked into the upper Snake, Southeast, and Magic Valley regions encompassing the study area. Records indicate that stocking ranged from a few dozen fish for one or two events to several millions of fish over multiple years (Supplemental Table 1). The spatial extent of translocations varied widely across the watershed and encompassed 278 locations (Fig. 9). The South Fork of the Snake River and surrounding areas appear to have received the greatest intensity, whereas the Raft River watershed and Portneuf River watershed appear to have much lower levels of stocking for cutthroat trout (Fig. 9). Of the 30 streams sampled for this study, only 4 of the streams had records of fish introduced into those named streams. The outgroup Pine Creek, in the South Fork of the Snake River, had the most recorded cutthroat trout translocated out of all the sampled populations. In the Raft River subbasin, no records were reported to have occurred in any of the sampled streams; only high mountain lakes were reported to have had cutthroat trout translocated into them (Fig 9). In the Portneuf River, 3 northern populations had 1 or 2 stocking events (Gibson Jack, Pebble, and Toponce creeks) with most occurring in mainstem rivers, high elevation lakes, and reservoirs. Average nearest neighbor distance between cutthroat trout streams and reported stocking location was 44.72 km, with a range of 0 to 121.0 km.

Spatial analyses of stocking data and genetic data indicated significant associations between some variables and not others. The number of events and the total number of fish did not explain a significant amount of the variability of allelic richness, number of alleles, or heterozygosity (all p-values>0.05). However, the distance from the nearest stocking locations did explain a significant amount of the variability in all three genetic measures; allelic richness (r^2=0.196, p=0.014, $F_{1,28}$= 6.81), number of alleles (r^2=0.171, p=0.023, $F_{1,28}$= 5.78), and heterozygosity (r^2=0.266, p=0.0035, $F_{1,28}$=10.16; Fig. 10). Multiple regression models indicated distance from stocking was the only significant factor explaining variability in allelic richness and number of alleles. Average heterozygosity measures did not show a significant relationship with any variables in the multiple regression model.

Discussion

In this study, I investigated whether cutthroat trout distribution patterns in the Bonneville-Yellowstone contact zone are explained by natural pathways of dispersal or stocking events of nonnative subspecies. My results indicate that historical geographic features have played a dominant role in the formation and organization of cutthroat trout diversity. Interestingly, the analyses also suggested that stocking of non-native species had minimal influence on natural distribution patterns, despite the intensity of stocking. Analysis of genetic diversity points to natural dispersal of the three major lineages of cutthroat trout between the Bonneville and Yellowstone ranges, providing evidence in support of

historical watershed connections. Such connections have been proposed by geologists and are of continued interest to biologists as an explanation for current distributions of fish fauna across the landscape (Link et al. 1999; Campbell et al. 2011; Loxterman and Keeley 2012; Campbell et al. 2017).

As a slowly evolving molecule of the genome, mitochondrial DNA (mtDNA) is often used to estimate deep evolutionary divergence between and within taxa (Avise et al. 1987; Walker and Avise 1998). For aquatic taxa, mtDNA has been particularly useful for uncovering the occurrence of movement barriers that naturally isolate watersheds over long periods of time. When geographic isolation is sustained, the distribution of mtDNA haplotypes can be used to identify historical barriers and connections (Zamudio et al. 2003; Johnson et al. 2004); however, secondary contact between lineages (Latta and Mitton 1999; Pinceel et al. 2005) and human-mediated translocation of taxa can obscure their natural extent, limiting the application of mtDNA data alone (Rawlings et al. 2007; Kolbe et al. 2013; Merson et al. 2017). Across cutthroat trout populations, significant evolutionary divergence is reflected in distinct lineages that can be defined by mtDNA haplotypes. Coastal, Westslope, Lahontan, and Rio Grande cutthroat trout, all have mtDNA haplotypes that appear to diagnose specific geographic areas and can therefore be used to define subspecies boundaries (Loudenslager and Gall 1980; Smith et al. 2002; Loxterman and Keeley 2012; Campbell et al. 2017). In other subspecies, intermixed mtDNA haplotypes may be a result of natural admixture from historical events or from more recent translocations. While it would be logical to conclude that haplotypes from geographically distant

locations are from non-native introductions, if admixed populations are in adjacent watersheds then one must be cautious when inferring whether the population is introduced or not. In this study, the distribution of the three mtDNA lineages is restricted in the contact zone and follows a natural progression from south to north (Fig. 3); however, translocations are not an impossibility based on these results alone. While mtDNA can be used to infer evolutionary patterns, the examination of contemporary distribution patterns and connectedness requires more polymorphic genetic markers.

Nuclear microsatellite loci are highly polymorphic and putatively selectively neutral, and thus are particularly useful for examining current geographic genetic structure among populations. In highly vagile species, microsatellite data reveals panmixia with little population structure except at very large spatial scales (Anderson et al. 2004). In contrast, dispersal-limited species exhibit population structure and increased genetic differentiation between neighboring populations (Palo et al. 2004). Contact zones, like that between subspecies of cutthroat trout, pose a unique situation when trying to determine whether the populations in these ranges overlap as a consequence of historical connections or recent migration. Codominant microsatellite data can provide information about current population structure and is complimentary to the slower evolving, deeper divergence estimates provided by mtDNA data. While studies of cutthroat trout have investigated contemporary distribution patterns with nuclear data (Wenburg et al. 1998; Taylor et al. 2003; Pritchard et al. 2009), secondary contact has not fully been explored with subspecies that exhibit an intermixed distribution

(Metcalf et al. 2007). In this study, microsatellite analyses suggest contemporary gene flow is restricted within watershed boundaries. These data, in conjunction with mtDNA analyses, supports the explanation that ancient connections provided natural avenues for dispersal between the Bonneville and Yellowstone basins and that current watershed boundaries have continued to limit gene flow since that dispersal event.

Extensive stocking of nonnative fishes has occurred in ecosystems worldwide and can have significant effects on native biodiversity through competition, predation and disease transmission (Allendorf 1991; Largiadèr and Scholl 1995; Matthews and A. Knapp 1999). When introduced populations have a close phylogenetic relationship with native taxa, hybridization and introgression can further complicate how to assess their status. Cutthroat trout are one of the most widely distributed freshwater fish in western North America, but they exhibit significant evolutionary diversification organized by geographic barriers not always understood or recognized (Wilson and Turner 2009; Loxterman and Keeley 2012). Hatchery propagation of cutthroat trout from a handful of sources and widespread stocking of fish has raised concerns that conservation populations may not represent native populations (Metcalf et al. 2007, 2012; AMEC Environment & Infrastructure Inc 2014). Given the admixed distribution of haplotypes and the extensive stocking history of cutthroat trout, one possible explanation for the biogeographic pattern observed in the study area is from hatchery introductions. Taken together, however, mtDNA and microsatellite data indicate an organized genetic population structure with little influence of

translocated fish. With records of more than 120 million cutthroat trout stocked into the study area, survival and success of those fish must have been extremely poor. In fact, hatchery propagated fish are well-known for their low survival rates when released into natural ecosystems (Reisenbichler and McIntyre 1977; Jonsson et al. 2003; Araki et al. 2008). For example, many millions of hatchery produced Pacific salmon and steelhead trout are released into the Columbia River every year to supplement natural populations, but often have much lower survival rates that their wild counterparts (Beamish et al. 2012). Hatchery populations of resident trout and char species also seem to have similarly low survival rates when released in lakes and streams (Brunner et al. 1998; Koskinen et al. 2002; Hansen 2002; Hansen and Mensberg 2009). Further evidence of low translocation success is reflected in the observation that no rainbow trout haplotypes were detected in our samples despite over 166 million rainbow trout being stocked in the study area over the last 49 years (https://idfg.idaho.gov/fish/stocking). In the populations we sampled, most were further from stocking locations in headwater streams, typically isolated by movement barriers, making it difficult for hatchery fish to interact with these native populations.

The correlation between genetic diversity and the proximity to stocking was not unexpected. The introduction of individuals into a population should add genetic variation (Roman 2006; Roman and Darling 2007) and has been used to supplement small populations at risk of genetic loss. However, translocation of individuals outside their native range, even when supplementing threatened

populations, must consider local adaptations and geographic structure, or such actions can be detrimental (Allendorf 1991; Reisenbichler and Rubin 1999; Levin et al. 2001). In my study, the correlation was not sufficient to disrupt the population genetic structure of cutthroat trout in the contact zone, indicating the natural organization is largely intact despite stocking. Future stocking activities should recognize local genetic diversity when identifying suitable source populations.

The exchange of aquatic taxa between neighboring watersheds illustrates how the gain and loss of natural connections over time can create distribution patterns that do not adhere to watershed boundaries. In particular, changes in climate and hydrological conditions affect the extent and degree of connectivity between populations of aquatic organisms (Smith 1978b; Hershler et al. 1999). Because they are restricted within the landscape, fish are reliant on aquatic connections to disperse and populations can become genetically distinct when natural connections are altered (Oberdorff et al. 1997). Cutthroat trout in the Bonneville-Yellowstone range have a long history of fluctuating aquatic connections that have influenced their distribution and population structure (Campbell et al. 2011; Loxterman and Keeley 2012; Campbell et al. 2017). Pluvial Lake Bonneville began to rise with the addition of the Bear River water to Lake Bonneville around 50 ± 10 ka (Bouchard et al., 1998). Around 17,400 years ago, Lake Bonneville overflowed and the flood passed through Marsh Valley and the Portneuf Valley before entering the Snake River Plain (Link et al. 1999). This flood created a temporary watershed connection from the Bonneville Basin to the

present day Portneuf River watershed, facilitating fish dispersal northward with the flow of water. The Bonneville flood provides a natural explanation for the current distribution of major the three lineages of cutthroat trout in the Portneuf River watershed. While the combinations of lineages in the Raft River watershed could also be attributed to the Bonneville flood, other studies have hypothesized that headwater transfer between the Raft River and rivers flowing southward into ancient Lake Bonneville is a likely avenue of dispersal (Campbell et al. 2011; Loxterman and Keeley 2012). Headwater transfer between the Raft River and rivers flowing to Lake Bonneville could provide the necessary historical watershed connections that explain the contemporary cutthroat trout distribution patterns in the Bonneville-Yellowstone contact zone.

Cutthroat trout are a species of conservation concern and efforts to improve their status have primarily focused on removing non-native competitors or predators, restoring habitat, and by reintroduction programs (Langlois 1983; Stuber et al. 1988; Coffin and Cowan 1995; Hilderbrand 2002). Management decisions for determining cutthroat trout subspecies are largely based on watershed boundaries despite the chance of secondary contact in transition zones. When a subspecies is found in a neighboring watershed, it is sometimes attributed to stocking practices without an exploration of possible natural avenues of dispersal (Metcalf et al. 2007). This study illustrates the importance of understanding the evolutionary history of cutthroat trout subspecies, in conjunction with contemporary gene flow. Consistent with other studies, my study suggests that conservation decisions should consider the genetic structure

between watersheds, as well as in neighboring populations (Taylor et al. 2011). While mtDNA haplotypes and genetic population structure may not align with all levels of intraspecific variation, they do describe primary axes of diversity that should inform how management plans proceed. To restore native populations, reintroduction efforts must consider localized adaptations, evolutionary lineages, secondary contact and differences between neighboring populations. By combining historical and contemporary genetic data, biologists are likely to provide the most comprehensive information to aid in conservation efforts.

Conclusions

Natural avenues of dispersal appear to be the primary factor influencing the distribution patterns of subspecies of cutthroat trout in the Bonneville-Yellowstone contact zone. Cutthroat trout populations have diversified into three major phylogenetic lineages intermixed in the Snake River and adjacent Bonneville Basin. Historical events have shaped the distribution of these closely related subspecies through geographic connections and isolation. However, translocations of cutthroat trout into neighboring populations have influenced the distribution of genetic diversity, adding complexity to their geographic structure. The mtDNA data supports ancient aquatic connections that allowed dispersal of subspecies into the Yellowstone region through the Bonneville Flood and headwater transfer with ancient Lake Bonneville. Microsatellite evidence identifies contemporary gene flow and migration that is primarily within watersheds and influenced by stream distance. While stocking events seem to have some impact, overall, these events had minimal influence on the natural

distribution in the populations sampled. The results of this study are in agreement with the growing body of evidence suggesting natural connections between watersheds in the Bonneville and Yellowstone range. My study illustrates how genetic data can be used to identify native or introduced populations in a contact zone. Importantly, this information will help identify where historical connections may have existed and allow mangers to prioritize populations of conservation concern.

Tables

Table 1. Population, population ID number, sample size (n), mean observed heterozygosity (Ho), mean Allelic richness (Ar), mean number of alleles, number of mitochondrial DNA haplotypes, and location information of 30 populations of cutthroat trout.

Population	Population ID	n	Mean Ho	Mean Ar	Mean No. of Alleles	No. of MtDNA haplotypes	UTM Zone	easting	northing
Basin Creek, ID	1	25	0.55	4.62	5.27	3	12	267903	4639520
Clear Creek, UT	2	25	0.48	3.41	4.00	1	12	301436	4643950
Dempsey Creek, ID	3	22	0.64	4.31	5.00	2	12	415889	4714419
E Bob Smith Creek, ID	4	25	0.39	3.31	3.82	1	12	411772	4722411
Eight Mile Canyon Creek, ID	5	25	0.25	3.25	4.18	3	12	321305	4669070
First Creek, ID	6	25	0.70	5.02	5.73	1	12	407254	4678708
Fish Creek, ID	7	25	0.54	5.62	6.91	2	12	419793	4718575
Garden Creek, ID	8	17	0.61	5.10	5.64	2	12	387831	4717333
George Creek, UT	9	25	0.66	5.75	7.55	2	12	298467	4642977
Gibson Jack Creek, ID	10	25	0.61	5.71	7.00	5	12	383034	4738756
Goodenough Creek, ID	11	25	0.65	5.50	6.91	4	12	394311	4723259
Harkness Creek, ID	12	22	0.45	3.66	4.18	2	12	405383	4724780
Inman Creek, ID	13	25	0.68	6.81	8.91	4	12	403566	4743461
Johnson Creek, UT	14	25	0.49	4.04	4.64	3	12	289113	4640137
LHF Marsh Creek, ID	15	25	0.59	4.60	5.64	2	12	416178	4699105
Maple Creek, ID	16	25	0.62	5.56	7.09	3	12	441496	4657545
Mill Creek, ID	17	25	0.67	5.97	7.45	3	12	395245	4689546
One Mile Creek, UT	18	25	0.63	4.78	5.55	3	12	298837	4649457
Pebble Creek, ID	19	25	0.55	5.87	8.00	2	12	414905	4731530
Pine Creek, ID	20	25	0.67	8.27	11.55	4	12	478060	4823821
Robbers Roost Creek, ID	21	25	0.56	4.49	5.45	3	12	401627	4728856
Second Creek, ID	22	25	0.61	4.13	4.82	2	12	406940	4675010
Six Mile Creek, ID	23	25	0.27	2.07	2.27	1	12	321638	4666072
Third Creek, ID	24	25	0.54	2.95	3.45	1	12	408787	4672530
Toponce Creek, ID	25	11	0.62	6.73	6.73	3	12	414919	4746856
Walker Creek, ID	26	25	0.66	5.38	6.27	2	12	397871	4730991
Wildcat Creek, UT	27	25	0.38	1.76	1.82	1	12	283570	4642956
Grape Creek	28	25	0.25	3.40	4.18	2	12	284737	4672669
Cassia Creek, ID	29	25	0.65	6.75	8.73	5	12	282795	4679521
Almo Creek, ID	30	21	0.52	3.99	4.45	2	12	279037	4670479

Table 2. The 18 identified ND2 haplotypes and their frequency among 30 populations of cutthroat trout within the Bonneville basin and upper Snake River. The lineages detected are represented by the red (Great Basin), black (Bonneville-Yellowstone clade A), and white (Bonneville-Yellowstone clade B).

Population	Haplotypes																		
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	Clades
Basin Creek	0	0	0	0	1	0	0	7	0	0	0	0	2	0	0	0	0	0	B, GB
Clear Creek	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B
Dempsey Creek	0	0	0	0	0	0	0	2	8	0	0	0	0	0	0	0	0	0	GB
E. Bob Smith Creek	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	GB
Eight Mile Creek	2	0	0	0	4	0	0	4	0	0	0	0	0	0	0	0	0	0	B, GB
First Creek	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	GB
Fish Creek	0	0	0	0	0	0	0	4	6	0	0	0	0	0	0	0	0	0	GB
Garden Creek	0	8	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	B
George Creek	0	0	0	0	7	0	0	0	0	0	0	0	0	0	3	0	0	0	A, B
Gibson Jack Creek	0	0	0	0	0	1	0	0	1	0	6	0	0	0	0	0	1	1	A, B, GB
Goodenough Creek	0	0	0	0	1	0	0	1	2	0	6	0	0	0	0	0	0	0	A, B, GB
Harkness Creek	0	0	0	0	0	0	0	8	2	0	0	0	0	0	0	0	0	0	GB
Inman Creek	2	0	0	0	3	0	0	4	0	1	0	0	0	0	0	0	0	0	B, GB
Johnson Creek	1	0	1	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	B, GB
LHF Marsh Creek	0	0	4	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	B, GB
Maple Creek	8	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B
Mill Creek	0	0	1	0	2	0	0	7	0	0	0	0	0	0	0	0	0	0	B, GB
One Mile Creek	6	0	1	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	B, GB
Pebble Creek	0	0	0	0	0	0	0	6	4	0	0	0	0	0	0	0	0	0	GB
Pine Creek	0	0	0	5	3	1	1	0	0	0	0	0	0	0	0	0	0	0	A, B, GB
Robbers Roost Creek	6	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	B, GB
Second Creek	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	GB
Six Mile Creek	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B
Third Creek	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	GB
Toponce Creek	0	0	0	0	0	0	0	3	5	0	2	0	0	0	0	0	0	0	A, GB
Walker Creek	0	0	0	0	2	0	0	0	0	8	0	0	0	0	0	0	0	0	B
Wildcat Creek	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	GB
Grape Creek	2	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	B, GB
Cassia Creek	1	0	1	0	5	2	0	0	0	0	0	1	0	0	0	0	0	0	A, B, GB
Almo Creek	1	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B, GB
Total	49	10	43	5	39	4	1	57	28	9	14	1	28	3	3	4	1	1	

Figures

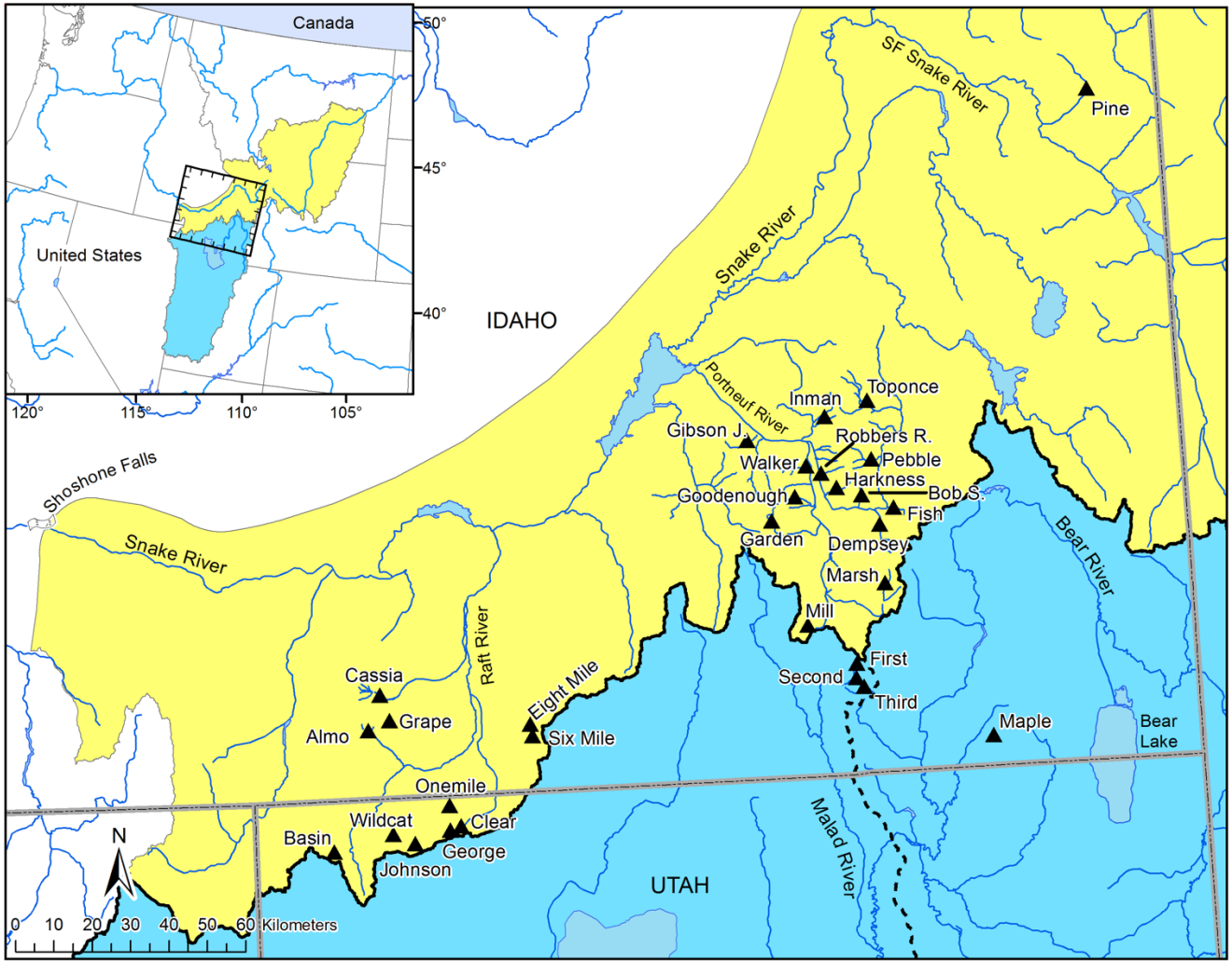

Figure 1. Sampling locations of cutthroat trout within the Bonneville basin and upper Snake River in western North America. Inset map indicates estimated boundary for the native range of Bonneville (blue polygon) and Yellowstone (yellow polygon) subspecies of cutthroat trout in the western United States. Dashed line represents the division between the Bear River and Malad River watersheds.

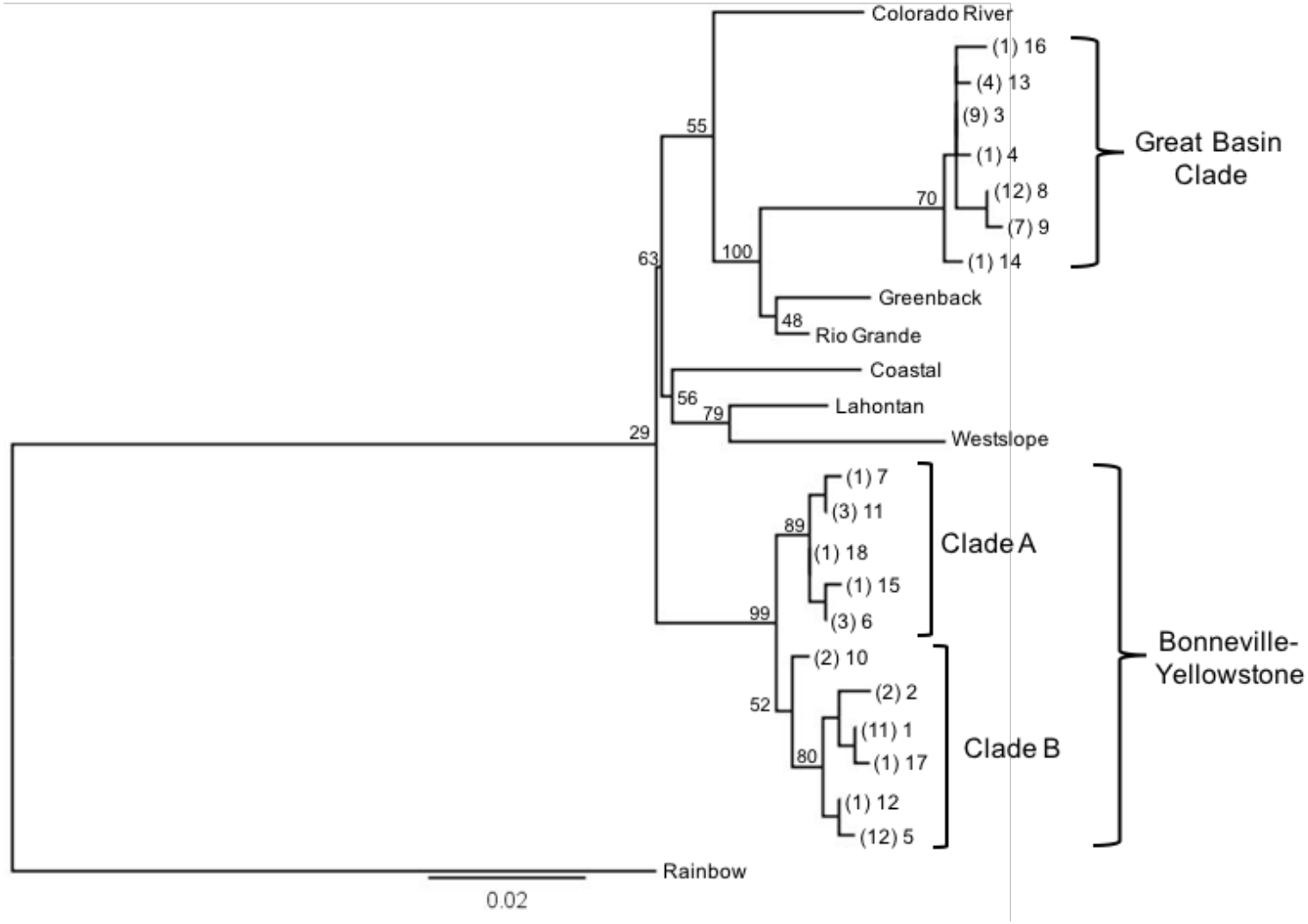

Figure 2. Maximum likelihood phylogeny of cutthroat trout within the Bonneville basin and upper Snake River based on the mitochondrial ND2 gene in reference to other subspecies of cutthroat trout obtained from Genbank. Numbers in parentheses represent the number of sampling locations where the haplotype was detected followed by the haplotype number. Numbers on branches indicate percent bootstrap support based on 1000 replicates. Scale bar represents the proportion of sequence divergence in the ND2 gene used to construct the phylogeny.

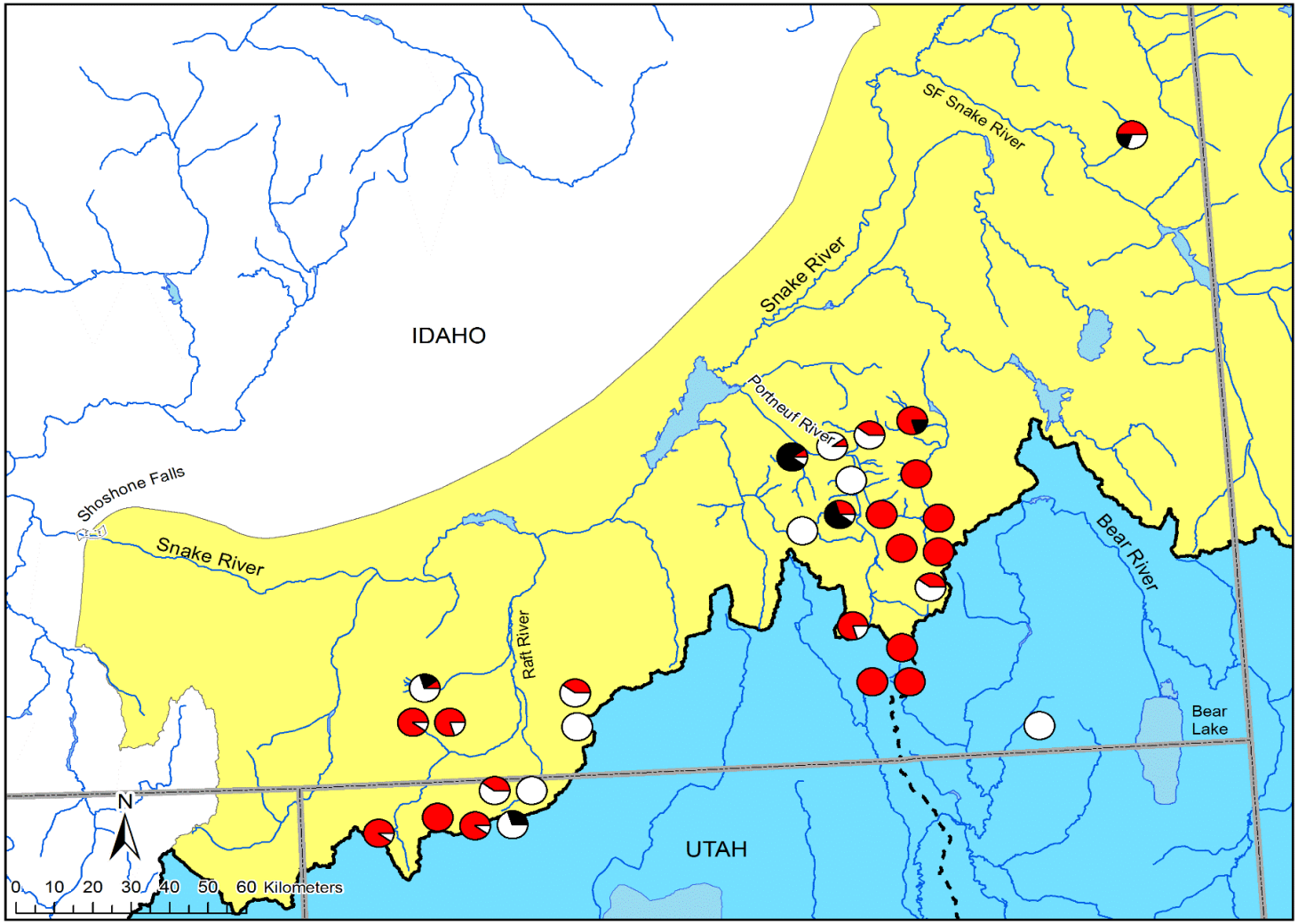

Figure 3. Geographic distribution of the three major lineages of Bonneville-Yellowstone and Great Basin cutthroat trout (see Figure 2) depicted by the red (Great Basin clade), white (Bonneville-Yellowstone clade B), and black (Bonneville-Yellowstone clade A) proportions of each circle. Colored polygons represent the estimated boundary for the native range of Bonneville (blue) and Yellowstone (yellow) subspecies of cutthroat trout. Dashed line represents the division between the Bear River and Malad River watersheds.

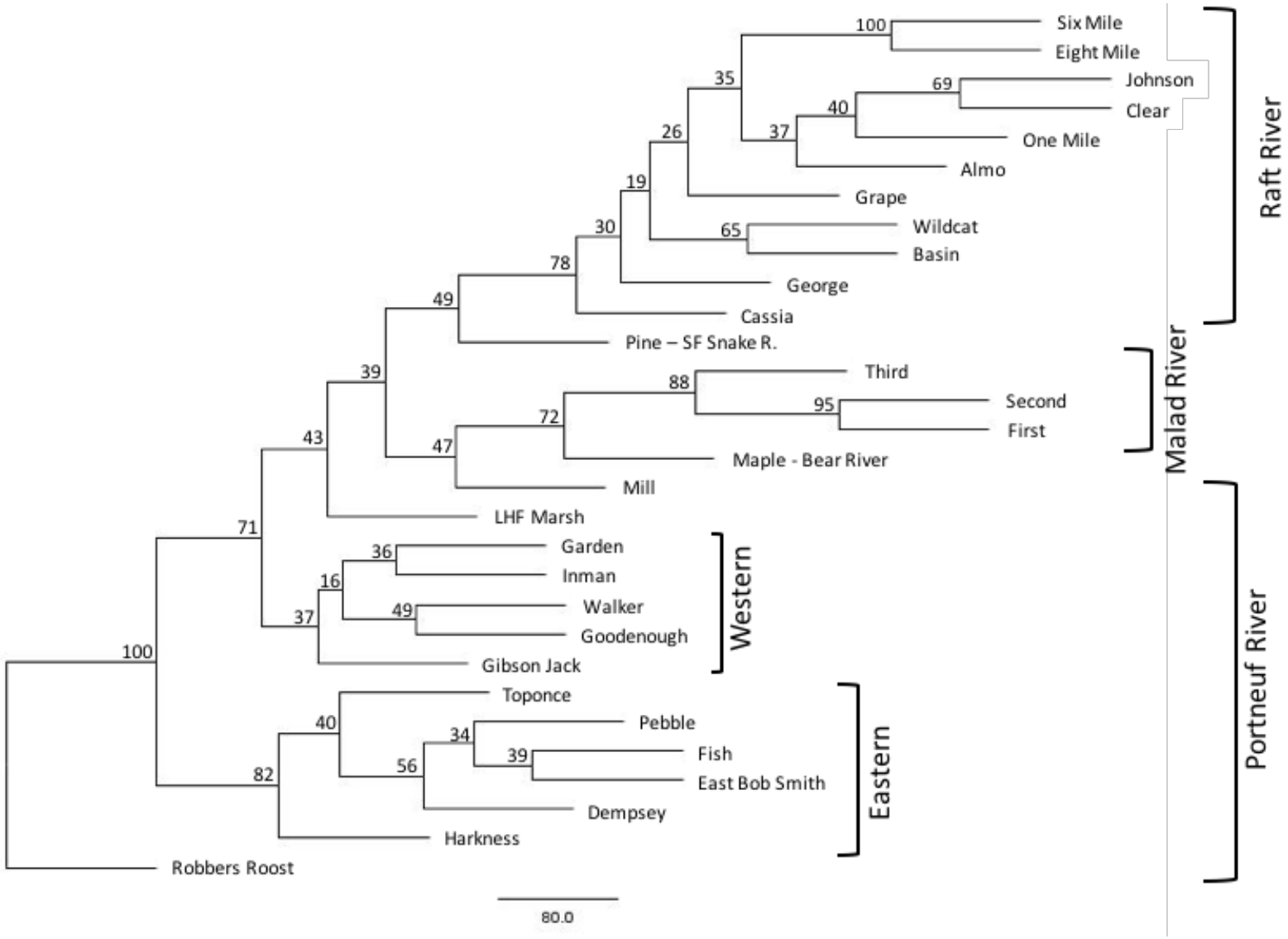

Figure 4. Neighbor joining tree of the 30 populations of cutthroat trout sampled from the Bonneville basin and upper Snake River based on Cavalli-Sforza chord distances. Groupings of major watersheds are displayed in brackets. Numbers represent the percentage of supported bootstraps.

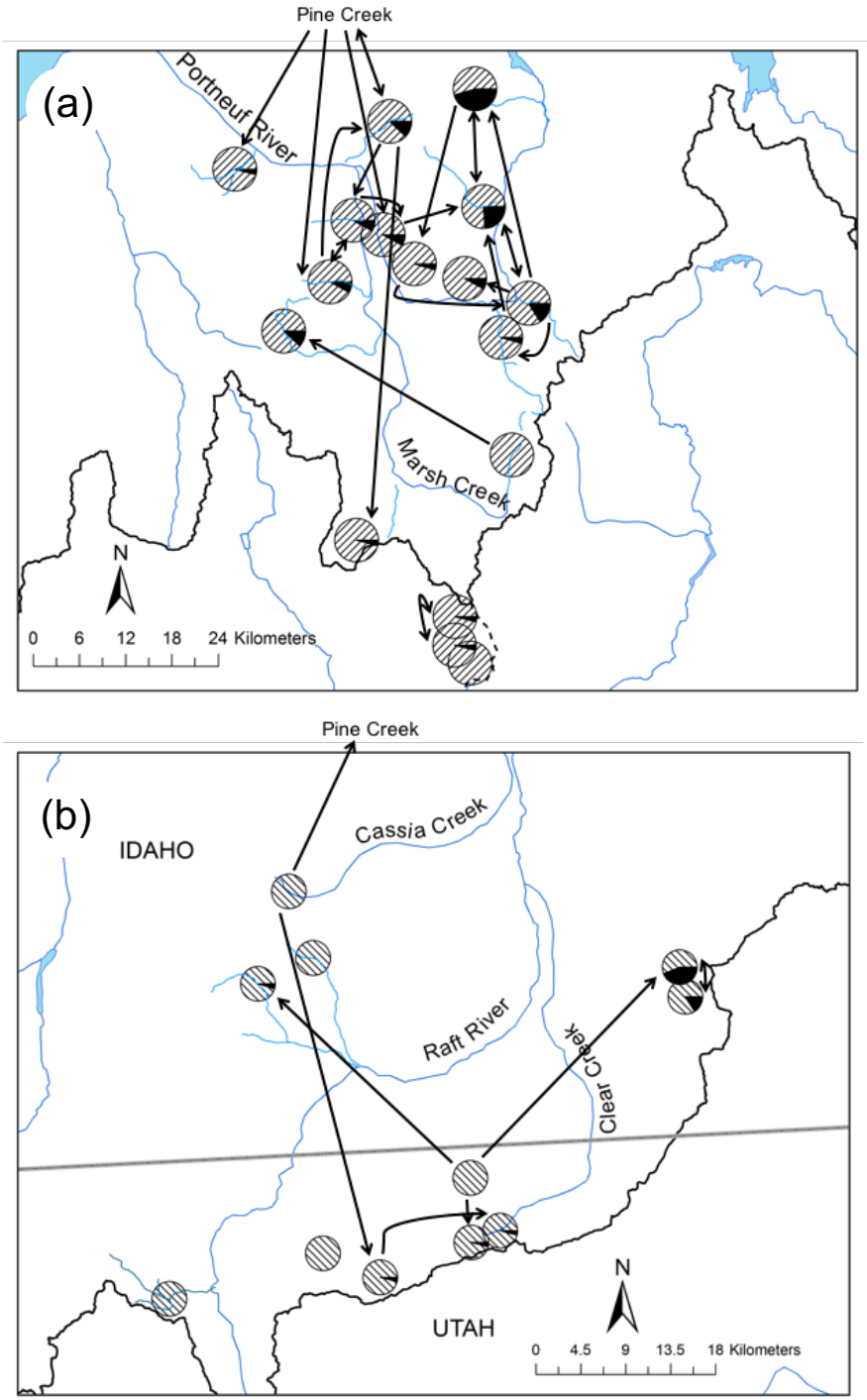

Figure 5. Location and assignments of cutthroat trout within the Bonneville basin and upper Snake River based on 11 microsatellite loci. Hatching represents the proportion of individuals that assigned to their sampled population. Solid black represents the proportion of individuals that assigned to a different population than what was sampled. Arrow direction points from the location where a misassigned individual originated to their sampled location within the **(a)** Portneuf and Malad River watershed and **(b)** the Raft River watershed.

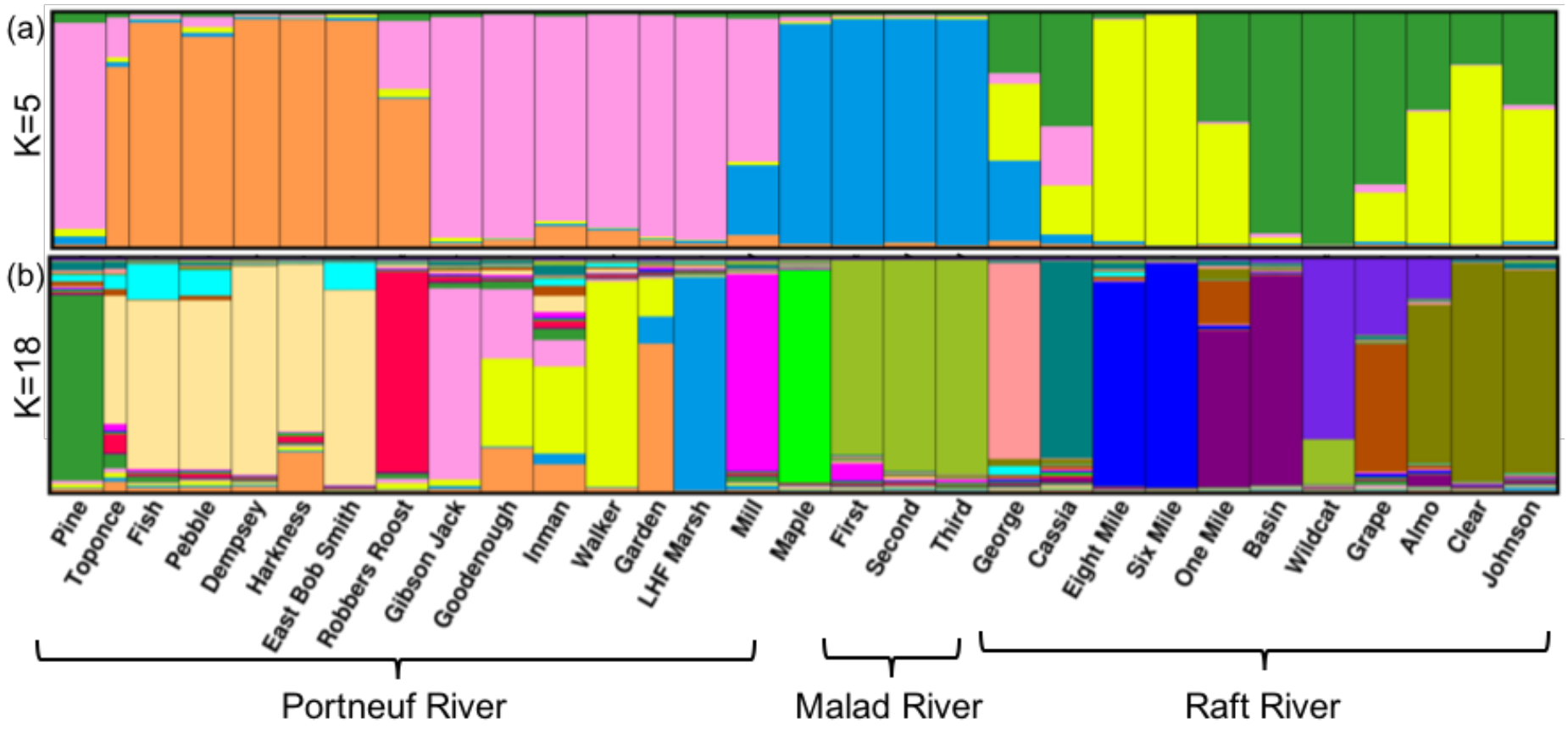

Figure 6. Proportions of assigned clusters for populations of cutthroat trout within the Bonneville basin and upper Snake River based on 11 microsatellite loci. Clusters and proportions were calculated in Structure with **(a)** K=5 and **(b)** K=18. Each color represents a different cluster. Groupings of watersheds are displayed within brackets.

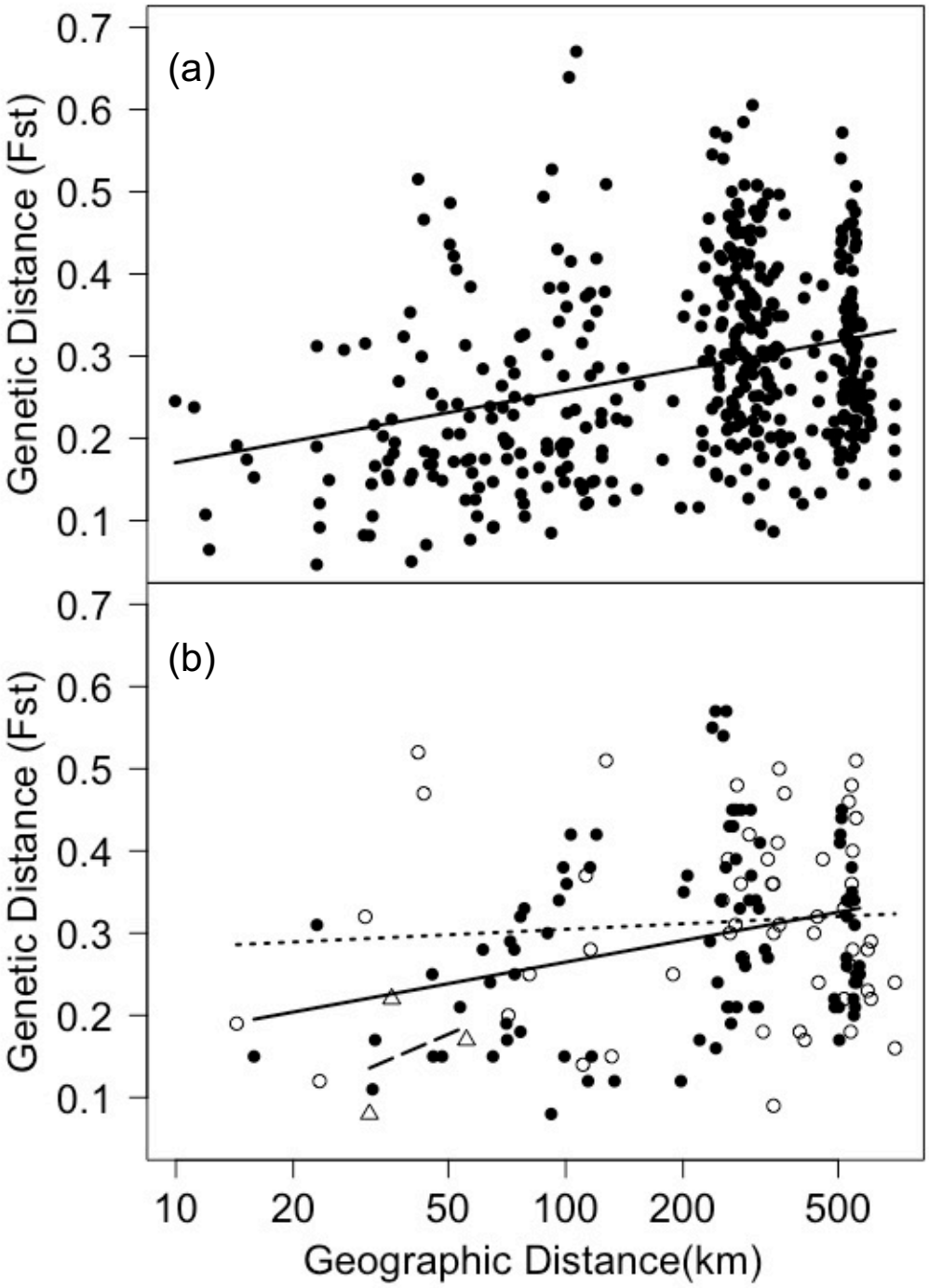

Figure 7. (a) The relationship between geographic distance (km) and genetic distance (Fst) based on populations of cutthroat trout within the Bonneville basin and upper Snake River. (Mantel test, r=0.31, p=0.0020). **(b)** The relationship between geographic distance (km) and genetic distance (Fst) based on populations of cutthroat trout in each watershed within the Bonneville basin and upper Snake River. Solid circles and solid line represent the Portneuf River watershed (Mantel test, r=0.30, p=0.022), open circles and dotted line represent the Raft River watershed (Mantel test, r=0.085, p=0.26, and open triangles and dashed line represent the Malad River watershed (Mantel test, r=0.37, p=0.52).

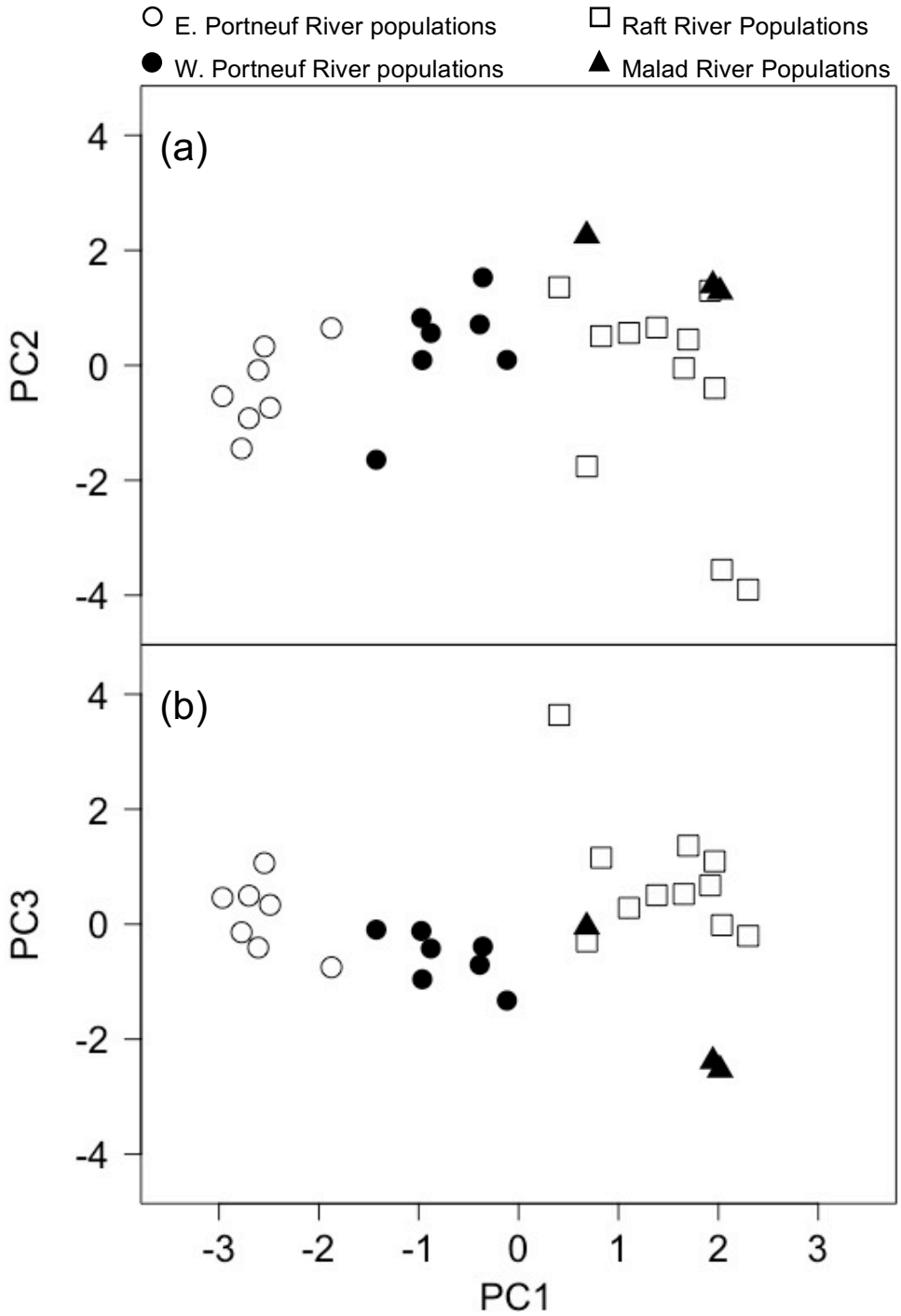

Figure 8. Principal component analysis for cutthroat trout within the Bonneville basin and upper Snake River based on 11 microsatellite loci. Populations grouped by major watersheds represented by different clusters. Open circles represent the eastern Portneuf River watershed populations, closed circles represent the western Portneuf River watershed populations, open squares represent the Raft River watershed, and closed triangles represent the Malad River watershed.

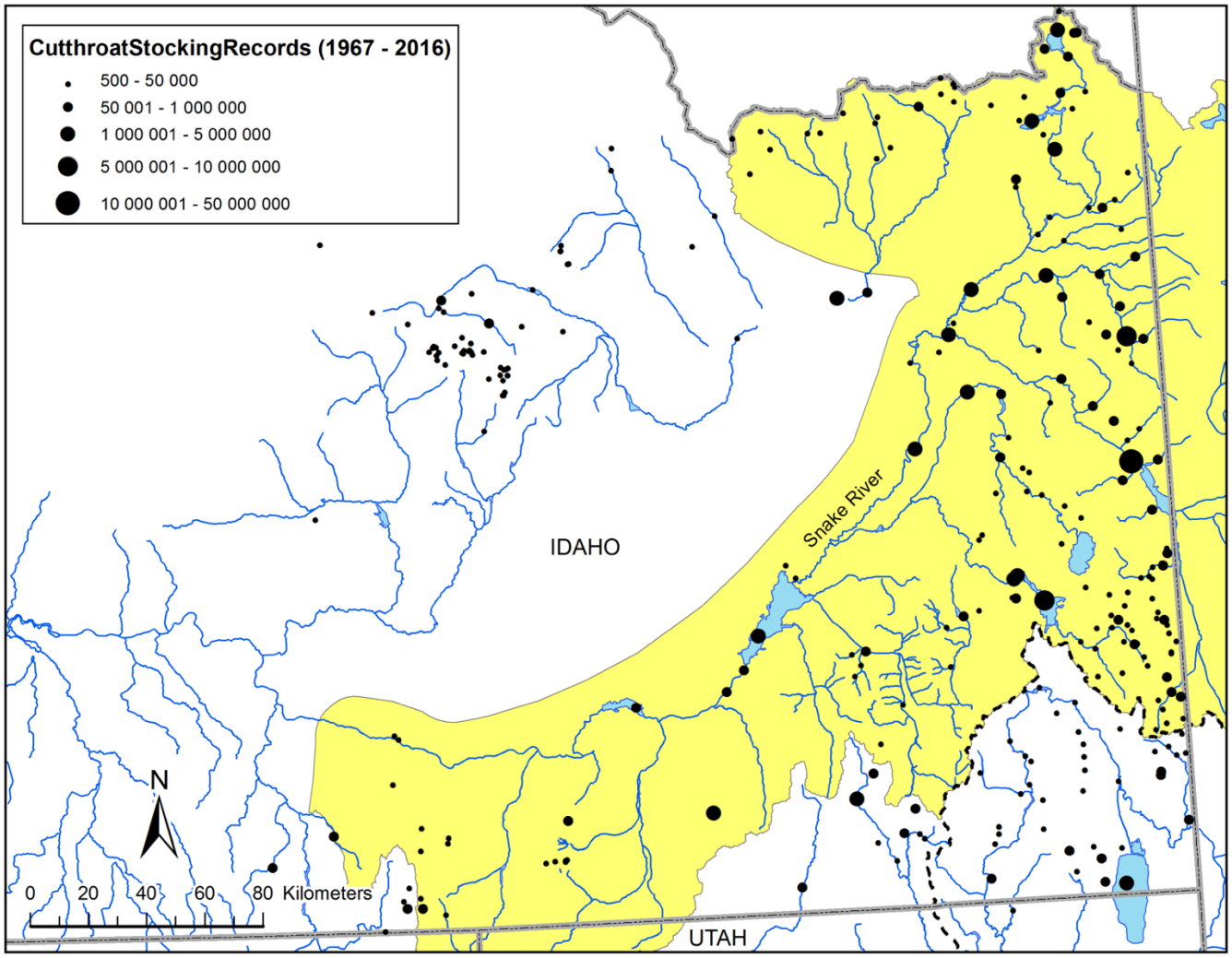

Figure 9. Total number of cutthroat trout stocked into the upper Snake, southeast Idaho, and Magic Valley watersheds according to locations reported in the Idaho Department of Fish and Game historical stocking records database from the years 1967 to 2016. See legend for estimated range of fish numbers stocked within each site. Dashed line represents the Bear River watershed boundary.

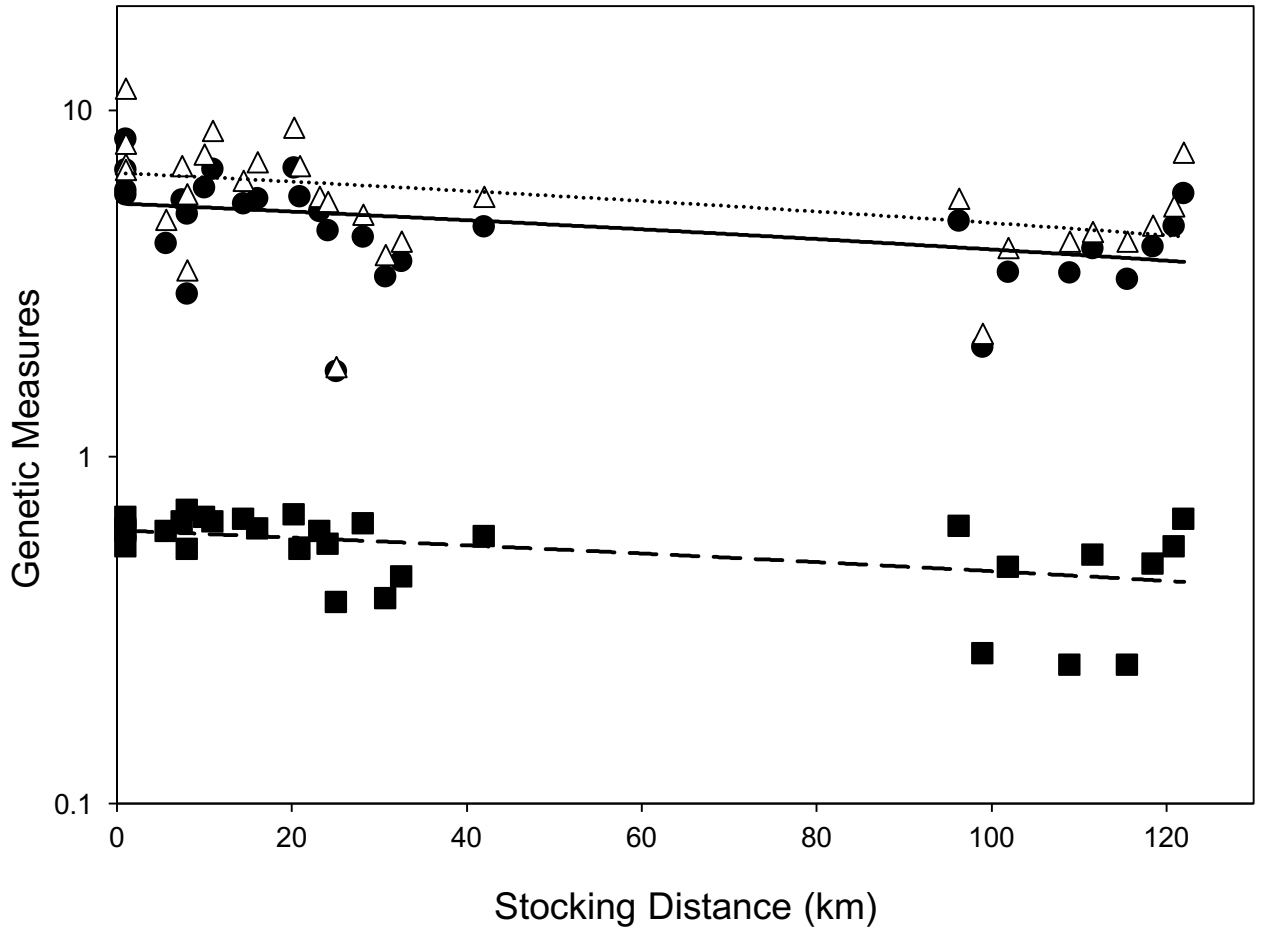

Figure 10. The relationship between nearest stocking location distance (km) and three genetic measures based on populations of cutthroat trout within the Bonneville basin and upper Snake River. Solid circles and solid line represent average allelic richness (r^2=0.196, n=30, p=0.014), open triangles and dotted line represent the average number of alleles (r^2=0.171, n=30, p=0.023), and solid squares and dashed line represent average heterozygosity (r^2=0.266, n=30, p=0.0035).

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Appendices

Supplemental Table 1. Location of cutthroat trout stocking events in the upper Snake, Southeast, and Magic Valley regions. Stocking records of cutthroat trout obtained from the Idaho Department of Fish and Game historical stocking records database (https://idfg.idaho.gov/fish/stocking) for the years 1967 to 2016.

Name	Watershed Location	Total Stocking Events	Total Fish stocked	UTM zone	northing	easting
Airplane Lake	Big Lost Basin	2	1812	11T	731745	4851804
Alder Creek	Tributary of Bear River	3	1767	12T	292090	4856571
Aldous Lake Mickeys Lake	Beaver-Camas Basin	14	21536	12T	432461	4930188
Alexander Reservoir	Bear Lake Basin	1	1250	12T	446212	4722668
American Falls Reservoir	American Falls Basin	21	3000852	12T	351378	4747507
Angel Lake	Big Lost Basin	1	650	11T	737306	4854023
Angus Creek	Tributary of Blackfoot River	6	21265	12T	472689	4741342
Angus Pond #3	Blackfoot Basin	5	14940	12T	467447	4741531
Antelope Creek	Tributary of S. Fork Snake River	1	10656	12T	457147	4819873
Arrowhead Lake	Big Lost Basin	8	13834	11T	734002	4847577
Ashton Reservoir	Upper Henrys Basin	4	42814	12T	461747	4883267
Bacon Creek	Tributary of Lanes Creek	5	11592	12T	477940	4741864
Badger Creek	Tributary of Teton River	2	203680	12T	477452	4862493
Bailey Creek	Tributary of Bear River	2	13215	12T	451542	4713448
Baptie Lake	Big Lost Basin	8	6559	11T	740220	4851374
Bear Canyon	Tributary of Diamond Creek	2	4780	12T	482818	4730444
Bear Creek	Tributary of Corral Creek	3	65040	12T	440552	4753763
Bear Creek	Tributary of Palisades Reservoir	5	95320	12T	480111	4791475
Bear Lake	Bear Lake Basin	38	1645273	12T	471220	4653497
Bear River	Tributary of Great Salt Lake	27	263453	12T	425038	4658397
Beaver Creek	Tributary of Camas Creek	1	1800	12T	404362	4919908
Beaver Creek	Tributary of Preuss Creek	4	3643	12T	488987	4699143
Beaver Dam Creek	Tributary of Crow Creek	4	5080	12T	485008	4705257

Bechler Creek	Tributary of Stump Creek	2	2800	12T	488720	4745466
Bellas Canyon Lake #1	Big Lost Basin	14	16015	12T	260065	4851857
Bellas Canyon Lake #2	Big Lost Basin	4	2539	12T	259696	4852567
Betty Lake	Big Lost Basin	17	39305	11T	740379	4852398
Big Creek	Tributary of Shoshone Creek	20	173953	11T	720897	4660751
Big Creek Lake	Little Lost Basin	2	4968	12T	454398	4658734
Big Elk Creek	Tributary of Palisades Reservoir	2	60375	12T	492621	4797652
Big Fall Creek Lake	Big Lost Basin	12	16317	11T	721170	4861542
Big Lake	Big Lost Basin	15	42343	12T	269843	4845800
Big Lost River	Tributary of Dry Channel	1	19910	12T	351612	4849767
Birch Creek	Tributary of Mink Creek	1	1005	12T	444134	4674092
Bitch Creek	Tributary of Teton River	3	502724	12T	490157	4867664
Black Springs Creek	Tributary of Henrys Fork	1	21000	12T	457335	4877655
Blackfoot Reservoir	Blackfoot Basin	111	8322559	12T	450174	4752352
Blackfoot River (Mouth-Blackfoot Dam)	Tributary of Snake River	13	1453370	12T	440354	4760436
Blackfoot River (Reservoir-Headwaters)	Tributary of Snake River	51	2158404	12T	441615	4761503
Bloomington Lake	Bear Lake Basin	18	341874	12T	452416	4666007
Blue Creek Reservoir	Lower Henrys Basin	14	51103	12T	451248	4897067
Bobber Lake	Big Lost Basin	11	8717	12T	269741	4836223
Boulder Creek	Tributary of Stump Creek	7	10272	12T	488217	4743382
Boulder Lake	Big Lost Basin	9	13933	11T	730882	4853459
Boulder Lake #1	Big Lost Basin	2	1019	11T	730062	4853893
Boulder Lake #2	Big Lost Basin	5	2511	11T	730062	4853893
Brockie Lake	Big Lost Basin	13	20640	12T	270263	4841386
Brockman Creek	Tributary of Grays Lake Outlet	4	37234	12T	459490	4784230
Browns Canyon	Tributary of Lanes Creek	3	4708	12T	477566	4748445
Brush Creek	Tributary of Rawlins Creek	9	46547	12T	429338	4774686
Brush Creek	Tributary of S. Fork Tincup Creek	5	7140	12T	487632	4756978
Buffalo River	Tributary of Henrys Fork	1	5700	12T	472345	4919893

Burns Canyon	Tributary of S. Fork Snake River	5	283583	12T	461769	4827693
Camas Creek	Tributary of Mud Lake	4	470232	12T	397361	4862208
Canyon Creek	Tributary of Teton River	3	161100	12T	464050	4855585
Cedar Creek Reservoir	Salmon Falls Basin	4	87738	11T	674631	4674906
Chesterfield Reservoir	Portneuf Basin	10	720540	12T	422152	4748926
Ching Creek	Tributary of Spring Creek	2	3000	12T	431832	4925243
Chippy Creek	Tributary of Lanes Creek	4	14288	12T	472561	4752634
Clark Creek	Tributary of Grays Lake Outlet	1	900	12T	464790	4779759
Clark Lake	Upper Henrys Basin	2	2000	12T	470246	4953541
Clear Lake	Big Lost Basin	12	8073	12T	260387	4850870
Conant Creek	Tributary of Fall River	1	1560	12T	466051	4874954
Condie Reservoir	Middle Bear Basin	1	6300	12T	428687	4673566
Copper Lake	Little Lost Basin	5	4275	12T	293546	4886008
Corral Lake	Big Lost Basin	14	14475	12T	278072	4859333
Cottonwood Creek	Tributary of Bear River	3	35361	12T	437177	4686725
Cottonwood Creek	Tributary of Warm Creek	2	1500	12T	427631	4928172
Cottonwood Creek	Tributary of Rock Creek	2	22376	11T	715848	4703403
Cottonwood Creek	Tributary of Shoshone Creek	5	11044	11T	719479	4663248
Crooked Creek	No connection to parent stream	1	2000	12T	360046	4905699
Crow Creek	Tributary of Salt River	18	221697	12T	491461	4717749
Cub River	Tributary of Bear River	2	7680	12T	431621	4647014
Dan Creek	Tributary of Hell Creek	1	1800	12T	448250	4796591
Daniels Reservoir	Lower Bear-Malad Basin	19	1030168	12T	381076	4689223
Deep Creek	Tributary of Jackknife Creek	1	3288	12T	493336	4767088
Deep Creek	No connection to parent stream	2	42000	12T	401544	4675520
Deer Creek	Tributary of Crow Creek	12	32089	12T	486609	4715306
Densmore Creek	Tributary of Bear River	2	3540	12T	434862	4705049
Devil Creek	Tributary of Malad River	6	117800	12T	396416	4676228
Devil Creek Reservoir	Lower Bear-Malad Basin	12	634400	12T	400858	4684308

Devils Corral Lake	Upper Snake-Rock Basin	1	800	11T	716401	4720190
Diamond Creek	Tributary of Blackfoot River	14	347789	12T	479890	4735070
Dike Lake	Blackfoot Basin	1	18900	11T	690959	4888665
Divide Creek Lake	Medicine Lodge Basin	12	11907	12T	355028	4918242
Draney Creek	Tributary of Tygee Creek	2	2530	12T	492255	4731476
Dry Beds	Tributary of Snake River	1	2941	12T	420365	4839981
Dry Creek	Tributary of Beaver Creek	1	1000	12T	403995	4907754
Dry Creek	Tributary of Thomas Fork	7	15296	12T	494699	4696508
Duck Creek	Tributary of Henrys Lake	3	311040	12T	464324	4940899
Eagle Spring Creek	Tributary of Cottonwood Creek	2	3960	11T	721435	4667883
East Fork Big Lost River	Tributary of Big Lost River	3	21558	11T	731796	4866981
East Fork Dry Creek	Tributary of Dry Creek	1	1920	11T	734910	4685105
East Harriman Fish Pond	Upper Henrys Basin	1	8377	12T	466519	4906636
East Shadow Lake	Little Lost Basin	1	1952	12T	295703	4879586
Eightmile Creek	Tributary of Bear River	2	19500	12T	458073	4716673
Fish Haven Creek	Tributary of Bear Lake	5	54858	12T	463958	4654509
Fishpole Lake	Big Lost Basin	19	35494	12T	270083	4836193
Fourth Fork Rock Creek	Tributary of Rock Creek	1	4860	11T	725358	4680612
Fritz Creek	Tributary of Med. Lodge Creek	1	1200	12T	364853	4919968
Gibson Jack Creek	Tributary of Portneuf River	2	7200	12T	382901	4738722
Giraffe Creek	No connection to parent stream	6	12669	12T	493841	4703146
Goat Lake	Big Lost Basin	17	28290	11T	739776	4851611
Golden Lake	Upper Henrys Basin	4	30371	12T	461673	4911638
Goodheart Creek	No connection to parent stream	1	2070	12T	474999	4725468
Goose Creek	Tributary of Milner Lake	7	58726	11T	726111	4660795
Grant Creek Lake	Big Lost Basin	8	6758	12T	261674	4871886
Grays Lake Outlet	Tributary of Willow Creek	5	283288	12T	438713	4802439
Green Lake	Big Lost Basin	10	12149	12T	269482	4843200
Grizzly Creek	Tributary of Corral Creek	3	9560	12T	439236	4754128

Haderlie Creek	Tributary of Tincup Creek	2	7000	12T	491343	4762104
Hancock Lake	Beaver-Camas Basin	12	10192	12T	432107	4931237
Hatchery Creek	Tributary of Henrys Lake	12	1587094	12T	469364	4947079
Hawkins Reservoir	Portneuf Basin	9	27807	12T	390555	4707250
Hell Creek	Tributary of Grays Lake Outlet	2	11050	12T	446061	4798178
Henrys Fork (Mesa Falls-Island Park Dam)	Tributary of Snake River	60	3450967	12T	465316	4906492
Henrys Fork (Upstream from Reservoir)	Tributary of Snake River	14	399818	12T	468588	4925517
Henrys Lake	Upper Henrys Basin	336	52584648	12T	468680	4943193
Henrys Lake Outlet (Lake-Big Springs)	Tributary of Snake River	30	861810	12T	472160	4937777
Homer Creek	Tributary of Grays Lake Outlet	2	21060	12T	446874	4790165
Horse Creek	Tributary of Rawlins Creek	2	3000	12T	430451	4776380
Horse Creek	Tributary of Stump Creek	5	13160	12T	491929	4737904
Horseshoe Creek	Tributary of Teton River	6	209010	12T	478124	4841759
Hot Creek	Tributary of Shoshone Creek	1	2376	11T	713262	4652989
Howard Creek	Tributary of Henrys Lake	6	407065	12T	475849	4945711
Hyde Canyon	Tributary of Stump Creek	5	11280	12T	490641	4743220
Independence Lake #1	Raft Basin	9	10975	12T	280253	4675551
Independence Lake #2	Raft Basin	16	20545	12T	279702	4675362
Independence Lake #3	Raft Basin	10	10474	12T	279633	4674879
Iron Bog Lake	Big Lost Basin	13	26579	12T	270555	4837199
Island Park Reservoir	Upper Henrys Basin	16	1776252	12T	458173	4916659
Jackknife Creek	Tributary of Salt River	7	98489	12T	493488	4765335
Jim Moore Pond	Idaho Falls Basin	1	1748	12T	410231	4837070
Johnson Creek	No connection to parent stream	3	9560	12T	466521	4724914
Kane Lake	Big Lost Basin	17	26942	11T	728536	4851945
Kendall Creek	No connection to parent stream	5	45835	12T	476340	4736224
Lake Cleveland	Lake Walcott Basin	25	50387	12T	281571	4688866
Lake Creek	Tributary of Star Hope Creek	2	2500	12T	264499	4851644
Lake Creek Lake #11	Big Lost Basin	1	500	12T	271978	4842909

Lake Creek Lake #12	Big Lost Basin	3	2500	12T	271965	4842935
Lake Walcott	Lake Walcott Basin	4	650559	12T	307779	4725901
Lander Creek	Tributary of Lanes Creek	3	9800	12T	478949	4752999
Lanes Creek	Tributary of Blackfoot River	8	195675	12T	475023	4743829
Lau Creek	Tributary of Tincup Creek	3	8000	12T	483803	4757638
Lava Creek	Tributary of Grays Lake Outlet	1	900	12T	452012	4788548
Left Hand Fork Georgetown Canyon	Tributary of Georgetown Canyon	1	3000	12T	472816	4706527
Little Beaver Creek	Tributary of Montpelier Creek	3	2163	12T	484102	4697810
Little Blackfoot River	Tributary of Grays Lake	1	2090	12T	464635	4755714
Little Lost River	No connection to parent stream	1	15000	12T	312645	4910387
Little Malad River	Tributary of Malad River	1	21000	12T	393236	4667001
Little Valley Reservoir	Bear Lake Basin	6	23805	12T	457437	4771261
Long Lake	Big Lost Basin	7	15700	12T	271573	4844993
Lower Deep Creek Reservoir	Upper Snake-Rock Basin	13	556500	11T	695591	4685612
Lower Palisades Lake	Palisades Basin	3	25088	12T	487056	4808635
Lower Swauger Lake	Little Lost Basin	4	3875	12T	293162	4884139
Mackay Reservoir	Big Lost Basin	3	11750	12T	282831	4871569
Mahogany Creek	Tributary of Teton River	1	3600	12T	481846	4836115
Marsh Creek	Tributary of Portneuf River	1	1005	12T	399153	4720221
Marshall Canyon	Tributary of Tincup Creek	3	8000	12T	488038	4760998
McCoy Creek	Tributary of Palisades Reservoir	5	142720	12T	489425	4780659
McTucker Pond #8	American Falls Basin	2	2980	12T	365615	4766205
Middle Creek	Tributary of Med. Lodge Creek	1	1000	12T	380947	4918088
Mill Canyon	Tributary of Stump Creek	3	6120	12T	491600	4740878
Mill Creek	Tributary of Ovid Creek	10	23458	12T	458545	4686155
Mill Creek	Tributary of Goose Creek	1	3404	12T	276087	4675278
Mill Creek Lake	Little Lost Basin	11	31191	12T	313339	4917977
Miners Creek	Tributary of Beaver Creek	1	1000	12T	405239	4921984
Mink Creek	Tributary of Portneuf River	1	750	12T	385779	4734726

Montpelier Creek	Tributary of Bear River	4	181571	12T	485769	4690704
Montpelier Reservoir	Bear Lake Basin	16	393905	12T	485672	4689592
Moody Creek	Tributary of S. Fork Teton River	1	2400	12T	454576	4838080
Moose Creek	Tributary of Henrys Fork	1	2000	12T	477238	4925428
Mud Lake	Medicine Lodge Basin	18	1090496	12T	386953	4861001
Muldoon Creek	Tributary of Star Hope Creek	2	2500	12T	262541	4824336
North Creek	Tributary of Ovid Creek	9	10099	12T	459671	4693209
North Fork Bellas Creek Lake	Big Lost Basin	5	3037	12T	259177	4852288
North Fork Big Lost River	Tributary of Big Lost River	62	122443	11T	732683	4869602
North Fork Lake	Big Lost Basin	3	5800	11T	708933	4865431
North Fork Stump Creek	Tributary of Stump Creek	5	18880	12T	487135	4750264
Ovid Creek	Tributary of Bear River	1	1040	12T	470902	4688582
Packsaddle Lake	Teton Basin	9	18148	12T	472591	4846433
Palisades Creek	Tributary of S. Fork Snake River	2	33282	12T	482673	4805008
Palisades Reservoir	Palisades Basin	456	10358080	12T	483591	4797776
Pass Creek	Tributary of Birch Creek	1	1000	12T	346951	4892210
Pass Creek Lake	Birch Basin	14	22710	12T	338451	4882354
Paul Reservoir	Beaver-Camas Basin	2	3876	12T	393650	4924164
Pearl Creek	Tributary of Bear River	10	17840	12T	458505	4706461
Pebble Creek	Tributary of Portneuf River	1	25500	12T	416435	4732026
Pine Creek	Tributary of S. Fork Snake River	9	490874	12T	471785	4817611
Pit Creek	Tributary of East Fork Dry Creek	1	12000	11T	734600	4683224
Portneuf River	Tributary of Ross Fork	6	90038	12T	387838	4739319
Preuss Creek	Tributary of Geneva Ditch	9	28803	12T	491477	4696101
Rainey Creek	Tributary of S. Fork Snake River	14	839831	12T	478526	4811889
Ramey Lake	Big Lost Basin	2	1000	12T	260222	4854887
Ririe Reservoir	Willow Basin	111	248743	12T	440639	4823998
Robinson Creek	Tributary of Warm River	4	139078	12T	480102	4885183
Rock Creek	Tributary of Crow Creek	18	53274	12T	494409	4715929

Rock Creek	Tributary of Snake River	4	18677	11T	725558	4688349
Rough lake	Big Lost Basin	15	33630	12T	270884	4844912
Round Lake	Big Lost Basin	7	13476	12T	272227	4845360
Sage Creek	Tributary of Crow Creek	10	60700	12T	490198	4722935
Saint Charles Creek	Tributary of Spring Creek	7	217937	12T	463217	4662582
Salamander Lake	Beaver-Camas Basin	8	7283	12T	427865	4933626
Sand Creek WMA Pond #4	Lower Henrys Basin	9	46650	12T	450840	4894506
Sellars Creek	Tributary of Willow Creek	2	24360	12T	436278	4790208
Sheep Creek	Tributary of Hotel Creek	1	1000	12T	456084	4925069
Sheep Creek	Tributary of Lanes Creek	5	17600	12T	472638	4745606
Sheridan Creek	Tributary of Island Park Reservoir	2	2500	12T	453751	4917100
Sheridan Reservoir	Upper Henrys Basin	2	5023	12T	444511	4923065
Shingle Creek	Tributary of Cottonwood Creek	1	1005	12T	423857	4694933
Skinner Creek	Tributary of Stauffer Creek	1	515	12T	459793	4702199
Slug Creek	Tributary of Blackfoot River	1	2280	12T	466317	4734525
Smoky Creek	Tributary of Tygee Creek	3	3550	12T	492209	4730935
Snake River (Gem Lk Dam-Henrys Fork)	Tributary of Columbia River	64	1379225	12T	409814	4807524
Snake River (Lake Walcott-A. Falls Dam)	Tributary of Columbia River	28	479811	12T	345524	4736014
Snow Creek	Tributary of Robinson Creek	1	11400	12T	484481	4887678
South Fork Snake River	Tributary of Snake River	53	3568825	12T	424228	4845734
South Fork Tincup Creek	Tributary of Tincup Creek	4	8720	12T	486645	4757920
Spring Creek	Tributary of Bear Lake Outlet	1	1088	12T	470417	4665721
Spring Creek	No connection to parent stream	2	4100	12T	494678	4708394
Spring Creek	Tributary of Teton River	1	69300	12T	483571	4851007
Spring Creek	Tributary of Camas Creek	2	17345	11T	689229	4794331
Springfield Reservoir	American Falls Basin	3	13873	12T	362425	4770897
Squirrel Creek	Tributary of Conant Creek	1	520	12T	485994	4877346
Star Hope Creek	Tributary of E. Fork Big Lost Riv.	73	60833	12T	267035	4861180
Star Hope Lake	Big Lost Basin	3	3580	12T	265499	4842184

Stauffer Creek	Tributary of Bear River	2	1059	12T	459827	4697767
Stone Reservoir	Curlew Valley Basin	3	82224	12T	360090	4660153
Strawberry Creek	Tributary of Mink Creek	1	1005	12T	444543	4684133
Stump Creek	Tributary of Salt River	67	240438	12T	490770	4742598
Sublett Reservoir	Raft Basin	46	1114744	12T	331609	4687986
Summit Creek	Tributary of Goose Creek	1	1702	12T	272902	4674813
Surprise Valley Lake #2	Big Lost Basin	2	1150	11T	739729	4856859
Targhee Creek	Tributary of Henrys Lake	3	120159	12T	474820	4945662
Teardrop Lake	Upper Henrys Basin	2	2977	12T	489681	4896651
Teton Creek	Tributary of Teton River	8	326971	12T	490786	4839304
Teton River (Canyon)	Tributary of Henrys Fork	35	2473726	12T	459182	4863524
Teton River (Mouth-Canyon)	Tributary of Henrys Fork	29	1955629	12T	433134	4860631
Teton River (Upper Teton Valley)	Tributary of Henrys Fork	56	5846055	12T	485092	4840611
Tex Creek	Tributary of Bulls Fork	1	9250	12T	442043	4809072
Texas Slough	Tributary of Henrys Fork	1	2080	12T	426094	4849500
Thomas Fork Bear River	Tributary of Bear River	7	88660	12T	494131	4673532
Threemile Creek	Tributary of Rattlesnake Creek	2	32252	12T	408897	4911187
Timber Creek	Tributary of Diamond Creek	4	13930	12T	483895	4727411
Timothy Creek	Tributary of Diamond Creek	4	8300	12T	479331	4739927
Tincup Creek	Tributary of Salt River	38	778469	12T	491694	4761258
Toponce Creek	Tributary of Portneuf River	1	25500	12T	416005	4745553
Trail Creek	Tributary of Blackfoot River	6	23187	12T	461541	4737345
Trail Creek	Tributary of Teton River	1	50000	12T	486035	4831227
Treasureton Reservoir	Middle Bear Basin	4	15350	12T	428878	4676100
Trout Creek	Tributary of Bear River	13	19157	12T	441527	4697552
Trout Creek	Tributary of Goose Creek	4	35206	11T	733937	4658615
Twentyfour Mile Creek Reservoir #1	Portneuf Basin	16	42549	12T	427553	4750491
Tygee Creek	Tributary of Stump Creek	2	2530	12T	494233	4734989
Upper Deep Creek Reservoir	Lower Bear-Malad Basin	1	2649	12T	403220	4673871

Upper Pleasantview Reservoir	Lower Bear-Malad Basin	1	15000	12T	387185	4673585
Upper Swauger Lake	Little Lost Basin	17	36282	12T	293314	4884220
Vineyard Lake	Upper Snake-Rock Basin	2	5284	11T	717769	4718811
Warm Creek	Tributary of Crow Creek	3	3565	12T	488006	4712051
Warm River	Tributary of Henrys Fork	3	14220	12T	475355	4885508
Washington Lake	Big Lost Basin	3	1655	11T	729654	4853364
Webber Creek	Tributary of Med. Lodge Creek	1	2056	12T	367629	4913621
West Camas Creek	Tributary of Camas Creek	4	148507	12T	419651	4924481
West Fork Indian Creek	Tributary of Indian Creek	2	1618	12T	385215	4917905
West Fork Mink Creek	Tributary of Mink Creek	1	2100	12T	383372	4731116
West Shadow Lake	Little Lost Basin	2	1375	12T	295389	4879530
Whiskey Creek	Tributary of Bear River	9	9931	12T	439660	4699614
White Dugway Creek	Tributary of Crow Creek	4	8160	12T	488905	4707192
Wild Horse Creek	Tributary of E. Fork Big Lost Riv.	3	4127	11T	733485	4865709
Wildhorse Lake #1	Big Lost Basin	1	816	11T	731279	4849196
Wildhorse Lake #2	Big Lost Basin	1	1632	11T	730978	4850697
Williams Creek	Tributary of Bear River	1	3000	12T	440447	4689670
Willow Creek	Tributary of S. Fork Willow Creek	23	1231488	12T	429212	4825642
Winder Reservoir	Middle Bear Basin	3	22015	12T	427120	4670194
Winecup Creek	Tributary of Goose Creek	1	1104	11T	725266	4664340
Worm Creek	Tributary of Unnamed Stream	2	1648	12T	460742	4666777
Wright Creek	Tributary of Daniels Reservoir	6	424800	12T	387303	4697392
Yellowjacket Creek	Tributary of Diamond Creek	4	19760	12T	480884	4735261

Supplemental Table 2. Pairwise Fst (below diagonal) and geographic distance (km) (above diagonal) for cutthroat trout populations within the Bonneville basin and upper Snake River. Population ID's are defined by Table 1 and listed in the same order of the first column.

Pop ID	30	28	29	1	2	3	4	5	6	7	8	9	10	11	12
30	0	30.6	108.7	56.7	115.7	299.1	290.3	90.8	544.7	299.7	305.5	45.8	237.5	284.3	274.5
28	0.32	0	105.7	55.3	112.6	296.0	287.2	87.7	541.6	296.7	302.5	42.7	234.4	281.3	271.4
29	0.15	0.23	0	133.4	97.0	259.1	250.3	72.0	504.7	259.8	265.6	114.1	197.5	244.4	234.5
1	0.23	0.31	0.12	0	140.4	323.8	315.0	115.4	569.4	324.4	330.2	70.4	262.1	309.0	299.1
2	0.28	0.37	0.19	0.29	0	287.3	278.5	52.4	532.9	288.0	293.8	121.0	225.7	272.6	262.7
3	0.33	0.38	0.23	0.31	0.39	0	15.2	262.4	553.1	12.2	98.6	304.4	70.1	77.4	31.8
4	0.42	0.51	0.34	0.41	0.47	0.17	0	253.6	544.3	15.9	89.8	295.6	61.3	68.6	23.0
5	0.38	0.49	0.29	0.38	0.41	0.47	0.54	0	508.0	263.0	268.8	96.1	200.8	247.6	237.8
6	0.28	0.36	0.18	0.25	0.34	0.24	0.36	0.41	0	553.8	559.5	550.0	491.5	538.3	528.5
7	0.29	0.38	0.21	0.28	0.35	0.06	0.15	0.43	0.22	0	99.3	305.1	70.8	78.1	32.4
8	0.29	0.37	0.19	0.27	0.36	0.18	0.30	0.43	0.22	0.15	0	310.9	76.5	35.1	74.0
9	0.18	0.30	0.12	0.19	0.29	0.25	0.34	0.34	0.19	0.21	0.21	0	242.8	289.7	279.8
10	0.24	0.31	0.12	0.21	0.29	0.20	0.28	0.35	0.20	0.17	0.18	0.16	0	55.3	45.5
11	0.27	0.36	0.15	0.23	0.31	0.16	0.26	0.39	0.18	0.12	0.15	0.19	0.12	0	52.8
12	0.41	0.46	0.29	0.37	0.47	0.14	0.31	0.55	0.32	0.17	0.25	0.33	0.25	0.24	0
13	0.23	0.30	0.12	0.22	0.28	0.13	0.21	0.34	0.16	0.11	0.09	0.15	0.08	0.07	0.20
14	0.15	0.32	0.15	0.24	0.23	0.37	0.45	0.38	0.32	0.34	0.33	0.21	0.24	0.29	0.45
15	0.30	0.36	0.22	0.29	0.39	0.25	0.38	0.48	0.23	0.22	0.23	0.25	0.21	0.20	0.32
16	0.27	0.36	0.17	0.25	0.34	0.26	0.35	0.42	0.19	0.21	0.24	0.20	0.21	0.20	0.32
17	0.27	0.35	0.16	0.22	0.33	0.22	0.34	0.41	0.14	0.19	0.17	0.20	0.19	0.14	0.28
18	0.16	0.27	0.08	0.15	0.19	0.31	0.39	0.28	0.26	0.27	0.26	0.15	0.17	0.23	0.38
19	0.31	0.40	0.23	0.30	0.36	0.08	0.17	0.43	0.24	0.05	0.15	0.23	0.19	0.16	0.17
20	0.24	0.32	0.12	0.21	0.30	0.20	0.31	0.37	0.16	0.17	0.18	0.13	0.13	0.09	0.25
21	0.30	0.37	0.19	0.30	0.36	0.22	0.31	0.44	0.27	0.19	0.22	0.24	0.18	0.18	0.24
22	0.31	0.37	0.21	0.26	0.35	0.26	0.38	0.41	0.11	0.24	0.25	0.22	0.22	0.22	0.34
23	0.43	0.53	0.32	0.42	0.44	0.50	0.57	0.11	0.44	0.45	0.45	0.36	0.37	0.42	0.57
24	0.40	0.48	0.29	0.34	0.46	0.34	0.46	0.54	0.19	0.30	0.31	0.29	0.30	0.26	0.42
25	0.31	0.41	0.19	0.26	0.36	0.09	0.24	0.45	0.22	0.08	0.14	0.20	0.18	0.15	0.16
26	0.30	0.39	0.21	0.28	0.34	0.17	0.24	0.41	0.22	0.16	0.17	0.24	0.16	0.12	0.22
27	0.47	0.52	0.35	0.35	0.51	0.51	0.61	0.64	0.44	0.47	0.47	0.38	0.42	0.42	0.58

Pop ID	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
30	259.3	39.8	341.6	543.2	330.3	40.4	319.0	445.4	265.2	542.0	95.2	544.4	352.9	263.8	43.3
28	256.3	38.4	338.6	540.1	327.2	37.3	315.9	442.3	262.1	538.9	92.2	541.4	349.8	260.8	41.8
29	219.4	116.5	301.7	503.2	290.3	91.8	279.0	405.4	225.2	502.0	76.5	504.5	312.9	223.9	120.0
1	284.0	64.0	366.3	567.9	355.0	65.1	343.6	470.0	289.8	566.7	119.9	569.1	377.5	288.5	39.9
2	247.6	123.5	329.9	531.4	318.5	98.7	307.2	433.6	253.4	530.2	50.4	532.7	341.1	252.1	126.9
3	58.6	306.9	134.7	551.6	123.4	282.1	31.4	356.1	35.8	550.4	266.9	552.8	65.3	56.9	310.3
4	49.9	298.1	125.9	542.8	114.6	273.4	35.1	347.3	27.0	541.6	258.1	544.1	69.0	48.1	301.5
5	222.6	98.6	304.9	506.5	293.6	73.8	282.3	408.7	228.5	505.3	31.9	507.7	316.2	227.1	102.0
6	513.3	552.5	595.7	277.2	584.3	527.7	573.0	699.4	519.2	11.9	512.5	14.3	606.9	517.8	555.9
7	59.3	307.5	135.4	552.2	124.0	282.8	23.0	356.8	36.4	551.1	267.5	553.5	56.9	57.6	311.0
8	65.1	313.3	73.4	558.0	62.0	288.6	118.5	362.5	64.7	556.9	273.3	559.3	152.4	44.6	316.8
9	264.7	53.6	347.0	548.5	335.6	45.7	324.3	450.7	270.5	547.3	100.6	549.8	358.2	269.2	57.0
10	30.3	245.2	112.7	490.0	101.3	220.5	90.0	294.5	36.2	488.8	205.2	491.2	123.9	34.8	248.7
11	43.9	292.1	71.3	536.8	59.9	267.4	97.3	341.3	43.5	535.7	252.1	538.1	131.2	23.4	295.6
12	34.0	282.2	110.1	527.0	98.7	257.5	51.6	331.5	11.1	525.8	242.2	528.2	85.6	32.3	285.7
13	0	267.1	101.2	511.8	89.8	242.4	78.5	316.3	24.7	510.7	227.1	513.1	112.4	23.4	270.6
14	0.25	0	349.4	551.0	338.1	48.2	326.8	453.2	273.0	549.8	103.0	552.2	360.7	271.6	50.6
15	0.17	0.35	0	594.1	45.4	324.7	154.6	398.7	100.8	593.0	309.4	595.4	188.5	80.7	352.9
16	0.18	0.31	0.24	0	582.8	526.2	571.5	697.9	517.7	274.6	511.0	277.0	605.4	516.3	554.4
17	0.14	0.31	0.17	0.20	0	313.3	143.2	387.3	89.4	581.6	298.1	584.0	177.2	69.3	341.5
18	0.18	0.15	0.30	0.26	0.25	0	302.0	428.4	248.2	525.1	78.3	527.5	335.9	246.9	51.6
19	0.10	0.34	0.26	0.24	0.22	0.28	0	376.0	55.6	570.3	286.7	572.7	40.2	76.8	330.2
20	0.09	0.28	0.18	0.19	0.13	0.21	0.20	0	322.2	696.7	413.1	699.1	409.9	320.8	456.6
21	0.15	0.33	0.23	0.23	0.19	0.26	0.17	0.14	0	516.5	232.9	518.9	89.6	23.0	276.4
22	0.18	0.34	0.25	0.21	0.20	0.27	0.27	0.21	0.28	0	509.8	10.0	604.2	515.2	553.2
23	0.36	0.42	0.51	0.45	0.44	0.33	0.45	0.39	0.47	0.44	0	512.2	320.7	231.6	106.5
24	0.25	0.43	0.28	0.26	0.24	0.37	0.34	0.24	0.36	0.25	0.57	0	606.6	517.6	555.7
25	0.12	0.35	0.25	0.22	0.17	0.26	0.05	0.17	0.19	0.25	0.49	0.29	0	110.7	364.1
26	0.09	0.32	0.25	0.23	0.20	0.27	0.13	0.18	0.19	0.25	0.43	0.33	0.14	0	275.1
27	0.42	0.49	0.50	0.45	0.40	0.42	0.50	0.39	0.49	0.48	0.67	0.51	0.47	0.48	0