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Barriers to movement and habitat availability influence genetic variation in populations of westslope cutthroat trout

by

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Committee Approval

To the Graduate Faculty:

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Dedication

As a member of my family, tribe, and community I am honored to represent the Matsaw and Fast Horse families, the Shoshone-Bannock and Oglala Nations, and local community as a father, husband, son, grandson, brother, cousin, friend, scientist, sundancer, and veteran. I dedicate this to all those who supported me over the years: my children and wife, my father and mother, siblings, aunties, uncles, cousins, grandparents, friends and mentors, and those who have gone on, and those to be. I'm happy about how this became our accomplishment through the blurs of defined lines because of what was ultimately important. We blurred the lines between one another's background to begin healing an age old wound. And, although, the path is difficult we are stronger for it.

I've come from homelessness, poverty, violence, and despair, and have been awakened by open hearts, homes, and dinner tables to share in a wealth of kindness, and opportunity towards a future of hope. I am truly grateful for those closest to me, who share a common vision of where we think and feel is our rightful place on our mother earth. Although, I use the words me, my, and I, to the conflicting idea of how this was accomplished by the we, us, and our. Please forgive me while I talk as a western scientist, but truly walk as Newe and Oyate person.

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Barriers to movement, and habitat availability influence genetic variation in populations of westslope cutthroat trout

Native fish populations have been isolated over time as a result of natural events; however, recent human activities have accelerated fragmentation and isolation of these populations. I compared populations of westslope cutthroat trout *(Oncorhynchus clarkii lewisi*) occurring above and below movement barriers and with varying amounts of habitat available to examine the temporal and spatial effects of habitat fragmentation on genetic population structure. I measured genetic variation in individuals from 31 populations of the Salmon River, Idaho, USA. Populations above artificial barriers had the lowest genetic diversity relative to other populations. Populations above barriers with <10 km of habitat available had lower genetic diversity and greater genetic differentiation than below barrier populations or above barrier populations with >10 km of habitat available. Results indicate that persistence of fish populations is affected not only by the presence of movement barriers, but an interaction of barriers and the amount of habitat available.

Introduction

Although the range of a species is often represented as a continuous distribution over a geographic area, species are usually distributed as a matrix of populations interconnected at varying levels in time and space (MacArthur & Wilson 1967; Levins 1969; Hanski 1999). Over geological time, vicariant events leading to mountain or island-building, or changes in hydrologic pathways can lead to allopatric speciation through the loss of physical connectivity between populations (Coyne & Orr 2004). Over a much shorter time frame, habitat fragmentation from road and dam building, urbanization, and agricultural expansion has isolated populations into increasingly smaller ranges that lack connectivity between habitat patches (Noss & Cooperrider 1994; Groom *et al.* 2006). Unlike historical isolation that can lead to speciation, human-induced isolation often leads to problematic effects associated with small population size resulting in a greater probability of extinction (Wilcox & Murphy 1985; Vitousek *et al.* 1997; Holsinger 2000).

Fragmented landscapes of once suitable habitat and barriers to movement that isolate migratory species, can influence their ability to persist (Fahrig 2003). In terrestrial ecosystems, the degree of isolation is related to the ability of an organism to pass over, around, or through unsuitable habitat (Forman & Alexander 1998; Loxterman 2011). In aquatic ecosystems, particularly in stream networks, barriers to movement often act as important isolating mechanisms, because many aquatic animals cannot move around physical barriers that extend across the land-water interface (Dunham *et al.* 2002). As a result, freshwater species are often some of the most imperiled taxa in ecosystems worldwide, frequently as a result of complications

stemming from habitat fragmentation (Nehlsen *et al.* 1991; Master *et al.* 1997; Stein *et al.* 2000).

Barriers to movement in aquatic ecosystems influence dispersal and population structure that are detectable by differences in allele frequencies among populations (Funk *et al.* 2005; Hughes 2007). Once a population is isolated, the probability of extinction increases in small populations because of increased inbreeding, as well as reductions in heterozygosity, effective population size, allelic richness, and gene flow (Frankham *et al.* 2002). In stream ecosystems, individuals downstream of a physical barrier may remain part of a series of interconnected populations, whereas individuals upstream of the barrier become isolated, prohibiting immigration from the larger matrix of populations. Upstream reaches from barriers are isolated into small disconnected fragments, and populations may experience a reduction in effective size, leading to increased inbreeding and even extinction (Hilderbrand & Kershner 2000; Harig & Fausch 2002; Allendorf & Luikart 2007; Guy *et al.* 2008).

Migratory species that move between distinct habitat patches, needed to complete critical life-history components, are often influenced by habitat fragmentation and barriers to movement between patches (Huey *et al.* 2008; Lindsay *et al.* 2008). Salmonid fishes are cool-water adapted species that commonly occur in streams and rivers world-wide (Behnke 2002). They typically penetrate into headwater streams to spawn and newly emerged juvenile fish often remain in natal areas to feed and grow before migrating to downstream habitat to achieve larger size (Northcote 1997; Quinn 2005). Given the propensity of salmonids to home to headwater streams for reproduction and early rearing, it is not surprising that they often exhibit significant population structure and differentiation even among streams within relatively localized watersheds (Taylor *et al.* 2003; Meeuwig *et al.* 2010). The common occurrence of

resource extraction activities related to forestry, irrigation, and mining in headwater habitats can also isolate salmonid populations above artificial barriers by isolating cohorts during movement to spawning areas. As a result, salmonid populations are often separated above artificial movement barriers in small habitat fragments, disconnected from a larger matrix of populations (Neville *et al.* 2006a; Northcote & Hartman 1988; Morita & Yamamoto 2000; Wofford *et al.* 2005; Deiner *et al.* 2007; Whiteley *et al.* 2013).

Past studies have noted significant impacts of headwater isolation of salmonids, resulting in reductions in genetic diversity as well as deformities and changes in life-history characteristics such as decreasing size and age at maturity (Northcote & Hartman 1988; Morita & Yamamoto 2000; Meyer *et al.* 2003). Despite the problems associated with habitat fragmentation and isolation of populations, salmonids also exhibit significant behavioral, morphological, and life-history diversification as a result of local adaptation to specific environmental conditions when naturally isolated (Snorrason *et al.* 1994; Keeley *et al.* 2005). In contrast to artificial barriers, isolation of populations above barriers over geological time frames can promote diversification, but presumably only if adequate habitat is available to support a sufficiently large effective population size.

Genetic studies of salmonids have detected the influence of headwater isolation in the form of decreased levels of genetic variability in populations isolated above barriers in some cases (Neville *et al.* 2006a). Whereas other studies have either found no effect on genetic diversity (Guy *et al.* 2008) or only in instances that have occurred by recent fragmentation above barriers with little stream habitat available (Wofford *et al.* 2005; Deiner *et al.* 2007). Although naturally isolated populations may exhibit decreased levels of diversity, their persistence over long time periods suggests that

sufficient variability exists to prevent extinction, even if reduced in comparison to interconnected populations (Whiteley *et al.* 2010). In contrast, artificially isolated populations may become isolated above barriers with varying degrees of habitat availability, and suffer severe decreases in genetic diversity when isolated in relatively small habitat patches (Neville *et al.* 2006a; Wofford *et al.* 2005; Deiner *et al.* 2007). Evaluating the effect of headwater isolation has become increasingly important because headwater streams are often the only remaining pieces of habitat protected from further degradation, and are used to create refuges for critically endangered salmonid populations (Hilderbrand & Kershner 2000; Young *et al.* 2005; Fausch *et al.* 2009).

The objective of this study was to examine the effects of barriers to movement on the genetic population structure of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). In the western United States, natural populations exist above waterfalls and other natural barriers, but like many freshwater fish species, habitat fragmentation and loss have greatly influenced connectivity between populations (Northcote & Hartman 1988). Headwater isolation of cutthroat trout populations is expected to reduce genetic diversity; however, the amount of available habitat may mitigate the effects of isolation such that headwater populations are sufficiently large to persist over time. In this study, I compare genetic diversity between populations located above and below both artificial and natural barriers to movement. Hence, I tested the effects of habitat fragmentation on population genetic structure, and examine the interaction between movement barriers, barrier types, and habitat available to cutthroat trout.

Materials and Methods

Study sites and sampling design

In order to examine the effects of barriers to movement on the population genetic structure of westslope cutthroat trout, I sampled locations in the Salmon River watershed of central Idaho, USA (Fig. 1). Although the watershed represents a core area for native cutthroat trout, numerous human-related activities have fragmented many different streams within the area. Some of the most common artificial movement barriers for fish include: culverts for road crossings, water diversions for irrigation, and catchment ponds for mining activities (Fausch *et al.* 2009). In addition to artificially isolated populations, westslope cutthroat trout also occur in continuously connected habitat in the Salmon River watershed that range from large mainstem river locations to smaller tributary streams (Schoby & Keeley 2011). Finally, native cutthroat trout populations in the Salmon River also occur above natural movement barriers such as waterfalls or cascades. Naturally isolated populations of trout occur throughout western North America and are commonly thought to occur as a result of glacial rebound and uplifting of land sufficient to create fish movement barriers (Northcote & Hartman 1988).

I identified locations for cutthroat trout populations that fell into three categories by conducting a survey of the watershed for possible movement barriers on streams. The three categories I considered to evaluate the influence of movement barriers were those populations above 1) artificial movement barriers (culverts, dams, or irrigation diversions), 2) above natural barriers (waterfalls or cascades) or 3) below barrier populations where no movement barrier was present to mainstem river locations. Using mapping software and fine scale U.S. Geological Survey topographic maps, I noted all

areas of constriction including steep topography, landslides, mining sites, and other related disturbances as potential barriers to fish movement. This survey created a list of candidate streams with potential barriers and their locations within each of the sub basins of the Salmon River. Once a barrier was verified by having a greater than two meter drop above to below a stream, I sampled 30 to 50 cutthroat trout from sites above and below the barrier. Whenever possible, I used sites directly below a barrier as the nearest below barrier population; however, in instances where no cutthroat trout were found, I used the next nearest stream without a barrier as the below barrier population. A variety of nonlethal sampling techniques were employed to collect fish across a full range of age and size classes. Streams were sampled with a backpack electro-shocker, by seining or by hook and line. From each fish a 3-5 mm piece of tissue was clipped from a pelvic or caudal fin and stored in 95% ethanol. After sampling, all animals were released near the point of capture.

Genetic analyses

Genomic DNA was extracted from each tissue sample using a Chelex protocol (Small *et al.* 1998). Extracted DNA samples were kept frozen at -30° C until polymerase chain reaction (PCR) amplification. Individuals were genotyped at 12 microsatellite loci including Fgt3 (Sakamoto *et al.* 1994), J14, H220 (Pritchard *et al.* 2007), OCH6, OCH13, OCH14, OCH16, OCH17 (Peacock *et al.* 2004), Oki10 (Smith *et al.* 1998), Omy77 (Morris *et al.* 1996), Ots101 (Small *et al.* 1998), and Ssa85 (O'Reilly *et al.* 1996). PCR amplification was performed in 10 μL reaction volumes containing 5 μL 2X ReddyMix™ PCR Master Mix (1.5mM $MgCl_2$), 2 μL sterile water, 0.5 μL of forward primer, and 0.5 μL of fluorescently labeled reverse primer, and 2 μL DNA. The thermal profile included an initial denaturing step at 94°C for 3 minutes

followed by 35 cycles at 94˚C for 30 s, 30 s at annealing temperatures ranging from 50˚C - 60˚C and 60 s at 72˚C, and a final 30 minute extension at 72˚C in a Bio-Rad C1000 Thermal Cycler. Genotyping was conducted with an Applied Biosystems 3130XL Genetic Analyzer. Loci for each individual fish were visually scored within GENEMAPPER software version 4.0 (Applied Biosystems).

Data analyses

In order to disentangle the effects of movement barriers not only by type, but to assess how habitat availability influences the population genetic structure of cutthroat trout in isolated streams, I calculated the amount of stream habitat above each barrier. Earlier studies examining the viability of isolated cutthroat trout populations have found that the probability of persistence increased dramatically when greater than 10 km of stream habitat was available above a barrier (Hilderbrand & Kershner 2000; Young *et al.* 2005; Fausch *et al.* 2009). Hence, in addition to categorizing barrier types, I also categorized populations by amount of stream length available above barriers as; <10km in length or as 10-50km in length, as well as in comparison to below barrier populations. I expected populations categorized as having <10 km of stream above a barrier to reflect the greatest influence of reduced population size and increased genetic drift (Hedrick 1999). To estimate the amount of stream habitat above a barrier I projected the geographic location of a confirmed movement barrier, and then measured stream distance available above the barrier based on a GIS layer of the stream channel using GIS software (ArcMap ver. 10.0, Redlands CA).

To gather initial genetic measures I used ARLEQUIN version 3.5 (Excoffier & Lischer 2010) and screened for deviations from Hardy-Weinberg Equilibrium (HWE) within and among sampling locations and linkage disequilibrium to determine

independence of loci. For all populations I included sample size (n), estimated number of alleles (N_a), allelic richness, expected heterozygosity (H_E), and observed heterozygosity (H_O) using ARLEQUIN 3.5 and FSTAT 2.9.3.2 (Weir & Cockerham 1984). To examine the effect of barrier type and stream habitat available I compared number of alleles, allelic richness, and observed heterozygosity by barrier type and habitat available using an analysis of variance (ANOVA), and identified which categories were different from each other using a Tukey's Studentized Range (HSD) test adjusted for un-equal sample sizes (*SAS Institute Inc.* 2011).

I estimated genetic differentiation between populations using F_{ST} (Weir & Cockerham 1984) and an analysis of molecular variance (AMOVA) to hierarchically examine genetic variation as implemented in ARLEQUIN. To examine the effect of barriers to movement and habitat available above a barrier, for the AMOVA I grouped populations into categories by barrier type and by habitat available. Statistical significance for the AMOVA was determined using a permutation procedure with 10,000 permutations in ARLEQUIN 3.5.

To test for isolation by distance between populations I compared pairwise F_{ST} to geographic distance, and then to examine the relationship of increased genetic drift and small population size (Hedrick 1999) I compared pairwise F_{ST} to average observed heterozygosity (H_O). For both of these relationships I used a likelihood-based analysis as described by Yang (2004) to account for non-independence of pairwise comparisons across all populations. Isolated populations are expected to lose genetic variability at a faster rate as a consequence of small population size and increased genetic drift (Hedrick 1999) relative to populations connected by gene flow. For these analyses, I grouped populations by barrier type and by amount of stream habitat available. If genetic drift is having an effect on genetic diversity, I expected a negative correlation

between F_{ST} and genetic distance. I modified a SAS program written by Yang (2004) to test for isolation by distance and genetic drift using a mixed model analysis of variance. To determine the possible effect of non-independence of error terms to model fit, I fit four different covariance structures of residuals to each regression model. The four modeled error terms included independent structure, correlated structure, first-order autoregressive structure, and autoregressive moving-average error structure (ARMA). The fit of the four models was then ranked by Akaike's Information Criterion (AIC) to choose the best-fit model for our dataset. In all cases the ARMA method provided the best model fit and was used to report the results of comparing F_{ST} and stream distance according to barrier type as well as F_{ST} and heterozygosity.

Results

Genetic diversity

A total of 1183 cutthroat trout were genotyped for the 12 microsatellite loci, representing 31 different populations. Of these populations, two were above natural barriers, nine were above artificial barriers, and 20 were below barrier populations (Fig. 1, Table 1). For above barrier populations, four were classified with < 10 km of available habitat and seven were classified with 10-50 km of available habitat (Fig. 1, Table 1). All 12 microsatellite loci were polymorphic with the number of alleles ranging from two to 20 with a mean of nine alleles per locus. I did not detect any significant departures from either Hardy-Weinberg equilibrium (HWE) or linkage disequilibrium so all loci and populations were retained for analyses. The average observed heterozygosity for all populations was 0.62, ranging from 0.26-0.77. The average allelic richness (A_R) ranged from 2.65 to 8.84 within populations with a mean of 5.11 (Table 1).

Cutthroat trout populations above natural barriers, artificial barriers, and in below barrier populations exhibited differences in levels of genetic diversity. I detected differences in the number of alleles present (Fig. 2a; $F_{2, 28} = 5.64$, $p = 0.00881$) and allelic richness (Fig. 2b; $F_{2, 28} = 4.23$, $p = 0.0247$), but not in levels of heterozygosity (Fig. 2c; $F_{2, 28} = 1.15$, $p = 0.331$). Populations isolated above natural barriers had the highest number of alleles and levels of allelic richness. Below barrier populations had intermediate levels that were similar to the lowest levels observed in cutthroat trout populations isolated above artificial movement barriers (Fig. 2a and b).

When I compared measures of genetic diversity according to the amount of habitat available above movement barriers, populations with 10 to 50 km of stream habitat had similar levels of diversity to below barrier populations, whereas above-barrier populations with less than 10 km tended to have the lowest levels of genetic diversity (Fig. 3). While the pattern of variability was similar to comparisons by barrier type, in contrast to differences between populations isolated by artificial and natural barriers, I did not detect significant differences in number of alleles (Fig. 3a; $F_{2, 28} = 1.49$, $p = 0.243$) or allelic richness (Fig. 3b; $F_{2, 28} = 2.91$, $p = 0.0710$) for populations with varying levels of habitat available, but did detect differences in the level of heterozygosity (Fig. 3c; $F_{2, 28} = 3.83$, $p = 0.0337$). Hierarchical analysis of cutthroat trout microsatellite variance showed significant division within populations by barrier type, and amount of habitat available (Table 2). In both scenarios there were significant divisions among populations within groups. However, delineations among groups by barrier type was not significantly divided ($p = 0.064$), whereas, by habitat available they were significantly different ($p < 0.001$).

Geographic stream distance between populations ranged from 2 km to 570 km and pairwise tests of genetic differentiation indicated significant differentiation between almost all populations of westslope cutthroat trout (Table 3). Of the 465 pairwise population comparisons, the average F_{ST} was 0.14, and ranged from 0.02 to 0.43. With the exception of two comparisons, all other populations were significantly different from one another ($p < 0.001$, Table 3). For the analysis of isolation by distance, F_{ST} increased significantly with increasing stream distance (partial $r = 0.16$, $p < 0.001$, Fig. 4). Genetic differentiation was slightly higher for populations above barriers than for those below barrier comparisons (partial $r = -0.22$, $p = 0.17$). However, when compared according to the type and number of barriers present between populations, the presence

of natural barriers was significantly related to the amount of genetic differentiation between populations (partial r = -0.54. p <0.001; Fig. 5).

When genetic differentiation and heterozygosity was compared between populations I found a significant decrease in F_{ST} with increasing levels of heterozygosity (H_O). The level of genetic differentiation between pairwise comparisons of F_{ST} decreased significantly with increasing H_O ($r = -0.75$, $p < 0.001$). When compared by the type of barrier present between populations, pairwise estimates of F_{ST} and H_O indicated that populations above barriers had some of the highest levels of genetic differentiation with corresponding low levels of heterozygosity producing a steeper inverse relationship that were marginally higher than below barrier populations (partial r = 0. 21, p = 0.059; Fig. 6a). Similarly, populations isolated above a barrier with less than 10 km of stream available had the highest values of F_{ST} with the lowest corresponding values of heterozygosity; whereas populations above barriers with 10-50 km or below barrier populations had significantly shallower declines (partial r = 0.25, p = 0.021; Fig. 6b).

Discussion

In this study, I examined the influence of barriers to movement and habitat availability on the population genetic structure of westslope cutthroat trout. My study revealed that the level of genetic variation maintained in cutthroat trout populations is related to the amount of stream habitat available above a barrier to movement. I observed the effect on genetic diversity was more pronounced the more stream habitat was limited to cutthroat trout populations occurring in streams above barriers in comparison to below barrier populations. However, populations classified with a greater amount of habitat (10-50 km) above barriers appear to maintain substantial genetic variation; whereas, less variation occurs within smaller sections of isolated streams (<10 km). In previous studies a similar relationship was reported between stream length and genetic variation with coastal cutthroat trout (*O. clarkii clarkii*), and habitat patch size in brook trout (*Salvelinus fontinalis*) populations (Whiteley *et al.* 2010, 2013).

Across the three main classes of cutthroat trout populations I sampled, below barrier populations did not have appreciably higher levels of genetic variation. Given that I selected populations based on their apparent access to surrounding streams within the watershed, I expected below barrier populations might exhibit the highest level of diversity as a result of their ability to exchange alleles with neighboring populations. Although levels of genetic variation were lower in populations above artificial barriers, they were not significantly different from diversity estimates for below barrier populations. Interestingly, populations above natural barriers to movement exhibited the highest levels of genetic differentiation. Previous studies have also detected

increased between-population genetic variability in conjunction with reduced within-population genetic variability in salmonid populations (Neville *et al.* 2006a; Taylor *et al.* 2003; Wofford *et al.* 2005; Guy *et al.* 2008; Meeuwig *et al.* 2010). However, the amount of habitat available above a barrier may interact with the presence of a movement barrier, such that the pattern of genetic diversity is only impacted when barriers isolate small patches of stream habitat (Deiner *et al.* 2007; Whiteley *et al.* 2010, 2013).

Interestingly, cutthroat trout populations with the highest levels of genetic variation were the two above natural barrier stream reaches. This result suggests that above-barrier streams can retain populations with important levels of genetic variation relative to below barrier populations that are often assumed to have the benefit of connectivity across stream networks. Furthermore, the observed relationship between stream length and genetic variation in the below-barrier populations, suggests connectivity is low, degraded, or nonexistent in the absence of visual barriers in the stream channel. Although cutthroat trout populations occur below barriers, management plans should not assume apparent connectivity to main stem habitats because of unknown barriers to movement. Cutthroat trout are known to occur in headwater locations where gene flow to these reaches from neighboring streams can be reduced by factors other than physical barriers considered in this study. Populations below barriers could be isolated because of other anthropogenic influences such as livestock activity and management leading to loss of cover/vegetation and undercut banks, temporary de-watering from irrigation, lethal stream temperatures (Hillyard & Keeley 2012), or from competition by invasive species.

In this study, when more than 10 km of stream habitat was available above a barrier, populations of cutthroat trout retained significant levels of genetic variation.

Above barrier populations with <10 km of habitat available had the lowest levels of genetic diversity. Observed heterozygosity was highest in populations with 10-50 km of habitat and was similar to populations below barriers. Above barrier populations with <10 km of habitat exhibited the lowest levels of heterozygosity among the three categories. Although, I did not detect a difference with two measures of genetic diversity, the pattern was relatively consistent across all three genetic diversity measures. I analyzed the distribution of the molecular variance to further explain the pattern of variation between classifications by barrier type, and the amount of habitat available. Whiteley et al. (2010, 2013) also found the amount of habitat available above a barrier was an important factor in explaining levels of genetic variation. In both brook trout (*S. fontinalis*) and coastal cutthroat trout (*O. c. clarkii*), the amount of genetic variation was positively correlated with the amount of habitat available for populations of these species. Greater habitat availability corresponds to a larger population size and supports greater levels of genetic diversity. Conversely, small habitat patches can only support smaller populations with limited genetic diversity, probably a result of increased inbreeding and genetic drift.

Populations that become isolated, with limited habitat available are susceptible to increased genetic drift. A strong negative relationship between F_{ST} and paired genetic diversity is expected in populations exhibiting drift in isolation (Hedrick 1999). This relationship has been observed in natural populations of Galápagos lava lizard (*Microlophus albemarlensis* complex), tidewater goby (*Eucyclogobius newberryi*), and coastal cutthroat trout (Jordan & Snell 2008; McCraney *et al.* 2010; Whiteley *et al.* 2010). In this study, I detected a significant relationship between genetic differentiation and heterozygosity in populations of westslope cutthroat. The relationship was more pronounced in populations above barriers and those with < 10km

of habitat available. This significant negative relationship is likely a direct consequence of increased genetic drift. In the analysis of isolation by distance and F_{ST} estimates indicate greater scatter and increased divergence among populations above barriers relative to below barrier populations. These results support the premise that the observed increase in genetic differentiation between populations of westslope cutthroat trout isolated above barriers is largely influenced by genetic drift; whereas gene flow is a more important factor influencing genetic structure between populations below barriers.

Across westslope cutthroat trout populations studied, I detected significant genetic differentiation for almost all population pairs. Salmonid fishes in streams typically home to headwater reaches to spawn and rear (Northcote 1997), and as a result genetic differentiation between tributaries is expected, and often observed (Neville *et al.* 2006a; Dunham & Rieman 1999; Guy *et al.* 2008; Pritchard *et al.* 2009). There was a significant pattern of isolation by distance for all populations of westslope cutthroat trout, however, an additional proportion of the variation in F_{ST} was accounted for by the presence of a barrier. Isolation by distance in my study area for westslope cutthroat trout suggests barriers to movement interact with the amount of suitable habitat available above a barrier to increase differentiation or preserve genetic diversity of populations (Guy *et al.* 2008).

Populations of westslope cutthroat trout in the upper Salmon River watershed of central Idaho exhibit significant population genetic structuring. In this study, I identified a number of different factors influencing levels of genetic diversity within cutthroat trout populations and the connectedness among populations. While geographic distance and barriers to movement are important factors influencing the degree of genetic differentiation, the amount of habitat available affects population size,

and in turn, the amount of genetic diversity. Populations of cutthroat trout isolated above barriers with < 10 km of available habitat were the most genetically depauperate, and may be at greatest risk of extinction.

Conservation Implications

Habitat fragmentation and loss are often the most common threats to both terrestrial and aquatic ecosystems (Wilcox & Murphy 1985; Nehlsen *et al.* 1991; Master *et al.* 1997; Vitousek *et al.* 1997; Holsinger 2000; Stein *et al.* 2000; Fahrig 2003). Many populations of cutthroat trout have declined in North America from a variety of human-related activities that have fragmented or altered aquatic habitats (Behnke 2002; Pritchard *et al.* 2007; Trotter 2008; Fausch *et al.* 2009; Rahel 2013). Remaining cutthroat trout populations have been the focus of intense conservation efforts in some areas because so few populations exist. The success of such conservation efforts will require an understanding of factors that influence the probability of remaining populations persisting over time. In this study, I demonstrate that barriers to movement often occurring as a result of habitat fragmentation can have a significant influence, especially true where limited habitat is available above those barriers.

The long-term consequence of reduced genetic variation may be a decrease in the probability of persistence for a population. Past studies have found that approximately 10 km of stream habitat is needed for translocation success in the reintroduction of cutthroat trout populations (Hilderbrand & Kershner 2000; Harig & Fausch 2002; Young *et al.* 2005; Rahel 2013); whereas, other studies have found that population persistence above man-made barriers was a positive function of stream length and a negative relationship with time since isolation, as observed in white-

spotted charr (*S. leucomaenis*) populations (Morita & Yamamoto 2002). I observed a strong relationship between stream length and genetic variation for populations recently isolated by man-made barriers to movement. My results further support the idea that a minimal measure of stream length could be a useful criterion in maintaining sufficient genetic diversity and probability of persistence for westslope cutthroat trout populations where barriers to movement may occur as a result of habitat alteration. This could also be useful to other headwater fishes with a similar life history as salmonids.

Some research has suggested using barriers as a conservation management tool to protect native species. Fausch et al. (2009) and Rahel (2013) provide trade-offs and strategies for intentionally fragmenting or connecting populations of fishes in aquatic systems. A downside would be to subject a population to the demographic and genetic effects of small population size by not providing at least 10 km of stream habitat as found in translocation studies (Young *et al.* 2005). These results provide a mechanism of habitat length to consider for a successful barrier installation, if this type of management is needed, however, there remains the concern to the quality and diversity of habitat located above a barrier (Neville *et al.* 2006b). The quality and diversity of habitat above a barrier should provide opportunities for individuals to seek refuge when catastrophic events occur or stream conditions are less than suitable. Management decisions to use barriers need to be included in management plans and coordinated to insure those actions are followed up to rescue populations at risk. When certain future events (i.e. climate change related conditions; forest fire, increased stream temperatures, etc.) occur those management plans should trigger a rescue of at risk populations to translocate. Furthermore, management plans should include simulating metapopulation dynamics to increase genetic diversity or to reestablish a population in suitable habitat when using barriers to protect native populations. Of course this is a

case-by-case situation, however, the effect of my study can add to the evaluation of barriers to movement and habitat availability on the population genetic structure of westslope cutthroat trout.

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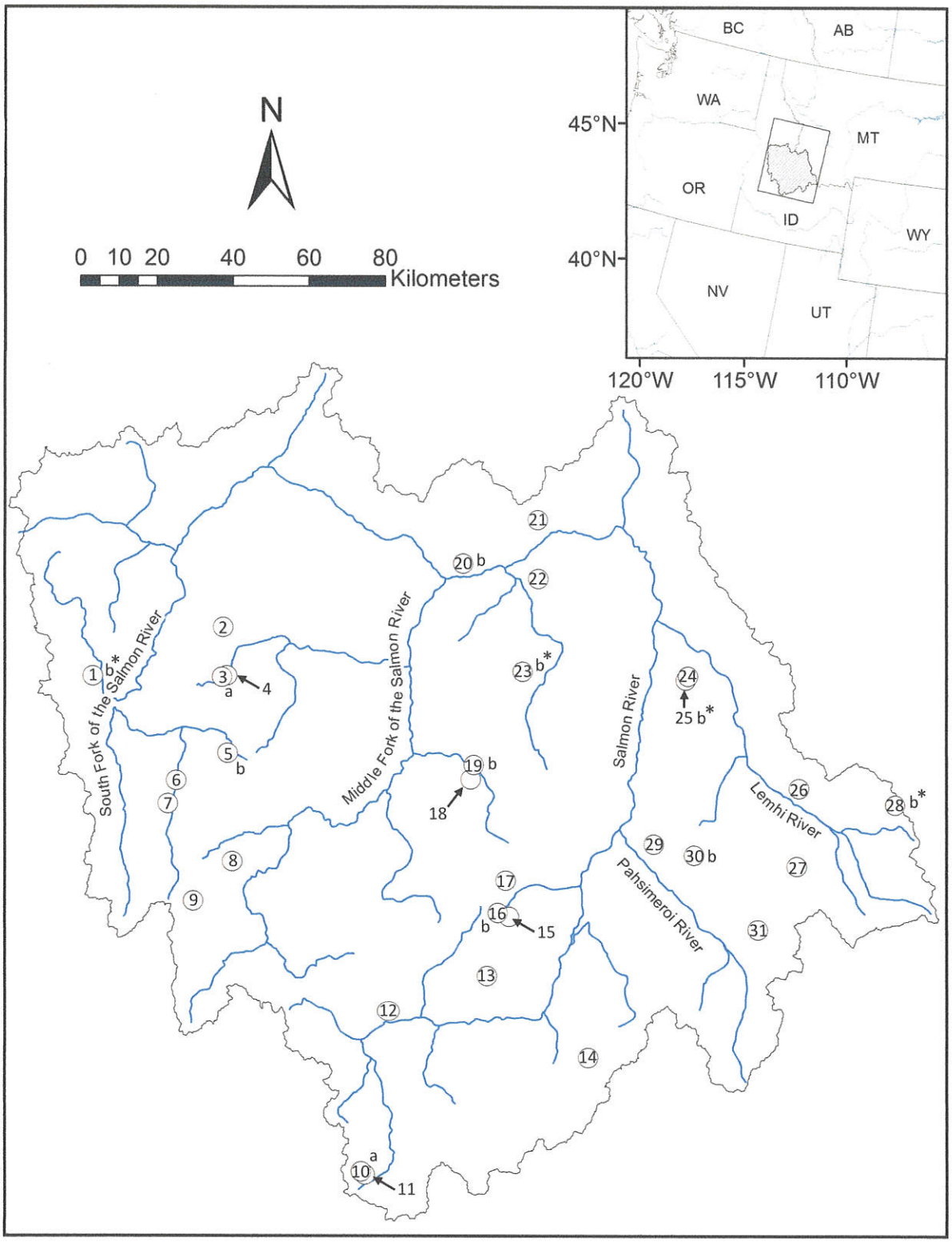

Figure 1. Location of sampling sites for populations of westslope cutthroat trout in the Salmon River, Idaho, USA. Populations marked by a lower case 'a' indicate sites that were above a natural movement barrier, whereas populations marked by a 'b' were

above an artificial movement barrier. Those above barriers with less than 10 kilometers of habitat were marked with a star '*', and those above barriers with 10-50 kilometers of habitat available have no other markings. Populations are identified as: 1 = Split Creek, 2 = North Fork Smith Creek, 3 = Big Creek (Middle Fork Salmon), 4 = Jacob's Ladder Creek, 5 = Meadow Creek, 6 = Bear Creek, 7 = Halfway Creek, 8 = North Fork Elkhorn Creek, 9 = East Fork Sulfur Creek, 10 = Alpine Creek (above), 11 = Alpine Creek (below), 12 = Salmon River, 13 = Cinnabar Creek, 14 = Road Creek, 15 = Mill Creek, 16 = Challis Creek, 17 = Twin Creek, 18 = Flume Creek, 19 = Duck Creek, 20 = Colson Creek, 21 = Spring Creek, 22 = Beaver Creek, 23 = Blackbird Creek, 24 = Withington Creek (below), 25 = Withington Creek (above), 26 = Little Eightmile Creek, 27 = Middle Fork Little Timber Creek, 28 = Reservoir Creek, 29 = Morgan Creek, 30 = Morse Creek, 31 = Big Creek (Pahsimeroi). Inset map illustrates the location of the watershed within the State of Idaho, USA.

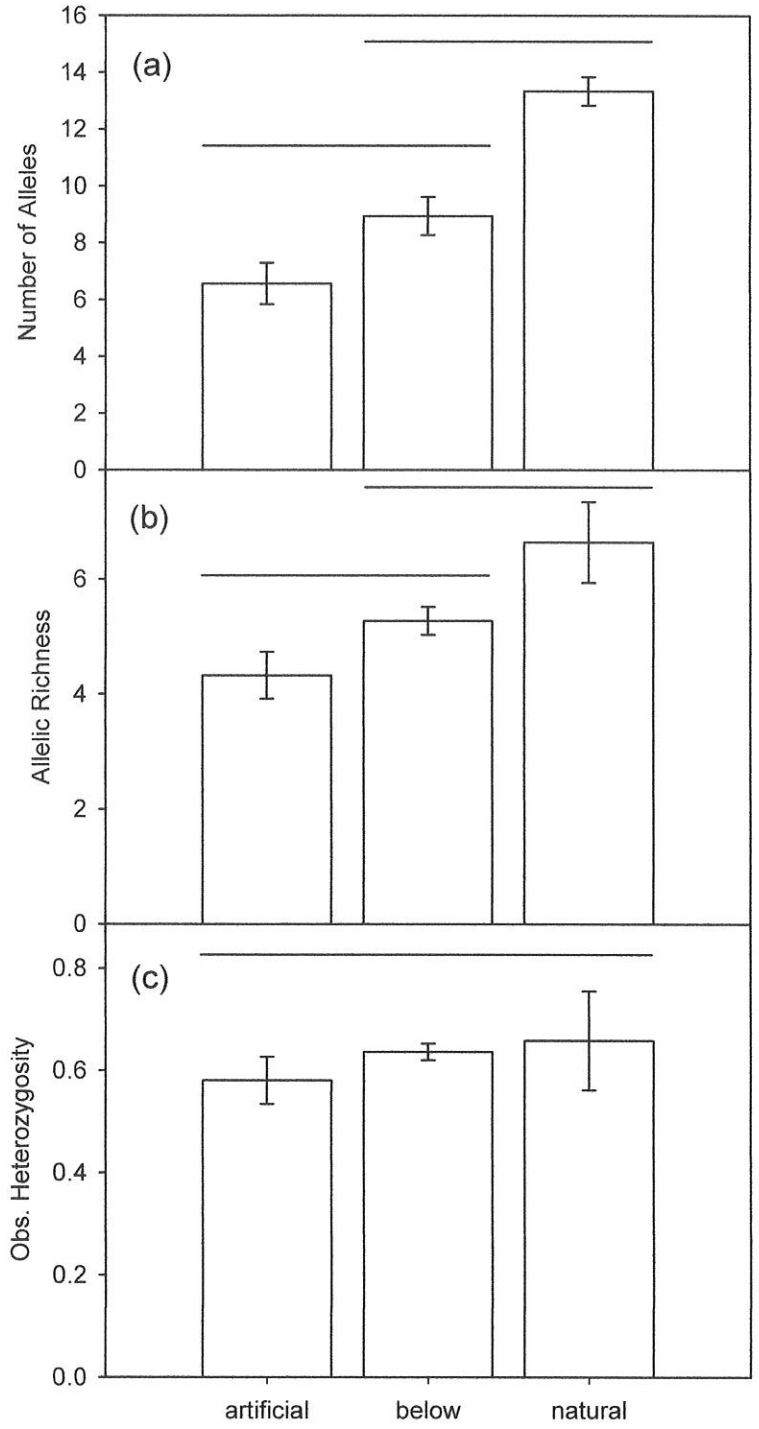

Figure 2. Mean (±1 SE) (a) number of alleles, (b) allelic richness, and (c) observed heterozygosity for westslope cutthroat trout populations above artificial or natural barriers or for below barrier populations in the Salmon River, Idaho, USA. Categories that were not significantly different share a solid horizontal bar (Tukey's HSD).

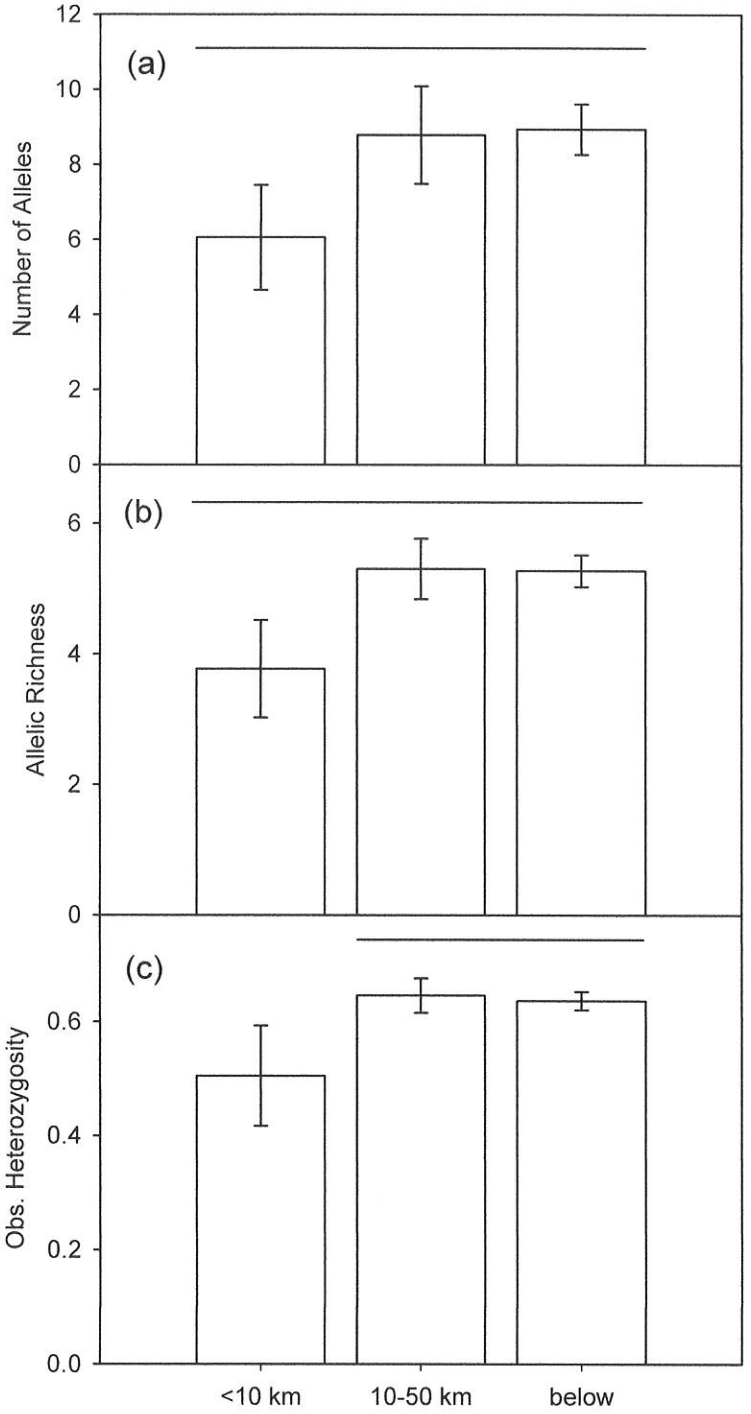

Figure 3. Mean (±1 SE) comparison of above barriers by stream length (<10 km and 10-50 km) to below barrier populations by response measures of (a) number of alleles, (b) allelic richness, and (c) observed heterozygosity in the Salmon River, Idaho, USA (2008-2009). Comparisons were not significantly different between groups by

ANOVA tests in (a) number of alleles $F_{2,28} = 1.49$, $p = 0.243$; (b) allelic richness $F_{2,28} = 2.91$, $p = 0.0710$; and significant for (c) observed heterozygosity $F_{2,28} = 3.83$, $p = 0.0337$. Categories were not significantly different from one another by Tukey's (HSD) post hoc procedure indicated by the solid horizontal bar.

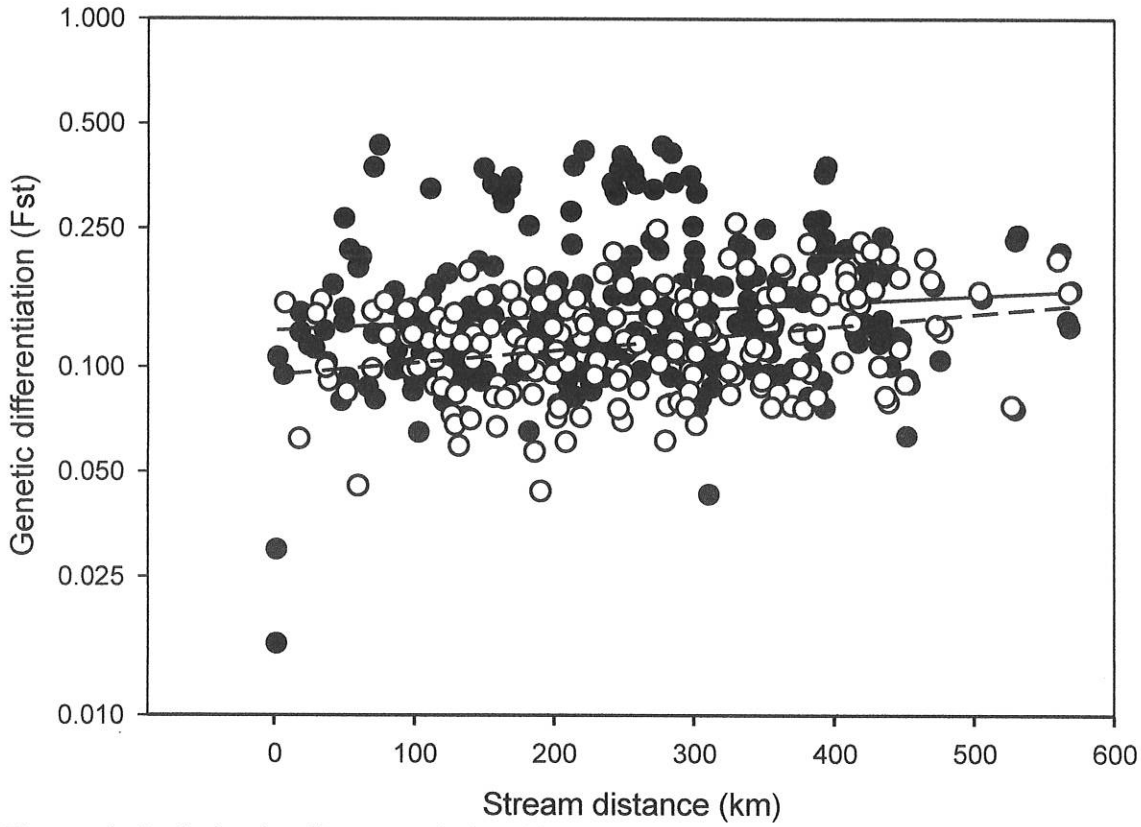

Figure 4. Isolation by distance relationship between pair-wise F_{ST} and stream distance (km) for populations of westslope cutthroat was significant ($r = 0.16$, $p < 0.001$) by Yang (2004) likelihood based analysis in the Salmon River, Idaho, USA (2008-2009). Differences between slopes of above barriers (dashed line, solid circles) and below barriers (solid line, white circles) are depicted.

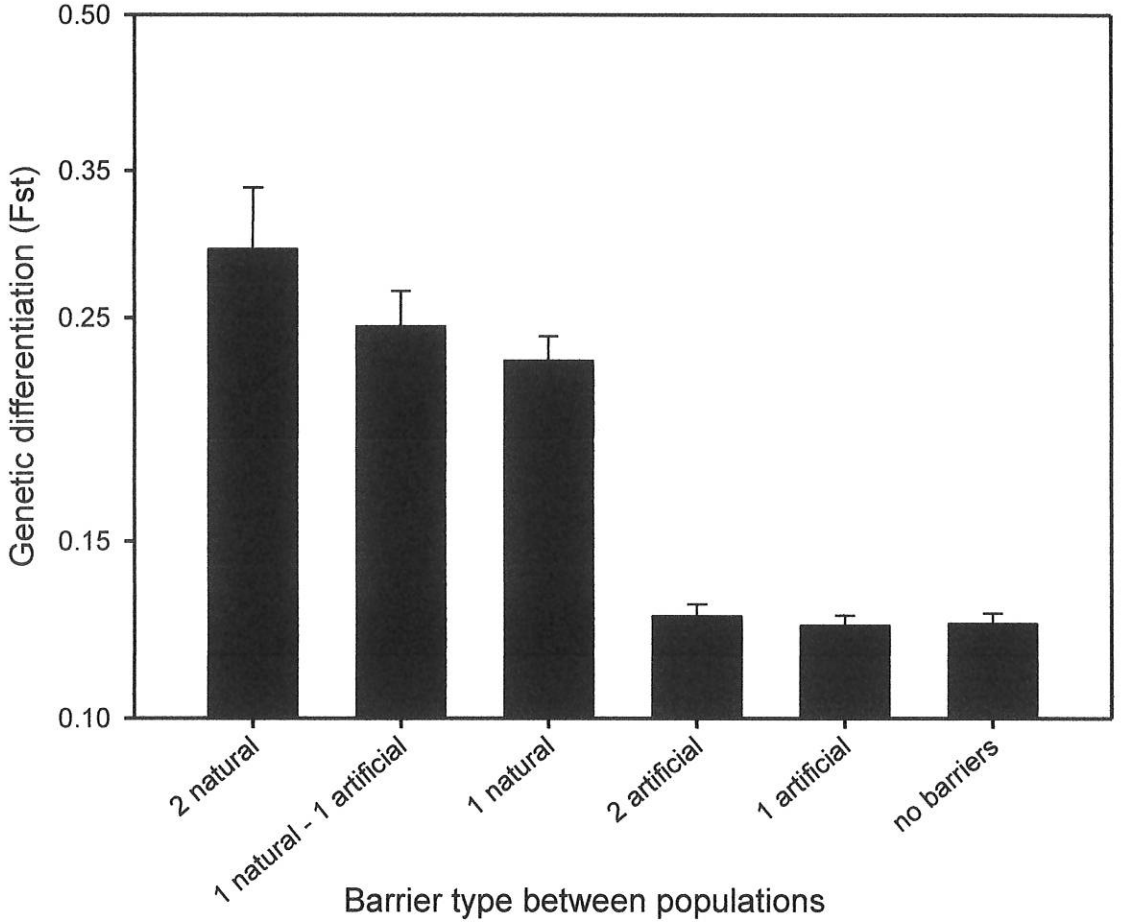

Figure 5. Pairwise genetic differentiation between populations of westslope cutthroat trout according to the number and types of barriers present.

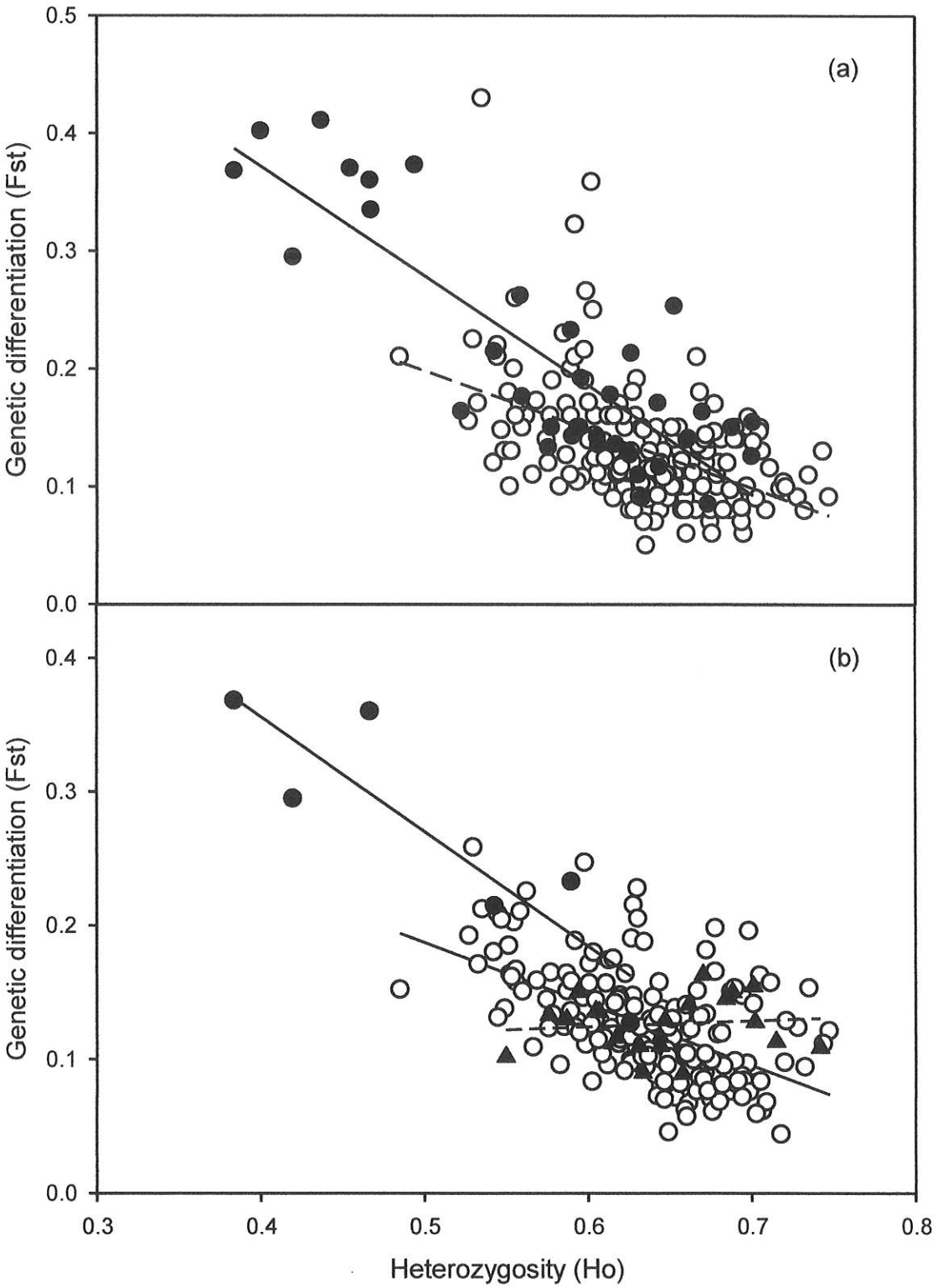

Figure 6. The relationship between pairwise F_{ST} and average observed heterozygosity for populations of westslope cutthroat in the Salmon River, Idaho, USA (2008-2009) that occurred above barriers (circles, solid line), and below barriers (open circles,

dashed line). (b) The relationship between pairwise F_{ST} and average observed heterozygosity for populations of westslope cutthroat in the Salmon River, Idaho, USA (2008-2009) that occurred above barriers with less than 10 km of stream habitat available (circles, solid line), below barriers (open circles, solid line) or above barrier with 10-50km of stream habitat available (triangles, dashed line).

Table 1. Tributary stream name where sites were sampled, abbreviated code of sites, the sub-basin within which the stream is located, sample size (n), average number of alleles across locus (A), average allelic richness across locus (AR), and average expected heterozygosity across locus (HE) in the Salmon River, Idaho, USA (2008-2009). The number before the tributary stream name is referenced to the map in Fig. 1 and the letters in parentheses are noting the same name as two different locations above (a), and below (b) barrier.

Site	Code	n	Barrier Type	Stream available	A (±SE)	A_R (±SE)	H_E (±SE)	H_O (±SE)	Zone Northing/Easting [a]
1. Split Cr.	SPL	33	artificial	< 10 km	3.8 (1.4)	3.0 (0.3)	0.51 (0.20)	0.51 (0.24)	11T 0595674 4992657
2. North Fork Smith Cr.	SMI	50	below	below	8.8 (3.5)	4.9 (0.4)	0.73 (0.10)	0.65 (0.13)	11T 0629902 5005329
3. Big Cr. (MF Salmon)	BCM	38	natural	10-50 km	13.8 (3.4)	7.3 (0.3)	0.87 (0.03)	0.76 (0.16)	11T 0629602 4992395
4. Jacob's Ladder Cr.	JLC	48	below	below	10.1 (2.8)	5.4 (0.3)	0.75 (0.08)	0.67 (0.15)	11T 0630872 4992806
5. Meadow Cr.	MDW	15	artificial	10-50 km	4.6 (1.0)	3.8 (0.3)	0.65 (0.12)	0.61 (0.18)	11T 0631043 4972629
6. Bear Cr.	BER	21	below	below	4.8 (2.0)	3.7 (0.3)	0.57 (0.20)	0.49 (0.18)	11T 0617575 4965502
7. Halfway Cr.	HWC	49	below	below	5.8 (2.9)	3.8 (0.4)	0.58 (0.18)	0.48 (0.16)	11T 0615358 4959447
8. North Fork Elkhorn Cr.	ELK	23	below	below	5.25 (2.0	4.0 (0.4)	0.62 (0.18)	0.62 (0.19)	11T 0632256 4944370
9. East Fork Sulfur Cr.	SUL	45	below	below	8.0 (2.6)	5.3 (0.4)	0.78 (0.12)	0.68 (0.16)	11T 0622037 4934021
10. Alpine Cr. (a)	AP1	42	natural	10-50 km	12.8 (2.4)	5.9 (0.3)	0.75 (0.06)	0.56 (0.14)	11T 0666047 4863599
11. Alpine Cr. (b)	AP2	48	below	below	13.9 (4.2)	6.8 (0.4)	0.82 (0.07)	0.62 (0.12)	11T 0666815 4863377
12. Salmon R.	SAL	62	below	below	12.1 (2.5)	6.2 (0.3)	0.80 (0.08)	0.71 (0.11)	various locations
13. Cinnabar Cr.	CIN	45	below	below	11.8 (2.7)	5.8 (0.3)	0.77 (0.09)	0.72 (0.09)	11T 0699299 4914505
14. Road Cr.	ROA	35	below	below	10.5 (2.7)	5.9 (0.4)	0.75 (0.14)	0.61 (0.16)	11T 0726019 4893188
15. Mill Cr.	MIL	42	below	below	7.4 (3.3)	4.8 (0.4)	0.72 (0.11)	0.63 (0.17)	11T 0705129 4929983
16. Challis Cr.	CHA	39	artificial	10-50 km	7.5 (3.0)	4.8 (0.4)	0.72 (0.10)	0.67 (0.15)	11T 0702144 4930968
17. Twin Cr.	TWN	33	below	below	10.8 (3.2)	6.6 (0.4)	0.83 (0.07)	0.77 (0.08)	11T 0704231 4939428

18. Flume Cr.	FLU	41	below	below	15.4 (3.0)	7.9 (0.4)	0.87 (0.06)	0.67 (0.08)	11T 0695088 4965701
19. Duck Cr.	DUK	44	artificial	10-50 km	7.4 (3.2)	4.8 (0.4)	0.73 (0.09)	0.65 (0.09)	11T 0695807 4969522
20. Colson Cr.	COL	29	artificial	10-50 km	9.3 (3.4)	6.1 (0.5)	0.80 (0.09)	0.73 (0.17)	11T 0693065 5021971
21. Spring Cr.	SPG	29	below	below	6.2 (2.0)	4.4 (0.3)	0.67 (0.13)	0.58 (0.19)	11T 0712783 5033370
22. Beaver Cr.	BVR	28	below	below	9.7 (2.7)	5.7 (0.5)	0.74 (0.14)	0.62 (0.17)	11T 0712897 5018049
23. Blackbird Cr.	BBD	51	artificial	< 10 km	3.6 (1.2)	2.0 (0.2)	0.38 (0.23)	0.26 (0.23)	11T 0708714 4993870
24. Withington Cr. (b)	WT2	39	below	below	7.3 (2.6)	4.4 (0.4)	0.65 (0.19)	0.57 (0.17)	12T 0279746 4991445
25. Withington Cr. (a)	WT1	39	artificial	< 10 km	8.9 (2.8)	5.0 (0.4)	0.70 (0.16)	0.58 (0.13)	12T 0279078 4990495
26. Little Eightmile Cr.	L8M	25	below	below	6.6 (2.2)	4.9 (0.4)	0.72 (0.12)	0.64 (0.13)	12T 0306724 4960140
27. MF Little Timber Cr.	TIM	44	below	below	11.1 (2.1)	5.8 (0.2)	0.79 (0.05)	0.69 (0.08)	12T 0304579 4939990
28. Reservoir Cr.	RES	43	artificial	< 10 km	8.0 (2.7)	5.0 (0.3)	0.73 (0.14)	0.67 (0.17)	12T 0331617 4954068
29. Morgan Cr.	MRG	23	below	below	5.3 (1.6)	4.2 (0.3)	0.69 (0.11)	0.70 (0.16)	12T 0267482 4948442
30. Morse Cr.	MRS	29	artificial	10-50 km	6.0 (3.0)	4.2 (0.5)	0.67 (0.12)	0.54 (0.14)	12T 0277881 4944801
31. Big Cr. (Pahsimeroi)	BGP	46	below	below	8.2 (3.2)	4.9 (0.3)	0.73 (0.11)	0.60 (0.15)	12T 0293201 4924204

a – Universal Transverse Location, North American Datum 1983.

Table 2. Analysis of molecular variance (AMOVA) for 12 microsatellite loci allele frequencies among and within populations of westslope cutthroat trout above and below barriers to movement, and by habitat available by measured stream length in the Salmon River, Idaho, USA (2008-2009).

Comparison	df	Source of variation	Percentage	P value
Barrier type				
(Natural vs. Artificial vs. below)	2	Among groups	0.56	0.064
	28	Among populations within groups	14.0	<0.001
	2336	Within populations	85.8	<0.001
Total	2366			
Habitat available				
(<10km vs. 10-50km vs. below)	2	Among groups	1.4	<0.001
	28	Among populations within groups	13.5	<0.001
	2336	Within populations	85.1	<0.001
Total	2366			

Table 3. Stream distance (km) between each of the 31 sampled sites and pairwise F_{ST} values (coded from Table 1) for westslope cutthroat trout within the Salmon River, Idaho, USA (2008-2009).

	AP1	AP2	BER	BVR	BCM	BCP	BBD	CHA	CIN	COL	DUK	SUL	FLU	HWC	JLC
AP1		2	562	353	454	252	395	196	122	355	435	529	441	570	453
AP2	0.02*		560	351	452	250	393	194	120	353	433	527	439	568	451
BER	0.21	0.20		236	300	439	278	426	470	213	280	375	287	8	299
BVR	0.12	0.11	0.18		128	230	50	217	260	29	108	203	114	244	127
BCM	0.09	0.06	0.19	0.10		331	170	318	362	105	115	210	121	308	3
BCP	0.11	0.10	0.21	0.10	0.13		271	116	159	232	311	406	317	447	330
BBD	0.38	0.36	0.43	0.27	0.35	0.32		259	302	71	150	245	156	286	168
CHA	0.12	0.11	0.17	0.10	0.11	0.09	0.33		103	219	299	393	305	434	317
CIN	0.10	0.09	0.18	0.09	0.09	0.07	0.32	0.06		263	342	437	348	477	360
COL	0.11	0.10	0.22	0.11	0.11	0.14	0.37	0.15	0.12		85	180	91	221	104
DUK	0.14	0.13	0.15	0.11	0.13	0.14	0.37	0.14	0.10	0.15		134	7	288	114
SUL	0.08	0.08	0.12	0.08	0.08	0.10	0.31	0.08	0.08	0.10	0.09		140	383	209
FLU	0.10	0.08	0.16	0.09	0.08	0.12	0.33	0.11	0.09	0.10	0.09	0.07		294	120
HWC	0.16	0.16	0.15	0.14	0.17	0.18	0.34	0.13	0.13	0.17	0.13	0.10	0.14		307
JLC	0.09	0.09	0.13	0.07	0.11	0.09	0.32	0.10	0.08	0.13	0.09	0.06	0.09	0.12	
L8M	0.13	0.11	0.23	0.12	0.12	0.15	0.38	0.11	0.12	0.12	0.16	0.09	0.12	0.15	0.14
MDW	0.13	0.14	0.17	0.11	0.14	0.12	0.41	0.12	0.10	0.16	0.11	0.08	0.13	0.13	0.08
TIM	0.10	0.08	0.16	0.07	0.08	0.07	0.32	0.09	0.06	0.10	0.08	0.08	0.08	0.15	0.07
MIL	0.10	0.10	0.21	0.13	0.09	0.12	0.36	0.10	0.10	0.13	0.16	0.08	0.11	0.17	0.12
MRG	0.13	0.12	0.19	0.10	0.14	0.05	0.34	0.10	0.07	0.14	0.13	0.10	0.12	0.16	0.09
MRS	0.10	0.08	0.22	0.13	0.13	0.09	0.40	0.14	0.10	0.09	0.15	0.10	0.11	0.17	0.11
ELK	0.16	0.16	0.16	0.11	0.17	0.17	0.42	0.16	0.13	0.19	0.11	0.09	0.14	0.16	0.08
SMI	0.12	0.11	0.16	0.10	0.13	0.10	0.32	0.12	0.08	0.14	0.10	0.08	0.10	0.11	0.06
RES	0.12	0.10	0.19	0.08	0.11	0.10	0.36	0.12	0.09	0.14	0.14	0.09	0.10	0.18	0.10
ROA	0.15	0.12	0.20	0.11	0.13	0.13	0.35	0.08	0.08	0.13	0.14	0.10	0.11	0.13	0.13
SAL	0.09	0.08	0.17	0.10	0.10	0.09	0.32	0.09	0.04	0.08	0.10	0.08	0.08	0.11	0.08
SPL	0.24	0.23	0.22	0.16	0.23	0.22	0.37	0.21	0.19	0.25	0.18	0.18	0.21	0.21	0.14
SPG	0.09	0.08	0.21	0.15	0.13	0.12	0.43	0.14	0.12	0.13	0.17	0.10	0.12	0.17	0.12
TWN	0.13	0.10	0.23	0.15	0.04	0.15	0.38	0.14	0.12	0.14	0.15	0.12	0.10	0.22	0.13
WT1	0.14	0.11	0.22	0.10	0.12	0.13	0.29	0.14	0.11	0.12	0.15	0.12	0.11	0.16	0.12
WT2	0.17	0.15	0.26	0.13	0.14	0.16	0.31	0.16	0.13	0.18	0.17	0.15	0.15	0.19	0.14

Table 3. Continued from page 40.

L8M	MDW	TIM	MIL	MRG	MRS	ELK	SMI	RES	ROA	SAL	SPL	SPG	TWN	WT1	WT2
343	569	37	191	222	229	506	448	386	144	283	531	328	189	293	292
341	567	370	189	220	227	504	447	384	143	281	530	326	187	291	290
382	42	411	421	409	416	352	294	424	465	279	54	243	419	332	330
172	242	202	212	200	207	180	122	215	256	70	205	34	210	122	121
274	307	303	313	301	308	186	19	316	357	171	270	135	311	224	223
220	445	249	111	60	67	383	325	262	155	160	408	205	109	170	169
214	284	243	253	241	249	221	164	257	298	111	247	75	251	164	163
207	433	236	38	86	93	370	312	250	99	147	396	192	19	157	156
250	476	280	98	129	136	413	356	293	52	191	439	236	96	200	199
175	219	204	214	202	209	157	99	217	259	72	182	36	212	125	124
254	287	283	293	281	288	111	109	297	338	151	250	115	291	204	203
349	382	378	388	376	383	39	204	391	433	246	345	210	386	299	298
260	293	289	299	287	295	117	115	303	344	157	256	121	297	210	209
390	50	419	429	417	424	360	302	432	473	287	62	251	427	339	338
273	305	302	312	300	307	185	18	315	356	170	268	134	310	222	221
	388	37	202	190	197	325	268	50	246	103	351	148	200	71	70
0.20		417	427	415	423	358	301	431	472	285	60	249	425	338	337
0.10	0.12		231	219	226	355	297	48	276	132	380	177	229	100	99
0.11	0.17	0.10		81	88	365	307	244	94	142	390	187	30	152	151
0.15	0.12	0.07	0.12		25	353	295	232	125	130	378	175	79	140	139
0.14	0.13	0.09	0.11	0.11		360	302	240	132	137	385	182	86	147	146
0.21	0.09	0.13	0.19	0.14	0.18		180	368	409	223	321	187	363	275	274
0.16	0.10	0.08	0.13	0.08	0.12	0.10		310	351	165	264	129	305	218	216
0.15	0.13	0.08	0.14	0.11	0.13	0.16	0.12		289	145	394	190	242	114	112
0.09	0.17	0.10	0.14	0.13	0.14	0.18	0.14	0.15		186	435	231	92	196	195
0.10	0.11	0.06	0.10	0.08	0.07	0.13	0.08	0.12	0.06		248	45	140	53	51
0.25	0.19	0.16	0.26	0.15	0.26	0.17	0.17	0.23	0.24	0.19		212	388	301	300
0.12	0.18	0.11	0.11	0.15	0.07	0.18	0.14	0.14	0.12	0.10	0.28		185	98	97
0.16	0.18	0.09	0.14	0.15	0.16	0.20	0.16	0.16	0.15	0.11	0.25	0.17		150	149
0.12	0.18	0.09	0.14	0.15	0.14	0.22	0.12	0.13	0.11	0.09	0.21	0.15	0.14		2
0.14	0.22	0.12	0.16	0.19	0.20	0.25	0.16	0.16	0.14	0.13	0.25	0.20	0.18	0.03*	