

Bull Trout Population Genetic Structure and Entrainment in Warm Springs Creek, Montana

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Introduction

The Clark Fork River system west of the Continental Divide in Montana historically contained one of the largest metapopulations of bull trout (*Salvelinus confluentus*) throughout the species range. Historically, bull trout were likely distributed throughout the upper Clark Fork River upstream of Milltown Dam (near Missoula, MT) as there are no major natural barriers excluding bull trout from major portions of the drainage. The Warm Springs Creek watershed likely provided a significant portion of the spawning and rearing habitat for bull trout in the upper Clark Fork River due to the large area of the drainage, the geology of the drainage, and the diversity of habitats. However, a century of mining and smelting polluted streams in the upper Clark Fork River system with toxic metals and other chemicals (MBTSG 1995), and such mining-related habitat degradation effectively extirpated migratory bull trout from much of the system. The US Fish and Wildlife Service (USFWS) designated seven local populations within the upper Clark Fork River core area (USFWS 2002). However, more recent information suggests that bull trout in this core area have been reduced to only three viable populations, including Warm Springs, Boulder, and perhaps Harvey Creeks (USFWS 2009). Currently these populations are isolated from one another. Of these three locations, Warm Springs Creek contains the most-upstream population of bull trout in the Clark Fork River, is comprised of multiple demes (discrete spawning units in individual tributaries), and is likely the largest among the upper Clark Fork bull trout populations in terms of number of individuals and extent of occupied habitat.

Elevations within the Warm Springs Creek drainage range from 1,524 to 3,139 meters (5,000-10,300 ft) and the drainage encompasses over 40,499 ha (100,077 acres). This system is unique in that both resident and migratory (adfluvial and fluvial) life history type bull trout occur over a range of habitats including a series of lakes (Upper and Lower Twin Lakes), reservoirs (Silver Lake), and tributaries (Foster, Twin Lake, Storm Lake, Warm Springs and Barker Creeks). Predictions about the response of bull trout to climate change (Rieman et al. 2007) suggest that local populations like Warm Springs Creek will represent important conservation units.

Since the early 1900s bull trout habitat within the Warm Springs Creek drainage has been extensively fragmented by development and utilization of a water supply system for large-scale smelting operations based in Anaconda, MT (Figure 1). Warm Springs Creek, Silver Lake,

Storm Lake Creek and Twin Lakes Creek contain an intricate water conveyance system, including diversion structures, aqueducts, exposed and buried pipes, and pumping stations (Figures 2-6). These structures clearly influence habitat connectivity within the system, and have caused the isolation of bull trout populations in Twin Lakes and Storm Lake Creeks. Moreover, bull trout from Storm Lake and Twin Lakes Creeks may also be entrained into Silver Lake, which could represent a demographic loss to source populations and constrain expression of a migratory life history if entrained individuals cannot return to spawn in their natal habitats.

Currently little is known about the effect of fragmentation or introduced fish species on bull trout in the system. Previous studies of bull trout populations in other systems have shown that habitat fragmentation and entrainment can cause reduced genetic diversity, loss of gene flow among populations and disruption of migratory corridors (Nerass and Spruell 2001; Costello et al. 2003; Whiteley et al. 2006; DeHaan et al. 2007). Habitat fragmentation may also result in genetic bottlenecks (Yamamoto et al. 2004) and life history changes in salmonid fishes (Morita et al. 2000).

The presence of non-native brook trout (*Salvelinus fontinalis*) represents another threat to bull trout in the Warm Springs Creek drainage. Hybridization between bull trout and brook trout has been documented where the two species occur sympatrically, including in a number of tributaries within the Clark Fork River system (Leary et al. 1995; Kanda et al. 2002). Although the majority of bull trout × brook trout hybrids appear to be first generation (F1) progeny, occurrence of post-F1 hybrids provides evidence that introgression can occur in some situations (Kanda et al. 2002; DeHaan et al. 2010). The threat of hybridization and introgression may be greater in areas where habitat has become degraded and fragmented (DeHaan et al. 2010). Additionally brook trout may display a competitive advantage over bull trout in areas where they are sympatric (Gunckel et al. 2002; McMahan et al. 2007).

Although the USFWS Draft Bull Trout Recovery Plan (USFWS 2002) identifies the collection of genetic data as a priority for recovery planning in many watersheds, no baseline genetic data currently exist for bull trout populations in the Warm Springs Creek drainage. Given the increased conservation concern for bull trout in Warm Springs Creek and the lack of genetic information pertaining to this population, we used a suite of microsatellite markers to conduct a fine-scale analysis of genetic variation in Warm Springs Creek bull trout populations. Our study had three specific objectives:

- 1) To determine the distribution of bull trout, brook trout, and bull trout x brook trout hybrids within the Warm Springs Creek drainage
- 2) To characterize levels of genetic variation both within and among remnant bull trout populations in the Warm Springs Creek drainage and determine the effect that habitat fragmentation has had on these populations.
- 3) To use genetic population assignment techniques to assign individuals of unknown origin entrained in Silver Lake to their most likely population of origin within the Warm Springs Creek drainage.

Methods

Sample Collection

Bull trout population surveys were conducted in Warm Springs Creek and its tributaries during the summers of 2008 and 2009. Juvenile bull trout were collected by backpack electrofishing in six spawning tributaries in the Warm Springs Creek drainage: Foster Creek, Twin Lakes Creek, Barker Creek, Storm Lake Creek, Warm Springs Creek, and West Fork Warm Springs Creek (Figure 1). Additionally sub-adult and adult bull trout were collected in gill nets set in upper and lower Twin Lakes and Silver Lake. To determine the distribution of brook trout and bull trout × brook trout hybrids within the Warm Springs Creek drainage, putative brook trout and hybrids were also collected from Warm Springs Creek, Twin Lakes Creek, Storm Lake Creek, and Cable Creek. Small tissue samples were taken from all *Salvelinus* captured and stored in 100% non-denatured ethanol or dried on blotter paper.

Laboratory methods

We extracted DNA from all samples using a modified chelex extraction protocol (Miller and Kapuscinski 1996). All individuals were genotyped at a suite of 16 microsatellite loci; *Omm1128*, *Omm1130* (Rexroad et al. 2001), *Sco102*, *Sco105*, *Sco106*, *Sco107*, *Sco109*, (Washington Dept. of Fish and Wildlife *unpublished*), *Sco200*, *Sco202*, *Sco212*, *Sco215*, *Sco216*,

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Sco218, *Sco220* (DeHaan and Ardren 2005), *Sfo18* (Angers et al. 1995) and *Smm22* (Crane et al. 2004). Several of these loci have diagnostic differences in allele size between bull trout and brook trout and can be used for species ID and to identify individuals with hybrid ancestry. We conducted PCR reactions in 10 μ L volumes containing 2 μ L of template DNA, 5 μ L of 2X QIAGEN Multiplex PCR Master Mix (final concentration of 3mM MgCl₂), and 0.2 μ L of oligonucleotide PCR primer mix. Primer mix concentrations and annealing temperatures for each multiplex are given in Appendix 1. PCR conditions were as follows: initial denaturation at 95°C for 15 minutes, then 29 cycles of 95°C for 30 seconds, 90 seconds at the multiplex specific annealing temperature and 60 seconds primer extension at 72°C, followed by a final extension at 60°C for 20 minutes. Following PCR, capillary electrophoresis was carried out on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) following the manufacturer's protocols. The G5 filter set was used to produce electropherograms, and electrophoresis data was analyzed using the program Genemapper v4.0 (Applied Biosystems Inc.).

Statistical Analyses

The genotype for each individual was compared to the distribution of alleles from known bull trout and brook trout from other drainages for species ID. All brook trout and hybrids were removed from the dataset for subsequent analysis. Bull trout were grouped into five putative populations according to their capture location: Twin Lakes Creek, Barker Creek, Storm Lake Creek, Warm Springs Creek, and West Fork (WF) Warm Springs Creek. Bull trout captured in these locations were primarily juveniles and sub-adults, so capture location was presumed to be synonymous with their natal tributary stream. Bull trout collected in the upper and lower Twin Lakes were grouped with the individuals from Twin Lakes Creek for analysis. Only two individuals were collected in Foster Creek and these samples were omitted from statistical analysis because they were determined to be hybrids (see below). Collections from the five spawning tributaries were tested for conformance to Hardy-Weinberg equilibrium (HWE) using the program GENEPOP v4.0.7 (Raymond and Rousset 1995). GENEPOP was also used to test each population for evidence of linkage disequilibrium (i.e., nonrandom association between alleles at two loci). Significance values for HWE and linkage disequilibrium tests were adjusted for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989).

We used the program GDA (Lewis and Zaykin 2001) to estimate levels of genetic variation including mean numbers of alleles per locus and observed and expected heterozygosity within each population. In addition we used the program HP-Rare v1.0 (Kalinowski 2005) to estimate allelic richness for each population based on a minimum sample size of 46 genes (two times the minimum sample size). This program provides estimates of allelic richness corrected for differences in sample size between populations. Populations were tested for evidence of recent (within the past few generations) genetic bottlenecks using the program BOTTLENECK (Cornuet and Luikart 1996) assuming a two-phased model of microsatellite mutation. This method tests for an excess of heterozygotes relative to the frequency of alleles in the population (Luikart and Cornuet 1998). Estimates of effective population size (N_e) can provide valuable information for determining the conservation status of small populations such as those in Warm Springs Creek. Because bull trout have overlapping generations and our sampling did not span an entire generation, we were unable to estimate the true N_e ; however, we were able to estimate the effective number of breeders (N_b) for the tributaries we sampled. We estimated contemporary N_b based on linkage disequilibrium (Waples 2006) using the program LDNe (Waples and Do 2008). Alleles with frequencies of less than 0.02 were excluded from this analysis and 95% confidence intervals for N_b estimates were based on a jackknife procedure.

We used the program FSTAT v2.9.3.2 (Goudet 2001) to estimate the overall level of genetic differentiation among spawning populations (F_{ST} ; Weir and Cockerham 1984) and the associated 95% confidence level based on 1,000 bootstrap replicates. We also used FSTAT to determine the level of genetic variation among all population pairs. A Bonferroni correction (Rice 1989) was used to adjust significance values of pairwise F_{ST} estimates for multiple comparisons. Using GENEPOP, we performed a chi-squared contingency analysis to determine if there were significant differences in allele frequencies among the different spawning tributaries. P-values were adjusted for multiple comparisons using a sequential Bonferroni correction (Rice 1989) as well as the B-Y FDR correction described in Narum (2006).

We used two methods to examine the spatial genetic relationship among populations. We first examined the multi-dimensional genetic relationship among populations by performing a factorial correspondence analysis (FCA) using the program GENETIX (Belkhir et al. 2004). This method is similar to a principal component analysis and provides an unbiased graphical approach for viewing the data where individuals that are more genetically similar cluster together on the

graph. Unknown origin bull trout collected in Silver Lake were included in the analysis to provide a preliminary test of population assignment. Second, we generated a consensus neighbor-joining (NJ) tree using the program Phylip v3.6 (Felsenstein 1993). The bootstrap procedure was first used to generate 1,000 replicate datasets based on our observed allele frequencies. We then estimated Cavalli-Sforza and Edwards (1967) chord distances between all population pairs and generated a consensus NJ tree based on these values.

We conducted genetic assignment tests to provide a more accurate means of determining the most likely source population(s) of bull trout captured in Silver Lake. First, in order to assess our ability to correctly assign unknown fish to their population of origin we performed a jackknife analysis of our baseline dataset using the program WHICHRUN v4.1 (Banks and Eichert 2000). With this procedure each individual fish is removed from the baseline dataset and treated as an unknown. The allele frequencies for each population are then recalculated without that individual, and the individual is assigned to its most likely population of origin based on a maximum likelihood algorithm. The number of individuals that are assigned to their true population of origin provides a means of estimating the statistical power of the baseline dataset to accurately assign unknown individuals. Once we had determined the ability of the baseline dataset to assign individuals, we used WHICHRUN to assign adults collected in Silver Lake to their first and second most likely population of origin. Confidence estimates for our assignments represent the likelihood ratio between the first and second most likely populations (i.e. likelihood individual originated from population #1/likelihood individual originated from population #2).

Results

We identified a total of 49 individuals that had two brook trout alleles at all diagnostic loci indicating that these fish were pure brook trout (Table 1; Appendix 2). These individuals were collected in Storm Lake Creek (n = 26), Twin Lakes Creek (n = 11), and Cable Creek (n = 12). All of these fish were identified in the field as brook trout with the exception of one fish collected in Storm Lake Creek that was identified in the field as a bull trout (ID number FWS-014-004). We identified a total of 34 individuals that possessed both bull trout and brook trout alleles providing evidence that hybridization does occur in the Warm Springs Creek drainage (Table 1; Appendix 2). Hybrid individuals were identified in Foster Creek (n = 2), Twin Lakes (n = 14), Silver Lake (n = 5), and Warm Springs Creek (n = 13). These individuals were identified

in the field as both hybrids and bull trout. All of the individuals we identified as hybrids appeared to be first generation hybrids (F1). Hybrids and brook trout were removed from subsequent statistical analyses. There was one individual (ID number FWS-017-022) that had hybrid genotypes at two loci but failed to amplify at several other loci after multiple DNA extractions. Due to the incomplete genotype of this fish, we did not classify it as a hybrid and we omitted it from further analysis.

One of the 16 loci we analyzed, *Sco215*, was fixed for a single allele (i.e. no variation was observed at this locus) in all populations and *Sco212* was fixed for a single allele in WF Warm Springs Creek. All of the baseline populations had at least one locus that deviated from HWE. Barker Creek, Twin Lakes Creek, Storm Lake Creek, and WF Warm Springs Creek all deviated from HWE at *Sco109* due to a heterozygote deficiency. We have observed this same trend in several other populations within the Clark Fork River system (USFWS *unpublished data*). Based on data from this study and other Clark Fork bull trout populations, deviations from HWE at *Sco109* appear to result from either a null allele (an allele which is not detectable due to a primer site mutation or failed amplification) or allelic dropout (an allele which is not detectable due to low copy numbers) at this locus rather than sampling issues. This locus was omitted from subsequent statistical analysis. Twin Lakes Creek also deviated from HWE at *Omm1128* due to an excess of heterozygotes and WF Warm Springs Creek also deviated from HWE at *Sco218* due to an excess of heterozygotes. Warm Springs Creek deviated from HWE at three loci: *Sco216* due to a heterozygote deficiency and *Smm22* and *Sco218* due to a heterozygote excess. The four baseline populations had the following number of locus pairs (out of 105) that showed evidence of linkage (i.e. linkage disequilibrium was observed): Barker Creek - six pairs of loci, Storm Lake Creek - four pairs of loci, Twin Lakes Creek – seven pairs of loci, Warm Springs Creek – seven pairs of loci, and WF Warm Springs Creek – four pairs of loci. The specific pairs of loci showing evidence of linkage varied among populations.

Estimates of genetic variation varied among the five spawning tributaries (Table 2). The mean number of alleles per locus (A), allelic richness (A_R), and expected heterozygosity (H_e) were lowest in WF Warm Springs Creek ($A = 2.933$, $A_R = 2.885$, $H_e = 0.466$) and observed heterozygosity was lowest in Barker Creek ($H_o = 0.507$). Mean number of alleles per locus, allelic richness, and expected heterozygosity were greatest in Twin Lakes Creek ($A = 5.000$, $A_R = 4.411$, $H_e = 0.533$) and observed heterozygosity was greatest in Warm Springs Creek ($H_o =$

0.586). Both Warm Springs Creek and West Fork Warm Springs Creek showed evidence of a recent genetic bottleneck (Wilcoxon test $P = 0.035$ and 0.004 respectively). Estimates of the effective number of breeders (N_b) was low in all populations and ranged from 6.0 in Warm Springs Creek to 10.4 for the combined sample from Twin Lakes Creek and upper and lower Twin Lakes (Table 3).

The overall level of genetic variation among populations (i.e. F_{ST}) was 0.205 and was found to be significantly different from 0.0 (95% C.I. = 0.158-0.257). Pairwise estimates of F_{ST} ranged from 0.128 (Warm Springs Creek and Barker Creek) to 0.249 (Twin Lakes Creek and WF Warm Springs Creek) and all pairwise estimates of differentiation were significantly different from 0.0 (Table 3). Chi-squared contingency tests revealed that there were significant differences in allele frequencies among all populations. The first axis on the FCA plot separated the individuals from the isolated populations in Storm Lake Creek and Twin Lake Creek from the individuals in Barker Creek, Warm Springs Creek, and WF Warm Springs Creek (Figure 7). The second axis on the FCA plot separated the individuals from Storm Lake Creek from those in Twin Lakes Creek and showed some separation between Barker Creek, Warm Springs Creek and WF Warm Springs Creek. Bull trout collected in Silver Lake clustered with the samples from Storm Lake Creek. The NJ tree showed that Warm Springs Creek was most similar to WF Warm Springs Creek, Twin Lakes Creek was most similar to Storm Lake Creek, and Barker Creek was intermediate to those two groupings (Figure 8). All branches on the NJ tree showed greater than 64% bootstrap support.

The proportion of individuals assigned to their population of origin in the jackknife analysis ranged from 0.905 to 1.00 (Table 5). There were three bull trout collected in Twin Lakes Creek that were assigned to Warm Springs Creek (ID numbers 1249-062, 1249-063, and FWS-017-023), and one individual from Twin Lakes Creek assigned to Barker Creek (ID number FWS-016-001). The individuals captured in Twin Lakes Creek that assigned to different tributary streams were captured downstream from the diversion structure (see Figures 1 and 2). One individual from Warm Springs Creek was assigned to Barker Creek (ID number 1249-037). All bull trout captured in Silver Lake were were assigned to Storm Lake Creek with a high likelihood (Table 6).

Discussion

Hybridization between bull trout and brook trout

Hybridization with brook trout (*Salvelinus fontinalis*) has been recognized as a threat to bull trout populations (Leary et al. 1993; Leary et al. 1995; Rieman et al. 1997; Kanda et al. 2002; DeHaan et al. 2010). Hybridization appears to be a threat to bull trout in the Warm Springs Creek drainage; pure brook trout were collected from two of the tributaries that contain spawning populations of bull trout and we identified 34 F1 bull trout x brook trout hybrids. Although we did not observe evidence of introgression, hybridization with brook trout still represents wasted reproductive effort for bull trout in the Warm Springs Creek drainage. Hybridization in the system appears to be influenced by habitat and stream characteristics. Several hybrids were found in Warm Springs Creek, perhaps because beaver ponds in the creek provide habitat suitable for brook trout. Hybridization appears to be less frequent in Barker, Storm Lake, and WF Warm Springs creeks, and genetic analysis did not identify any F1 hybrids in those streams. However, fish phenotypically characterized as bull trout × brook trout hybrids have been captured in the downstream reaches of both Barker and Storm Lake creeks (MFWP 2008).

Although hybrids collected in the tributaries presumably originated in those tributaries (especially in isolated systems like Twin Lakes Creek), determining the origin of hybrids collected in Silver Lake is difficult. Whereas we can genetically assign pure bull trout captured in Silver Lake to a tributary of origin with a high degree of confidence (Tables 5 and 6), we are unable to perform a similar assignment analysis for hybrid *Salvelinus*. Because bull trout captured in Silver Lake appear to be from Storm Lake Creek, hybrids in Silver Lake may have also originated from Storm Lake Creek. However, we did not detect any hybrids in Storm Lake Creek at any of the six sites we sampled in 2008 and 2009. This may be because sampling in Storm Lake creek was not sufficient to detect hybrids. Hybrids were also identified in Twin Lakes Creek, so we cannot completely discount the possibility that some of the hybrids collected from Silver Lake came from Twin Lakes Creek. It may also be that hybridization occurs somewhere within Silver Lake or within a small tributary to Silver Lake.

Our report focuses on the genetic threats to bull trout posed by brook trout. Ecological interactions between species, such as competition and predation by brook trout may also be important in some contexts (e.g., Gunckel et al. 2002; Rich et al. 2003). The general fish community within the Warm Springs Creek system has recently been surveyed (MFWP 2008),

and there is overlap in the distribution of bull trout and brook trout in some waters. However, the ecological threats posed by brook trout, and the likelihood of further invasion, have not been analyzed.

Genetic variation within and among populations

Warm Springs Creek deviated from HWE at three loci, two of which were due to a heterozygote excess. An excess of heterozygotes is often observed when a number of closely related individuals are collected from a tributary or population (i.e. half and full siblings; Balloux 2004). Given that estimates of genetic diversity and N_b were relatively low in Warm Springs Creek, the increased number of related individuals sampled is likely the result of a relatively small spawning population in this tributary.

Genetic diversity within Warm Springs Creek drainage bull trout populations was relatively low compared to other bull trout populations. In a recent analysis of genetic variation in 75 populations from across the species range, we observed that estimates of the mean number of alleles per locus, allelic richness and expected and observed heterozygosity (5.81, 4.55, 0.57, and 0.57, respectively; USFWS *unpublished data*) were all greater than estimates from the Warm Springs Creek drainage. This range-wide study included eight populations from the Clark Fork River system including tributaries to Lake Pend Oreille, the mainstem Clark Fork, the Flathead River, the Blackfoot River and the Bitterroot River. Mean estimates of variation within these eight Clark Fork River populations were also greater than those we observed in the Warm Springs Creek drainage: the mean number of alleles per locus, allelic richness, expected heterozygosity and observed heterozygosity were 7.60, 5.66, 0.686 and 0.690, respectively. Unlike populations in the Warm Springs Creek drainage, these eight Clark Fork River populations maintain some degree of connectivity with other populations in the basin. These data seem to corroborate findings that habitat fragmentation and isolation above barriers can lead to reductions in genetic variation within bull trout populations (Costello et al. 2003; Whiteley et al. 2006; DeHaan et al. 2007). Habitat availability may be another cause for the differences we observed in genetic variation between the Warm Springs Creek drainage and other bull trout populations. Whereas bull trout habitat in Warm Springs Creek has been reduced by fragmentation and other anthropogenic factors, other bull trout populations we have examined have a greater amount of available habitat. Populations with increased genetic diversity are likely

to have increased fitness and a lower risk of extinction (Quattro and Vrijenhoek 1989; Saccheri et al. 1998; Reed and Frankham 2003). Low levels of variation observed in Warm Springs Creek bull trout populations raise concerns for the long-term persistence of these populations.

Estimates of contemporary N_b we observed in Warm Springs Creek tributaries were quite low (Table 3). It is important to point out that for species such as bull trout, with overlapping generations, estimates of N_b are generally lower than the true effective population size, N_e (Luikart et al. 2010). It has been suggested that N_e less than 50 is cause for immediate concern over low genetic diversity (Franklin 1980; Allendorf and Luikart 2007). Rieman and Allendorf (2001) used computer simulations to examine the relationship between N_e and census population size in bull trout. These authors found that for bull trout, N_e ranged between 0.5 and 1.0 times the mean annual number of spawning adults. However, it is important to note, as these authors point out, that demographic and life history characteristics can greatly influence estimates of N_e in bull trout populations (Rieman and Allendorf 2001). The relationship between N_b and N_e in bull trout populations is unknown so it is difficult to say what the true N_e might be for the populations we surveyed in Warm Springs Creek. Regardless, low estimates of N_b along with low estimates of genetic variation suggest that few adults spawn annually in Warm Springs Creek tributaries and raise concerns for the long term persistence of these populations.

Reductions in genetic diversity may have occurred at different times for bull trout in different tributaries. Even though levels of genetic variation and effective population sizes were low in all tributaries, only Warm Springs Creek and WF Warm Springs Creek exhibited evidence of a recent genetic population bottleneck using the heterozygosity excess method. This suggests that that more recent events have affected bull trout in these tributaries, even if the cause of the bottleneck in Warm Springs Creek is unknown. The heterozygosity excess method is reportedly more sensitive to detecting very recent bottlenecks (Spear et al. 2006). We presume that bottlenecks in Storm Lake and Twin Lakes creeks, if they occurred, likely took place at least 50-75 years ago when habitat fragmentation altered those systems.

Bull trout tend to show a high level of genetic differentiation among populations throughout their range (Costello et al. 2003; Spruell et al. 2003). Furthermore, significant levels of differentiation have been observed among populations in relatively close geographic proximity (Spruell et al. 1999; Whiteley et al. 2006). Even so, the level of differentiation we observed among bull trout from different tributaries in the Warm Springs Creek drainage ($F_{ST} =$

0.205) was relatively high given the small spatial scale of this project. For example, in a study of bull trout populations distributed over hundreds of river kilometers in the Lake Pend Oreille and Clark Fork River system, DeHaan and Hawkins (2009) found that the overall estimate of F_{ST} was 0.132. DeHaan et al. (2007) observed a similar level of genetic variation ($F_{ST} = 0.136$) among four local populations of bull trout in the lower Flathead River, another Clark Fork tributary. The comparatively high level of differentiation observed among bull trout from different tributaries in the Warm Springs Creek drainage is likely due to a combination of limited gene flow among isolated populations and genetic drift within these small populations. Habitat fragmentation within Warm Springs Creek has limited gene flow among local spawning populations for nearly 100 years. The NJ tree and the FCA plot both showed that the two isolated populations, Storm Lake Creek and Twin Lakes Creek, were more highly differentiated than the other populations that maintained connectivity. Furthermore, the level of differentiation between the two isolated populations was nearly twice as great as the level of differentiation between creeks with connectivity (Table 4).

Patterns of movement and entrainment

Although water is diverted from both Storm Lake Creek and Twin Lakes Creek into Silver Lake, our data suggest that diversions primarily entrain bull trout from Storm Lake Creek; as all 15 bull trout genotyped from Silver Lake were assigned to Storm Lake Creek (Table 6). Because water diversions prevent entrained bull trout in Silver Lake from returning to Storm Lake Creek, bull trout in Silver Lake likely make little or no demographic or genetic contribution to the spawning population in Storm Lake Creek. Low levels of variation observed in Storm Lake Creek provide further support for this. Alternatively, it is possible (though less likely) that entrainment in Silver Lake does not represent a complete reproductive loss for bull trout. It is possible that bull trout: (a) occasionally ascend Storm Lake Creek during high flows, (b) spawn in stream inlets or outlets in Silver Lake, (c) are entrained a second time – either from Silver Lake or a diversion bypass from Storm Lake Creek – and sent into Warm Springs Creek where they stray into other tributaries to spawn, or (d) are entrained or migrate into Georgetown Lake (in the headwaters of Flint Creek) when the irrigation ditch between Silver Lake and Georgetown Lake is flowing. Additional genetic and distributional surveys of bull trout in the system may help address these uncertainties.

Maintaining multiple life history types within a population has been recognized as an important factor for bull trout persistence (Rieman and Dunham 2000). Large migratory fish have the potential to contribute significant numbers of offspring to a population, and migratory fish that disperse throughout the watershed may be protected against localized stochastic events (e.g. fires, floods, landslides, etc.; Rieman and Clayton 1997). Jackknife analysis provided evidence that fish still utilize migratory corridors within the Warm Springs Creek drainage when they are accessible. Individuals assigned to tributaries they were not collected from were all collected in areas that had connectivity to other spawning areas; four bull trout collected in Twin Lakes Creek below the water diversion structure were genetically assigned to either Warm Springs Creek or Barker Creek and one individual collected in Warm Springs Creek was assigned to Barker Creek. These data suggest that these individuals either migrated from other tributaries or had migratory parents. Bull trout in the Warm Springs Creek drainage would likely benefit from management activities aimed at maintaining existing connectivity and re-establishing connectivity among isolated populations and the mainstem of Warm Springs Creek provided that such actions did not increase the risk of hybridization with brook trout. Low N_b observed in the five tributaries surveyed indicates that management actions to increase genetic diversity and population size, such as passing migratory fish or facilitating dispersal among tributaries, may be warranted. Such actions could be implemented within a research or adaptive management framework to determine how best to conserve fragmented and partially isolated bull trout populations.

Conclusions

Bull trout habitat in Warm Springs Creek has been fragmented by a series of water diversions for nearly 100 years. Isolation of bull trout populations above barriers has apparently contributed to reduced levels of genetic variation for bull trout within different tributaries of Warm Springs Creek (relative to the Clark Fork River and elsewhere in the species range), low effective population sizes, and restricted gene flow among populations. Water diversions within the system have apparently led to the entrainment and loss of important migratory bull trout from Storm Lake Creek. Bull trout populations within the system would likely benefit from the re-establishment of migratory corridors as well as measures aimed at reducing the threats posed by non-native brook trout in the system.

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Table 1. Summary of genetic species ID for all *Salvelinus sp.* collected from the Warm Springs Creek drainage. The Twin Lakes collection includes fish from Twin Lakes Creek and the two Twin Lakes. All hybrids were determined to be first generation (F1).

Collection Location	Bull Trout	Brook Trout	Hybrids
Barker Creek	33	0	0
Cable Creek	0	12	0
Foster Creek	0	0	2
Silver Lake	15	0	5
Storm Lake Creek	39	26	0
Twin Lakes	42	11	14
Warm Springs Creek	25	0	13
WF Warm Springs Creek	37	0	0

Table 2. Estimates of genetic variation (based on 15 variable microsatellite loci) within five bull trout populations in Warm Springs Creek, MT. The Twin Lakes sample includes bull trout collected in Twin Lakes Creek as well as upper and lower Twin Lakes.

Population	n	A	A _R	H _e	H _o
Barker Creek	33	3.667	3.550	0.502	0.507
Storm Lake Creek	39	3.867	3.655	0.502	0.518
Twin Lakes Creek	42	5.000	4.411	0.533	0.519
Warm Springs Creek	25	3.400	3.367	0.529	0.586
WF Warm Springs Creek	37	2.933	2.885	0.466	0.510
MEAN		3.773	3.574	0.507	0.528

n = Sample size

A = Mean number alleles per locus

A_R = Allelic richness

H_e = Expected heterozygosity

H_o = Observed heterozygosity

Levels of variation observed in a range wide survey of 75 bull trout populations were: A=5.81, A_R=4.55, H_e=0.57, H_o=0.57

Levels of variation observed in eight other Clark Fork River system bull trout populations were: A=7.60, A_R=5.66, H_e=0.69, H_o=0.69

Table 3. Estimates of contemporary effective number of breeders (N_b) calculated based on linkage disequilibrium (Waples 2006) for five bull trout populations in Warm Springs Creek, MT.

Population	N_b	95% C.I.
Barker Creek	6.7	3.6-10.5
Storm Lake Creek	7.9	3.9-12.3
Twin Lakes Creek	10.4	6.7-15.2
Warm Springs Creek	6.0	3.2-9.1
WF Warm Springs Creek	6.7	3.4-11.8

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Table 4. Pairwise estimates of genetic variation (F_{ST}) among five bull trout populations in Warm Springs Creek, MT. All pairwise comparisons were found to be significantly different from 0.0 ($P < 0.05$).

	Barker Creek	Storm Lake Creek	Twin Lakes Creek	Warm Springs Creek	WF Warm Springs Creek
Barker Creek	---				
Storm Lake Creek	0.228	---			
Twin Lakes Creek	0.238	0.193	---		
Warm Springs Creek	0.128	0.184	0.234	---	
WF Warm Springs Creek	0.190	0.194	0.249	0.144	---

Table 5. Proportion of individuals assigned to each population during jackknife analysis using WHICHRUN. Values in bold represent the proportion of individuals correctly assigned to each population.

Collection Location	Assigned To:				
	Barker Creek	Storm Lake Creek	Twin Lakes Creek	Warm Springs Creek	WF Warm Springs Creek
Barker Creek	1.000	0.000	0.000	0.000	0.000
Storm Lake Creek	0.000	1.000	0.000	0.000	0.000
Twin Lakes Creek	0.024	0.000	0.905	0.071	0.000
Warm Springs Creek	0.040	0.000	0.000	0.960	0.000
WF Warm Springs Creek	0.000	0.000	0.000	0.000	1.000

Table 6. Genetic population assignments for unknown origin bull trout collected in Silver Lake at the headwaters of Warm Springs Creek.

ID	Collection Location	Most Likely Population #1	Likelihood Population #1	Most Likely Population #2	Likelihood Population #2	Likelihood Ratio
1259-072	Silver Lake	Storm Lake Creek	4.71×10^{-14}	Twin Lakes Creek	1.93×10^{-18}	2.40×10^{04}
1259-073	Silver Lake	Storm Lake Creek	1.72×10^{-12}	Twin Lakes Creek	2.11×10^{-21}	8.17×10^{08}
1259-074	Silver Lake	Storm Lake Creek	1.37×10^{-11}	Twin Lakes Creek	3.00×10^{-22}	4.58×10^{10}
1259-075	Silver Lake	Storm Lake Creek	7.32×10^{-12}	Twin Lakes Creek	9.53×10^{-22}	7.68×10^{09}
1259-078	Silver Lake	Storm Lake Creek	1.20×10^{-14}	Twin Lakes Creek	1.37×10^{-21}	9.00×10^{06}
1259-079	Silver Lake	Storm Lake Creek	1.51×10^{-12}	Twin Lakes Creek	8.08×10^{-16}	1.87×10^{03}
1259-080	Silver Lake	Storm Lake Creek	1.59×10^{-12}	Twin Lakes Creek	1.66×10^{-22}	9.59×10^{09}
1259-081	Silver Lake	Storm Lake Creek	1.06×10^{-15}	Twin Lakes Creek	2.56×10^{-18}	414.7
1259-082	Silver Lake	Storm Lake Creek	1.28×10^{-13}	WF Warm Springs Creek	4.05×10^{-17}	3.16×10^{03}
1259-084	Silver Lake	Storm Lake Creek	4.20×10^{-13}	Twin Lakes Creek	4.60×10^{-21}	9.13×10^{07}
1259-085	Silver Lake	Storm Lake Creek	1.79×10^{-14}	Twin Lakes Creek	1.43×10^{-19}	1.00×10^{05}
1259-086	Silver Lake	Storm Lake Creek	1.50×10^{-12}	Twin Lakes Creek	3.61×10^{-15}	416.5
1259-088	Silver Lake	Storm Lake Creek	9.30×10^{-14}	Twin Lakes Creek	1.93×10^{-24}	4.82×10^{10}
1259-089	Silver Lake	Storm Lake Creek	3.43×10^{-15}	Twin Lakes Creek	1.24×10^{-21}	3.00×10^{06}
2001-068	Silver Lake	Storm Lake Creek	2.97×10^{-12}	Warm Springs Creek	4.44×10^{-18}	7.00×10^{05}

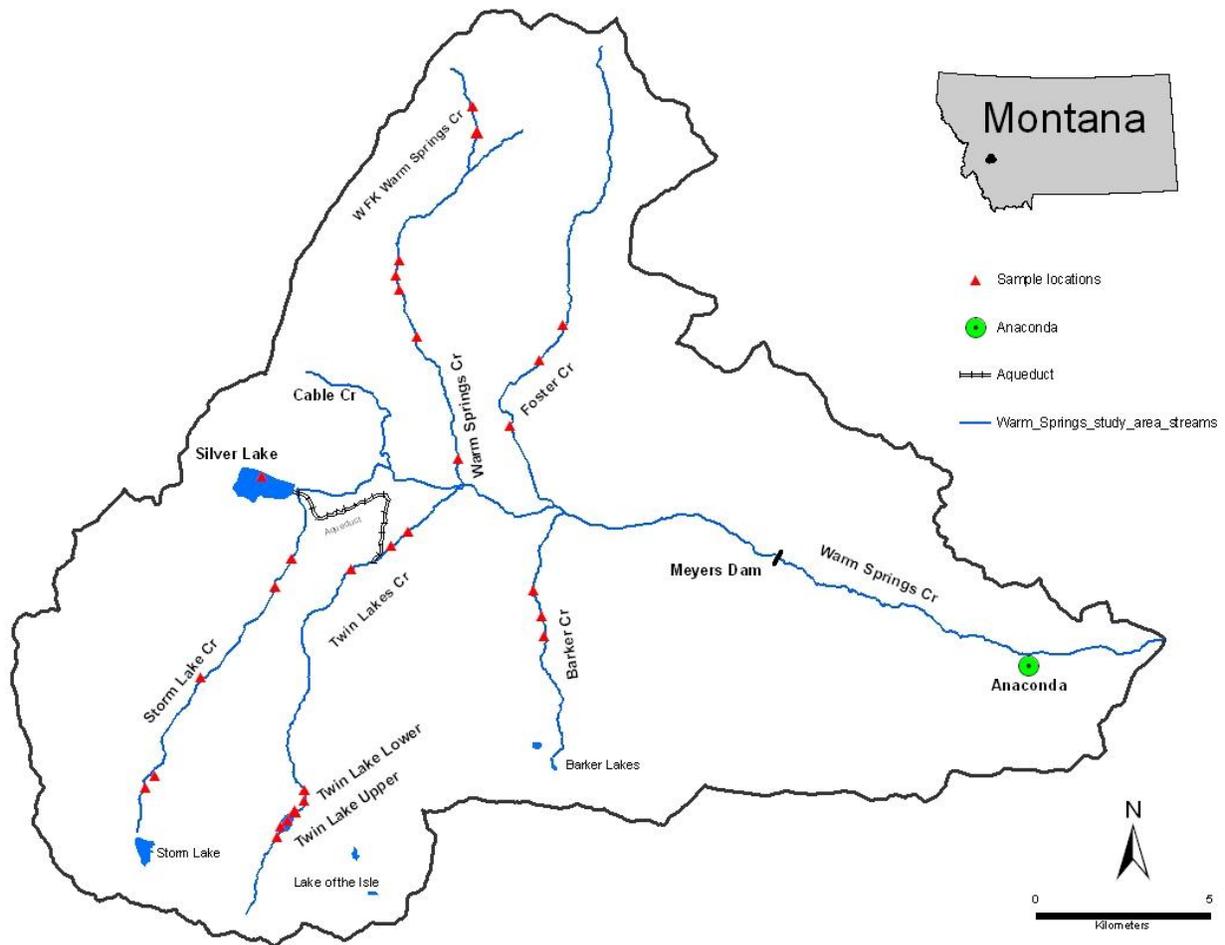


Figure 1. Warm Springs Creek drainage in western Montana, USA. Samples for this study were collected in Storm Lake Creek, Twin Lakes Creek (and lakes), Barker Creek, Foster Creek, Warm Springs Creek, and Silver Lake. Myers Dam is a barrier to upstream passage by fishes. Diversion structures on Storm Lake Creek and Twin Lakes Creek (upstream from the aqueduct) effectively isolate these habitats from the mainstem Warm Springs Creek.



Figure 2. Diversion structure on Twin Lakes Creek. Vertical boards at left are the entrance to a wooden aqueduct that diverts water approximately 4.5 miles (7.2 km) into Silver Lake.



Figure 3. A portion of the wooden aqueduct that transports water diverted from Twin Lakes Creek into Silver Lake.



Figure 4. Water control and bypass structure at the downstream terminus of Storm Lake Creek, where it enters Silver Lake Creek. Water flowing over the slot (at left) flows into short stream channel which empties into Silver Lake. When open, the headgate (at top) diverts water into a bypass pipe and channel which enters Warm Springs Creek (i.e., without first being diverted into Silver Lake).



Figure 5. Pumping station (at left) in Silver Lake that can transport water into Warm Springs Creek. Water leaving Silver Lake exits through a large pipe (at right) before entering a channel with a natural streambed.



Figure 6. Instream spillway structure at Myers Dam on Warm Springs Creek, just west of Anaconda, MT. The spillway structure is believed to be a barrier to upstream movement by fishes.

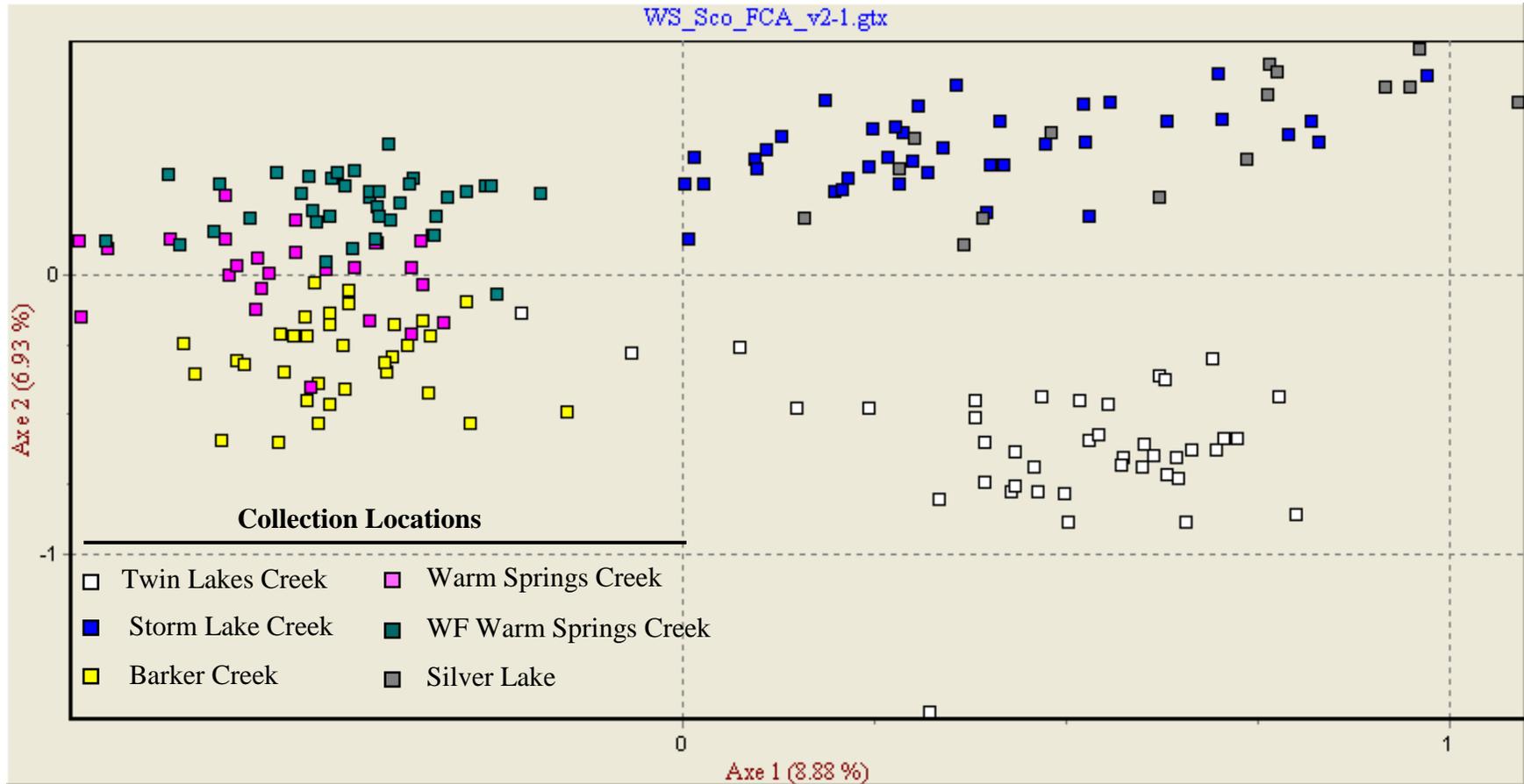


Figure 7. Factorial correspondence analysis (FCA) of bull trout collected in Warm Springs Creek, MT. Each point on the graph represents an individual bull trout in the analysis. Points that cluster closer together are more genetically similar.

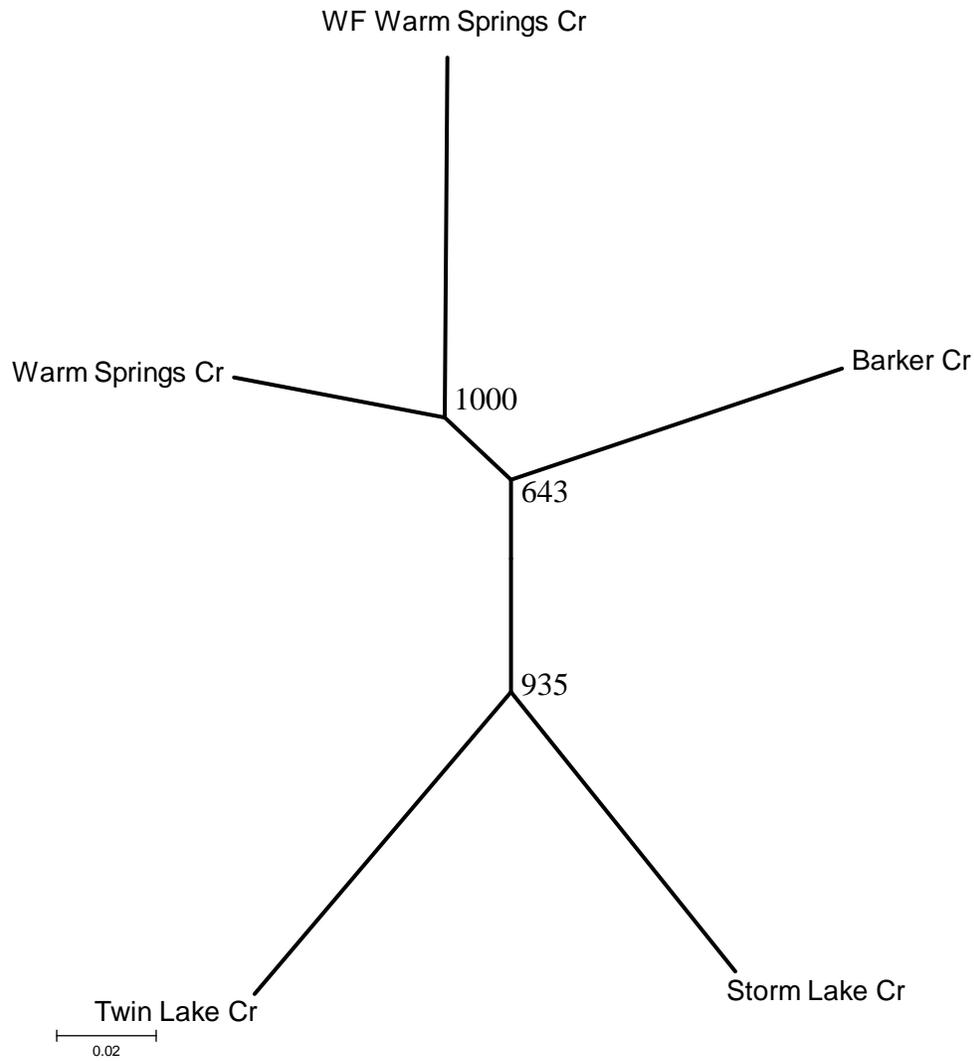


Figure 8. Neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance. Values at the nodes represent the number of bootstrap replicates (out of 1000) that showed the displayed topology.

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Appendix 1. Bull trout PCR multiplex primer concentrations and annealing temperatures.

Multiplex Set 1 $T_A = 54^\circ\text{C}$

Locus Name	Dye	Final Concentration
Sfo18	6FAM	0.3 μM
Sco212	VIC	1.0 μM
Sco220	NED	3.3 μM
Sco216	PET	4.0 μM
Sco109	6FAM	6.6 μM

Multiplex Set 2 $T_A = 59^\circ\text{C}$

Locus Name	Dye	Final Concentration
Sco202	6FAM	0.6 μM
Sco102	PET	1.0 μM
Sco215	PET	1.3 μM
Sco200	VIC	2.0 μM
Omm1128	VIC	2.0 μM
Sco105	NED	1.3 μM
Smm22	6FAM	4.6 μM

Multiplex Set 3 $T_A = 56^\circ\text{C}$

Locus Name	Dye	Final Concentration
Sco106	6FAM	1.0 μM
Sco107	VIC	2.6 μM
Omm1130	NED	5.3 μM
Sco218	PET	3.3 μM

$T_A =$ Annealing temperature

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Appendix 2. Collection location, morphological field identification, and genetic identification of individual *Salvelinus* collected in the Warm Springs Creek genetically determined to be brook trout and bull trout × brook trout hybrids. All hybrid individuals were determined to be first generation (F1).

Sample ID	Collection Location	Field ID	Genetic ID
FWS-019-001	Cable Creek	Brook Trout	Brook Trout
FWS-019-002	Cable Creek	Brook Trout	Brook Trout
FWS-019-003	Cable Creek	Brook Trout	Brook Trout
FWS-019-004	Cable Creek	Brook Trout	Brook Trout
FWS-019-005	Cable Creek	Brook Trout	Brook Trout
FWS-019-006	Cable Creek	Brook Trout	Brook Trout
FWS-019-007	Cable Creek	Brook Trout	Brook Trout
FWS-019-008	Cable Creek	Brook Trout	Brook Trout
FWS-019-009	Cable Creek	Brook Trout	Brook Trout
FWS-019-010	Cable Creek	Brook Trout	Brook Trout
FWS-019-011	Cable Creek	Brook Trout	Brook Trout
FWS-019-012	Cable Creek	Brook Trout	Brook Trout
FWS-014-001	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-002	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-003	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-006	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-007	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-009	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-010	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-011	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-012	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-013	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-014	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-015	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-016	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-017	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-018	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-019	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-020	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-021	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-022	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-023	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-024	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-025	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-027	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-029	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-030	Storm Lake Creek	Brook Trout	Brook Trout
FWS-014-004	Storm Lake Creek	Bull Trout	Brook Trout
FWS-017-004	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-005	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-006	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-008	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-009	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-013	Twin Lakes Creek	Brook Trout	Brook Trout

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Sample ID	Collection Location	Field ID	Genetic ID
FWS-017-014	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-015	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-016	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-018	Twin Lakes Creek	Brook Trout	Brook Trout
FWS-017-019	Twin Lakes Creek	Brook Trout	Brook Trout
1259-065	Foster Creek	Hybrid	Hybrid
1259-066	Foster Creek	Hybrid	Hybrid
1259-071	Silver Lake	Bull Trout	Hybrid
1259-076	Silver Lake	Bull Trout	Hybrid
1259-077	Silver Lake	Bull Trout	Hybrid
1259-083	Silver Lake	Hybrid	Hybrid
1259-087	Silver Lake	Hybrid	Hybrid
113-004	Twin Lakes	Bull Trout	Hybrid
113-055	Twin Lakes	Bull Trout	Hybrid
113-056	Twin Lakes	Bull Trout	Hybrid
113-057	Twin Lakes	Bull Trout	Hybrid
113-058	Twin Lakes	Bull Trout	Hybrid
113-061	Twin Lakes	Bull Trout	Hybrid
FWS-017-021	Twin Lakes Creek	Bull Trout	Hybrid
113-060	Twin Lakes	Hybrid	Hybrid
FWS-017-002	Twin Lakes Creek	Hybrid	Hybrid
FWS-017-007	Twin Lakes Creek	Hybrid	Hybrid
FWS-017-010	Twin Lakes Creek	Hybrid	Hybrid
FWS-017-011	Twin Lakes Creek	Hybrid	Hybrid
FWS-017-017	Twin Lakes Creek	Hybrid	Hybrid
FWS-017-020	Twin Lakes Creek	Hybrid	Hybrid
1249-004	Warm Springs Creek	Bull Trout	Hybrid
1249-008	Warm Springs Creek	Bull Trout	Hybrid
1249-009	Warm Springs Creek	Bull Trout	Hybrid
1249-015	Warm Springs Creek	Bull Trout	Hybrid
1249-026	Warm Springs Creek	Bull Trout	Hybrid
1249-028	Warm Springs Creek	Bull Trout	Hybrid
1249-001	Warm Springs Creek	Hybrid	Hybrid
1249-002	Warm Springs Creek	Hybrid	Hybrid
1249-003	Warm Springs Creek	Hybrid	Hybrid
1249-005	Warm Springs Creek	Hybrid	Hybrid
1249-006	Warm Springs Creek	Hybrid	Hybrid
1249-014	Warm Springs Creek	Hybrid	Hybrid
1249-017	Warm Springs Creek	Hybrid	Hybrid

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Appendix 3. Allele frequencies at 16 microsatellite loci for the bull trout collections analyzed in this study. Hybrid individuals and brook trout have been removed. Values in parentheses represent the sample size from each tributary.

Locus	Allele Size	Barker Cr (35)	Storm Lake Cr (39)	Twin Lakes (42)	Warm Springs Cr (25)	WF Warm Springs Cr (37)
Omm1128	273	0.152	0.000	0.012	0.000	0.000
	277	0.000	0.321	0.143	0.200	0.216
	302	0.030	0.000	0.000	0.000	0.000
	306	0.394	0.026	0.345	0.360	0.500
	319	0.136	0.000	0.036	0.000	0.000
	327	0.000	0.000	0.012	0.000	0.000
	335	0.288	0.128	0.095	0.440	0.284
	339	0.000	0.526	0.345	0.000	0.000
	347	0.000	0.000	0.012	0.000	0.000
Omm1130	197	0.000	0.000	0.012	0.000	0.000
	221	0.000	0.000	0.012	0.000	0.000
	302	0.106	0.000	0.024	0.000	0.000
	306	0.076	0.038	0.012	0.478	0.135
	312	0.000	0.179	0.049	0.000	0.000
	316	0.333	0.372	0.256	0.152	0.216
	320	0.000	0.295	0.000	0.000	0.000
	324	0.000	0.000	0.000	0.000	0.419
	328	0.000	0.000	0.122	0.000	0.000
	332	0.076	0.026	0.476	0.196	0.189
	336	0.409	0.090	0.037	0.152	0.041
	340	0.000	0.000	0.000	0.022	0.000
	Sco102	166	0.273	0.064	0.738	0.260
169		0.727	0.936	0.262	0.740	0.959
Sco105	170	0.000	0.064	0.000	0.000	0.000
	178	0.576	0.103	0.585	0.240	0.324
	182	0.000	0.218	0.341	0.000	0.000
	186	0.152	0.615	0.037	0.620	0.541
	190	0.000	0.000	0.024	0.140	0.135
	194	0.273	0.000	0.012	0.000	0.000
Sco106	196	0.242	0.538	0.024	0.220	0.000
	200	0.000	0.000	0.000	0.100	0.000
	212	0.061	0.321	0.024	0.120	0.392
	216	0.348	0.000	0.298	0.120	0.135
	220	0.182	0.000	0.024	0.020	0.000
	224	0.061	0.000	0.036	0.000	0.000
	228	0.106	0.141	0.595	0.420	0.473

Genetic Analysis of Bull Trout in Warm Springs Creek, MT

Appendix 3 continued

Locus	Allele Size	Barker Cr (35)	Storm Lake Cr (39)	Twin Lakes (42)	Warm Springs Cr (25)	WF Warm Springs Cr (37)
Sco107	269	0.303	0.141	0.061	0.380	0.000
	273	0.227	0.385	0.427	0.500	0.446
	277	0.394	0.269	0.439	0.020	0.554
	281	0.000	0.192	0.012	0.000	0.000
	297	0.076	0.000	0.000	0.100	0.000
	301	0.000	0.013	0.061	0.000	0.000
Sco109	250	0.033	0.000	0.025	0.040	0.000
	258	0.000	0.152	0.013	0.000	0.000
	262	0.000	0.000	0.000	0.060	0.000
	266	0.400	0.318	0.338	0.460	0.672
	270	0.000	0.015	0.550	0.200	0.250
	274	0.000	0.000	0.063	0.000	0.000
	296	0.017	0.000	0.000	0.000	0.000
	312	0.150	0.015	0.000	0.000	0.000
	320	0.000	0.106	0.000	0.000	0.000
	326	0.000	0.364	0.000	0.000	0.000
	350	0.000	0.030	0.000	0.000	0.000
	408	0.283	0.000	0.000	0.120	0.000
	412	0.117	0.000	0.013	0.120	0.078
Sco200	126	0.091	0.051	0.202	0.200	0.000
	130	0.000	0.295	0.679	0.100	0.068
	134	0.076	0.333	0.012	0.020	0.446
	167	0.833	0.321	0.107	0.680	0.486
Sco202	130	0.500	0.423	0.595	0.380	0.608
	134	0.500	0.577	0.405	0.620	0.392
Sco212	241	0.000	0.000	0.155	0.000	0.000
	249	0.167	0.064	0.143	0.040	0.000
	269	0.045	0.000	0.024	0.240	0.000
	295	0.788	0.936	0.679	0.720	1.000
Sco215	289	1.000	1.000	1.000	1.000	1.000
Sco216	225	0.076	0.000	0.244	0.040	0.000
	237	0.561	0.590	0.463	0.000	0.000
	241	0.000	0.064	0.000	0.000	0.000
	245	0.152	0.064	0.012	0.260	0.892
	257	0.212	0.282	0.280	0.700	0.108

Genetic Analysis of Bull Trout in Warm Springs Creek, MT

Appendix 3 continued

Locus	Allele Size	Barker Cr (35)	Storm Lake Cr (39)	Twin Lakes (42)	Warm Springs Cr (25)	WF Warm Springs Cr (37)
Sco218	221	0.000	0.000	0.024	0.000	0.108
	225	0.000	0.000	0.083	0.000	0.000
	233	0.015	0.141	0.000	0.000	0.000
	237	0.000	0.026	0.000	0.000	0.000
	245	0.515	0.013	0.048	0.280	0.270
	249	0.242	0.269	0.619	0.200	0.392
	253	0.136	0.423	0.190	0.200	0.203
	257	0.000	0.013	0.000	0.000	0.000
	269	0.000	0.013	0.000	0.000	0.000
	273	0.076	0.103	0.036	0.000	0.000
	277	0.015	0.000	0.000	0.000	0.000
	281	0.000	0.000	0.000	0.320	0.027
	Sco220	310	0.273	0.064	0.345	0.000
318		0.000	0.013	0.024	0.000	0.041
322		0.076	0.603	0.202	0.460	0.419
326		0.000	0.000	0.012	0.000	0.108
342		0.015	0.000	0.000	0.000	0.000
347		0.636	0.154	0.119	0.520	0.432
351		0.000	0.167	0.298	0.020	0.000
Sfo18	145	0.167	0.872	0.905	0.320	0.514
	151	0.833	0.128	0.095	0.680	0.486
Smm22	198	0.015	0.000	0.000	0.020	0.000
	202	0.000	0.013	0.000	0.180	0.041
	206	0.773	0.359	0.190	0.500	0.365
	210	0.015	0.038	0.012	0.000	0.000
	218	0.000	0.000	0.024	0.000	0.000
	230	0.000	0.000	0.012	0.000	0.000
	234	0.000	0.141	0.048	0.000	0.000
	238	0.000	0.000	0.298	0.020	0.000
	242	0.167	0.000	0.060	0.140	0.014
	246	0.030	0.000	0.071	0.000	0.095
	250	0.000	0.103	0.012	0.140	0.149
	254	0.000	0.346	0.250	0.000	0.000
	258	0.000	0.000	0.024	0.000	0.338