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

Draft Report Submitted
April 13, 2009

Final Report Submitted
June 2, 2009

Patrick DeHaan and Lindsay Godfrey
U.S. Fish and Wildlife Service
Abernathy Fish Technology Center
Conservation Genetics Program
1440 Abernathy Creek Road
Longview, WA 98632
(360) 425-6072
patrick_dehaan@fws.gov, lindsay_godfrey@fws.gov

In Collaboration With:
Doug Peterson and Dan Brewer
U.S. Fish and Wildlife Service
Montana Ecological Services Field Office
585 Shepard Way
Helena, MT 59601
(406) 449-5225
doug_peterson@fws.gov, dan_brewer@fws.gov

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 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. *in press*). The threat of hybridization and introgression may be greater in areas where habitat has become degraded and fragmented (DeHaan et al. *in press*). Additionally brook trout may display a competitive advantage over bull trout in areas where they are sympatric (Gunckel et al. 2002; McMahon 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 two specific objectives:

1) 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.

2) 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 summer of 2008. Juvenile bull trout were collected by backpack electrofishing in five spawning tributaries in the Warm Springs Creek drainage: Foster Creek (n=2), Twin Lakes Creek (n=26), Barker Creek (n=33), Storm Lake Creek (n=35) and Warm Springs Creek (n=38) (Figure 1). Additionally sub-adult and adult bull trout were collected in gill nets set in upper and lower Twin Lakes (n=11) and Silver Lake (n=19). Small tissue samples were taken from all bull trout captured and stored in 100% non-denatured ethanol.

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, 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 to identify individuals with hybrid ancestry. We conducted PCR reactions in 10µL volumes containing 2µL of template DNA, 5µL of 2X QIAGEN Multiplex PCR Master Mix (final concentration of 3mM $MgCl_2$), and 0.2µL 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

For statistical analysis, bull trout were grouped into four putative populations according to their capture location: Twin Lakes Creek, Barker Creek, Storm Lake Creek and 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 four spawning tributaries were tested for conformance to Hardy-Weinberg equilibrium (HWE) using the program GENEPOP v4.0 (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). We estimated contemporary

effective population size (N_e) 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_e 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. 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. The FCA analysis also provided a preliminary test of population assignment for bull trout captured in Silver Lake (see below). 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 27 individuals that possessed both bull trout and brook trout alleles providing evidence that hybridization does occur in the Warm Springs Creek drainage (Table 1). These individuals were identified in the field as both hybrids and bull trout. Hybrid individuals were identified in Foster Creek (n = 2), Twin Lakes (n = 7), Silver Lake (n = 5) and Warm Springs Creek (n = 13). All of the individuals we identified as hybrids appeared to be first generation hybrids (F1). Hybrids were removed from subsequent statistical analyses.

One of the 16 loci we analyzed, *Sco215*, was fixed for a single allele (i.e. no variation was observed at this locus). All four of the baseline populations had at least one locus that deviated from HWE. Barker Creek and Storm Lake Creek both deviated from HWE at *Sco109* due to a heterozygote deficiency and Twin Lakes Creek Deviated from HWE at *Omm1128* 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 Creek - four pairs of loci, Twin Lakes Creek – six pairs of loci and Warm Springs Creek – nine pairs of loci. The specific pairs of loci showing evidence of linkage varied among populations.

Estimates of genetic variation were similar among the four spawning tributaries (Table 2). The mean number of alleles per locus (A) and allelic richness (A_R) were lowest in Warm Springs Creek (A = 3.563, A_R = 3.532) and highest in Twin Lakes Creek (A = 4.500, A_R = 4.279). Observed (H_o) and expected heterozygosity (H_e) were lowest in Storm Lake Creek (H_o = 0.505, H_e = 0.496) and highest in Warm Springs Creek (H_o = 0.541, H_o = 0.592). Warm Springs Creek was the only tributary sampled that showed evidence of a recent genetic bottleneck (Wilcoxon test P = 0.030). Effective population sizes (N_e) were low in all populations and ranged

from 5.2 in Warm Springs Creek to 9.8 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 0.206 and was found to be significantly different from 0.0 (95% C.I. = 0.160-0.259). Pairwise estimates of F_{ST} ranged from 0.121 (Warm Springs Creek and Barker Creek) to 0.245 (Twin Lakes Creek and Barker 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 and 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. Pure 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 Barker Creek and Twin Lakes Creek was most similar to Storm Lake Creek (Figure 8). Branches on the NJ tree showed greater than 98% bootstrap support.

The proportion of individuals assigned to their population of origin in the jackknife analysis ranged from 0.93 to 1.00 (Table 5). There were two bull trout collected in Twin Lakes Creek (downstream from the Silver Bow County water diversion) that were assigned to Warm Springs Creek (ID numbers 1249-062 and 1249-063,) and one individual from Warm Springs Creek that was assigned to Barker Creek (ID number 1249-037). Bull trout captured in Silver Lake were all 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 many bull trout populations (Leary et al. 1993; Leary et al. 1995; Rieman et al. 1997; Kanda et al. 2002). Hybridization also appears to be a threat to bull trout in the Warm Springs Creek drainage, as we identified 27 F1 hybrids in the sample of 164 *Salvelinus* spp. we analyzed. 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. The

greatest number of hybrids were found in Warm Springs Creek (13 of 27 observed hybrids), perhaps because beaver ponds in the creek provide habitat suitable for brook trout. Hybridization appears to be less frequent in Barker and Storm Lake 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). Additional genetic analysis of *Salvelinus* in both streams could help confirm occurrence of hybrids, and characterize the associated threat to bull trout.

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, hybrids were genotyped in Twin Lakes Creek, so we cannot completely discount the possibility that some hybrids in Silver Lake came from that stream as well. A more thorough analysis of the distribution of brook trout and hybrids in the Warm Springs Creek drainage would be necessary to confirm the origin of hybrid *Salvelinus* in 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

Bull trout from both Barker Creek and Storm Lake Creek deviated from HWE at the locus *Sco109* due to a deficiency of heterozygotes, a trend we have observed 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. 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 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_e were all lowest 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). The spatial scale over which populations were sampled 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.

Estimates of contemporary N_e we observed in Warm Springs Creek tributaries were quite low (Table 3). It has been suggested that an N_e less than 50 is cause for immediate concern over low genetic diversity (Franklin 1980; Allendorf and Luikart 2007). Following this criteria,

populations of bull trout in the Warm Springs Creek drainage appear to be at an immediate risk of inbreeding depression. 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). Regardless, low estimates of N_e 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 the Warm Springs Creek sample 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 Warm Springs Creek, 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} = 0.206) 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 two populations that maintained connectivity (Barker and Warm Springs creeks). Furthermore, the level of differentiation between the two isolated populations was nearly twice as great as the level of differentiation between Barker and Warm Springs Creeks (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 13 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; two bull trout collected in Twin Lakes Creek below the water diversion structure were genetically assigned to Warm Springs Creek and one individual collected in

Warm Springs Creek was assigned to Barker Creek. The combination of these data suggest that bull trout in the Warm Springs Creek drainage would 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 effective population sizes (N_e<10) observed in the four 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 tributaires 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.

Acknowledgements

Funding for this project was provided by Northwestern Energy through an agreement with the US Fish and Wildlife Service (Reimbursable agreement 1946-6101) .We would like to thank the biologists with the U.S. Fish and Wildlife Service, U. S. Forest Service, and Montana Fish, Wildlife, and Parks for assistance with sample collections. Patty Crandell and Denise Hawkins provided helpful comments on earlier versions of this manuscript. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

References

Allendorf, F.W. and G. Luikart. 2007. Conservation and the Genetics of Populations. Blackwell Publishing, Oxford U.K.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook char reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology 47(supplement A):177-185.

Balloux, F. 2004. Heterozygote excess in small populations and the heterozygote-excess effective population size. Evolution 58:1891-1900.

Banks MA and Eichert W. 2000. WHICHRUN (version 3.2) a computer program for population assignment of individuals based on multilocus genotype data. Journal of Heredity, 91:87-89.

Cavalli-Sforza, L.L. and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550-570.

Cornuet, J.M. and G. Luikart. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Costello, A.B., T.E. Down, S.M. Pollard, C.J. Pacas, and E.B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). Evolution 57(2):328-344.

Crane, P.A., C.J. Lewis, E.J. Kretschmer, S.J. Miller, W.J. Spearman, A.L. DeCicco, M.J. Lisac and J.K. Wenberg. 2004. Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus*. Conservation Genetics 5:737-741.

DeHaan, P.W., and W.R. Ardren. 2005. Characterization of 20 highly variable tetranucleotide microsatellite loci for bull trout (*Salvelinus confluentus*) and cross-amplification in other *Salvelinus* species. Molecular Ecology Notes 5:582–585.

DeHaan, P.W. and D.K. Hawkins. 2009. Rapid response genetic identification of geographic origin of bull trout captured at Clark Fork River dams. Annual Report for Calendar Year 2008 submitted to Avista Corporation. March 16, 2009. 41 pages.

DeHaan, P.W., C.A. Barfoot and W.R. Ardren. 2007. Genetic analysis of bull trout populations on the Flathead Indian Reservation, Montana. Pages 145-153 *in* Carline, R.F. and LoSapio C., *eds*. Sustaining Wild Trout in a Changing World; Proceedings of Wild Trout Symposium IX. October 9-12, 2007. West Yellowstone, Montana. 308 Pages.

DeHaan, P.W., L.T. Schwabe, and W.R. Ardren. *In press*. Spatial patterns of hybridization between bull trout, *Salvelinus confluentus*, and brook trout, *Salvelinus fontinalis* in an Oregon stream network. Conservation Genetics.

Felsenstein, J. 1993. Phylip: phylogeny inference package. Ver. 3.5c. Department of Genetics, University of Washington, Seattle.

Franklin, I.A. 1980. Evolutionary changes in small populations. Pages 135-150 in M. Soule and B.A. Wilcox, editors. Conservation Biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Gunkel, S.L., A.R. Hemmingsen, and J.L. Li. 2002. Effect of bull trout and brook trout interactions on foraging habitat, feeding behavior, and growth. Transactions of the American Fisheries Society 131:1119-1130.

Kalinowski, S.T. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5:187-189.

Kanda, N., R.F. Leary, and F.W. Allendorf. 2002. Evidence of introgressive hybridization between bull trout and brook trout. Transactions of the American Fisheries Society. 131:772-782.

Leary, R.F., F.W. Allendorf and S.H. Forbes. 1993. Conservation genetics of bull trout in the Columbia and Klamath River drainages. Conservation Biology 7:856-865.

Leary, R.F., F.W. Allendorf, and G.K. Sage. 1995. Hybridization and introgression between introduced and native fish. Pages 91-101 in Schramm HL, Piper RG eds.Uses and Effects of Cultured Fishes in Aquatic Ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Lewis, P.O., and D. Zaykin. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html.

Luikart, G. and J.-M. Cornuet. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology 12:228-237.

McMahon T.E., A.V. Zale, F.T. Barrows, J.H. Selong, and R.J. Danehy. 2007. Temperature and competition between bull trout and brook trout: a test of the elevation refuge hypothesis. Transactions of the American Fisheries Society. 136:1313-1326.

Miller L.M., and A.R. Kapuscinski. 1996. Microsatellite DNA markers reveal new levels of variation in northern pike. Transactions of the American Fisheries Society 125:971-997.

MBTSG (Montana Bull Trout Scientific Group). 1995. Upper Clark Fork River drainage bull trout status report (including Rock Creek). Report prepared for the Montana Bull Trout Restoration Team. Montana Department of Fish, Wildlife and Parks, Helena. 40 pp.

MFWP (Montana Fish, Wildlife and Parks). 2008. An assessment of fish populations and riparian habitat in tributaries of the upper Clark Fork River basin. 294 pages.

Morita, K., S. Yamamoto, and N. Hoshino. 2000. Extreme life history change of white-spotted char (*Salvelinus leucomaenis*) after damming. Canadian Journal of Fisheries and Aquatic Science 57:1300-1306.

Narum, S.R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. Conservation Genetics 7:783-787.

Nerass, L.P and P.Spruell. 2001. Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork river system. Molecular Ecology 10:1153-1164.

Raymond M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rexroad, C.E., R.L. Coleman, A.M. Martin, W.K. Hershberger and J. Killefer. 2001. Thirty-five polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Animal Genetics 32: 317-319.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rich, C.F., T.E. McMahon, B.E. Rieman, and W.L. Thompson. 2003. Local-habitat, watershed, and biotic features associated with bull trout occurrence in Montana streams. Transactions of the American Fisheries Society 132:1053-1064.

Rieman, B.E. and F.W. Allendorf. 2001. Effective population size and genetic conservation criteria for bull trout. North American Journal of Fisheries Management 21:756-64.

Rieman, B.E. and J.B. Dunham. 2000 Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. Ecology of Freshwater Fishes 9:51-64.

Rieman, B., D.C. Lee, and R.F. Thurow. 1997. Distribution, status and likely future trends of bull trout within the Columbia and Klamath River basins. North American Journal of Fisheries Management 17:1111-1125.

Rieman, B., and J. Clayton. 1997. Wildfire and native fish: issues of forest health and conservation of sensitive species. Fisheries, **22**(11): 6-15.

Rieman, B.E, D. Isaak, S. Adams, D. Horan, D. Nagel, C. Luce and D. Myers. 2007. Anticipated climate warming effects on bull trout habitats and populations across the interior Columbia River Basin. Transactions of the American Fisheries Society 136:1552-1565.

Spear, S.F., C. R. Peterson, M. D. Matocq, and A. Storfer.. 2006. Molecular evidence for historical and recent population size reductions of tiger salamanders (*Ambystoma tigrinum*) in Yellowstone National Park. Conservation Genetics **7**(4): 605–611.

Spruell, P., B.E. Rieman, K.L. Knudsen, F.M. Utter and F.W. Allendorf. 1999. Genetic population structure within streams: microsatellite analysis of bull trout populations. Ecology of Freshwater Fishes 8:114-121.

Spruell, P., A.R. Hemmingsen, P.J. Howell, N. Kanda, and F.W. Allendorf. 2003. Conservation genetics of bull trout: geographic distribution of variation at microsatellite loci. Conservation Genetics 4:17-29.

USFWS (U.S. Fish and Wildlife Service). 2002. Bull Trout (*Salvelinus confluentus*) Draft Recovery Plan for the Columbia and Klamath River Distinct Population Segments. Portland Oregon. Available on-line at www.fws.gov/pacific/bulltrout/ .

USFWS 2009. Bull trout core area status update: upper Clark Fork River. March 9, 2009. Kalispell, Montana.

Waples, R.S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked loci. Conservation Genetics 7:167-184.

Waples, R.S. and C. Do. 2008. LDNe: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753-756.

Weir B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Whiteley, A.R., P. Spruell, B.E. Rieman, and F.W. Allendorf. 2006. Fine-scale genetic structure of bull trout at the southern limit of their distribution. Transactions of the American Fisheries Society 135:1238-1253.

Yamamoto S, Morita K, Koizumi I, and K Maekawa. 2004. Genetic differentiation of white spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. Conservation Genetics 5(4):529-538.

.

Table 1. Collection location and morphological field identification of individual *Salvelinus* collected in the Warm Springs Creek drainage genetically identified as bull trout × brook trout hybrids. All individuals were determined to be first generation (F1) hybrids.

Sample ID	Collection Location	Field identification
1259-065	Foster Creek	Hybrid
1259-066	Foster Creek	Hybrid
113-004	Twin Lakes	Bull Trout
113-055	Twin Lakes	Bull Trout
113-056	Twin Lakes	Bull Trout
113-057	Twin Lakes	Bull Trout
113-058	Twin Lakes	Bull Trout
113-060	Twin Lakes	Hybrid
113-061	Twin Lakes	Bull Trout
1259-071	Silver Lake	Bull Trout
1259-076	Silver Lake	Bull Trout
1259-077	Silver Lake	Bull Trout
1259-083	Silver Lake	Hybrid
1259-087	Silver Lake	Hybrid
1249-001	Warm Springs Creek	Hybrid
1249-002	Warm Springs Creek	Hybrid
1249-003	Warm Springs Creek	Hybrid
1249-004	Warm Springs Creek	Bull Trout
1249-005	Warm Springs Creek	Hybrid
1249-006	Warm Springs Creek	Hybrid
1249-008	Warm Springs Creek	Bull Trout
1249-009	Warm Springs Creek	Bull Trout
1249-014	Warm Springs Creek	Hybrid
1249-015	Warm Springs Creek	Bull Trout
1249-017	Warm Springs Creek	Hybrid
1249-026	Warm Springs Creek	Bull Trout
1249-028	Warm Springs Creek	Bull Trout

Table 2. Estimates of genetic variation (based on 15 variable microsatellite loci) within four 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	3.813	3.686	0.517	0.500
Storm Lake Creek	35	3.938	3.772	0.505	0.496
Twin Lakes	30	4.500	4.279	0.533	0.524
Warm Springs Creek	25	3.563	3.532	0.541	0.592
Mean		**3.953**	**3.817**	**0.518**	**0.525**

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 rangewide 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 population size calculated based on linkage disequilibrium (Waples 2006) for four bull trout populations in Warm Springs Creek, MT.

Population	**N_e**	**95% C.I.**
Barker Creek	7.8	5.4-10.5
Storm Lake Creek	8.5	6.2-11.1
Twin Lakes Creek	9.8	7.5-12.7
Warm Springs Creek	5.2	3.1-7.4

Table 4. Pairwise estimates of genetic variation (F_{ST}) among four 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
Barker Creek	---			
Storm Lake Creek	0.227	---		
Twin Lakes Creek	0.245	0.210	---	
Warm Springs Creek	0.121	0.184	0.226	---

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 Lake Creek	Warm Springs Creek
Barker Creek (n= 33)	**1.00**	0.00	0.00	0.00
Storm Lake Creek (n=35)	0.00	**1.00**	0.00	0.00
Twin Lakes Creek (n=30)	0.00	0.00	**0.93**	0.07
Warm Springs Creek (n=25)	0.04	0.00	0.00	**0.96**

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

ID	Most Likely Population	Likelihood Population #1	2nd Most Likely Population	Likelihood Population #2	Likelihood Ratio
1259-072	Storm Lake Creek	3.51	Twin Lakes Creek	$1.22X10^{-04}$	$2.90X10^{04}$
1259-073	Storm Lake Creek	3.63	Twin Lakes Creek	$8.87X10^{-09}$	$4.09X10^{08}$
1259-074	Storm Lake Creek	23.23	Twin Lakes Creek	$3.28X10^{-10}$	$7.09X10^{10}$
1259-075	Storm Lake Creek	12.11	Twin Lakes Creek	$9.36X10^{-10}$	$1.29X10^{10}$
1259-078	Storm Lake Creek	0.02	Twin Lakes Creek	$2.03X10^{-09}$	$8.00X10^{06}$
1259-079	Storm Lake Creek	37.12	Twin Lakes Creek	$2.50X10^{-04}$	$1.00X10^{05}$
1259-080	Storm Lake Creek	3.48	Twin Lakes Creek	$6.78X10^{-10}$	$5.14X10^{09}$
1259-081	Storm Lake Creek	0.01	Twin Lakes Creek	$1.13X10^{-06}$	$1.10X10^{04}$
1259-082	Storm Lake Creek	1.11	Warm Springs Creek	$4.48X10^{-08}$	$2.48X10^{07}$
1259-084	Storm Lake Creek	2.27	Twin Lakes Creek	$1.09X10^{-07}$	$2.08X10^{07}$
1259-085	Storm Lake Creek	0.06	Twin Lakes Creek	$8.17X10^{-08}$	$8.00X10^{05}$
1259-086	Storm Lake Creek	92.08	Twin Lakes Creek	$1.25 X10^{-03}$	$7.30X10^{04}$
1259-088	Storm Lake Creek	0.30	Twin Lakes Creek	$3.70X10^{-12}$	$8.18X10^{10}$
1259-089	Storm Lake Creek	$2.02X10^{-04}$	Twin Lakes Creek	$9.27X10^{-06}$	21.81

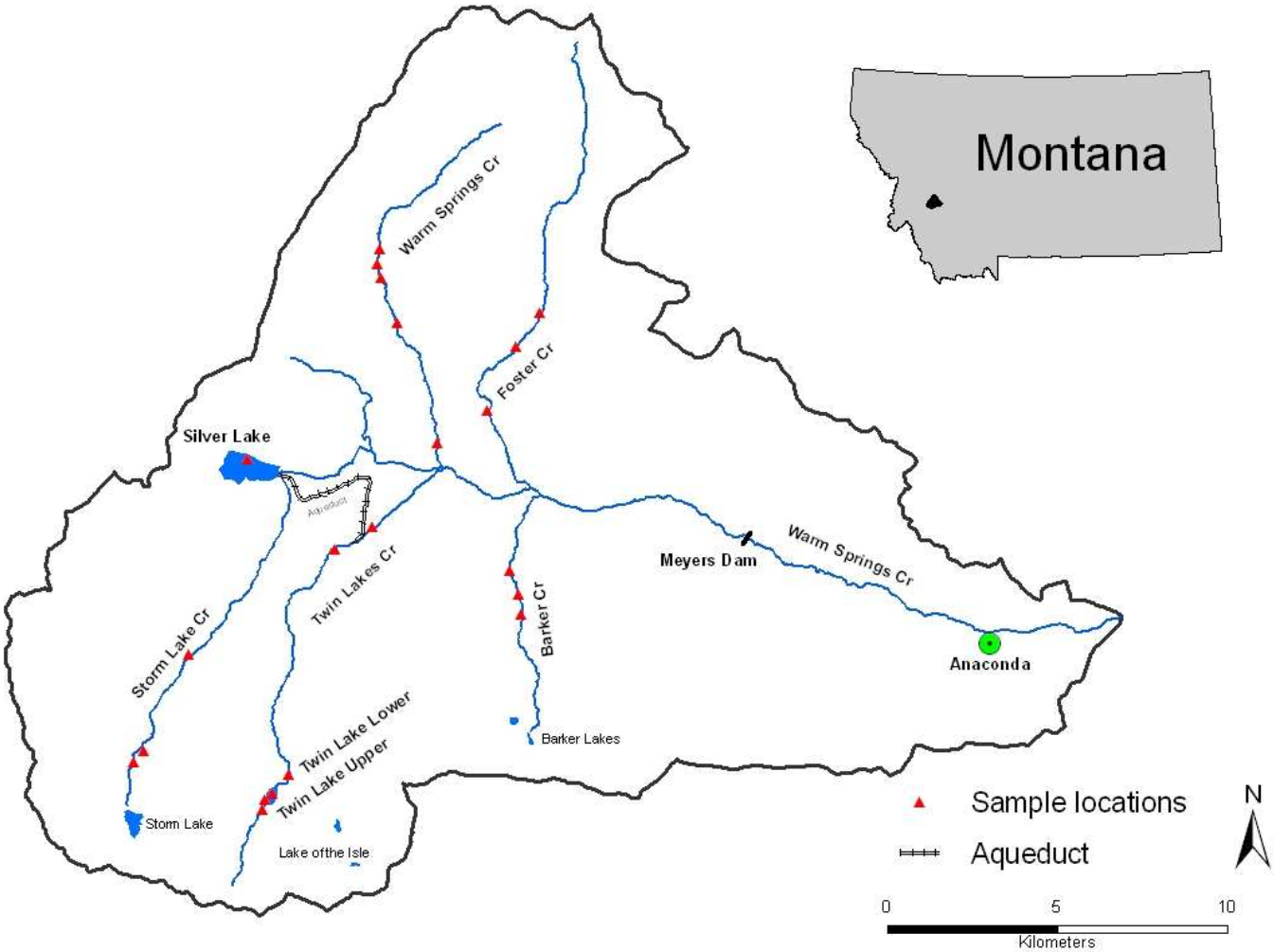

Figure 1. Warm Springs Creek drainage in western Montana, USA. Samples for this study were collected in Storm Lake Creek, Twin Lakes Creek (and lakes), Barker Creek, Foster Creek, Warm Springs Creek, and Silver Lake. Myers Dam is a barrier to upstream passage by fishes. Diversion structures on Storm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

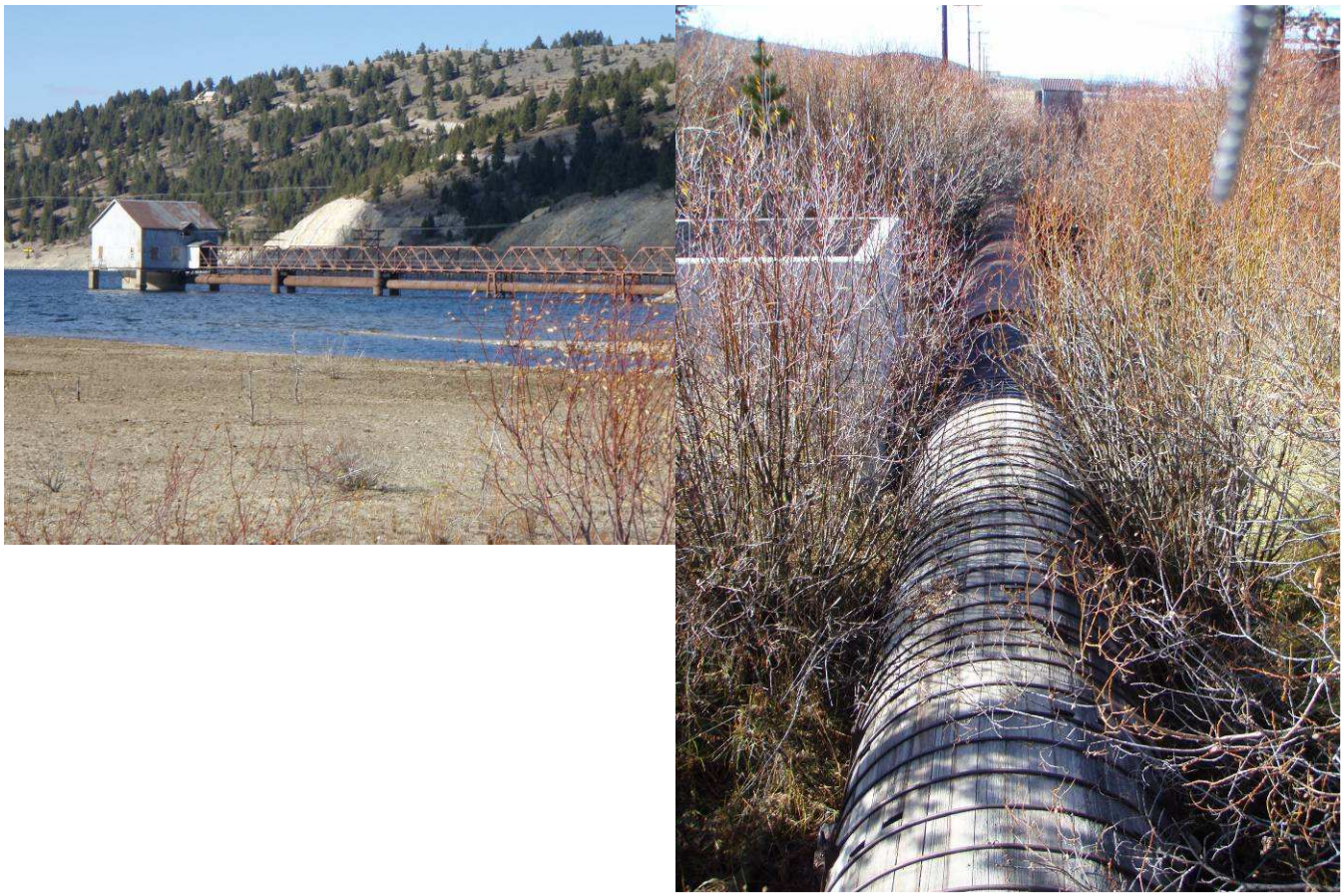

Figure 5. Pumping station (at left) in Silver Lake that can transport water into Warm Springs Creek. Water leaving Silver Lake exits through a large pipe (at right) before entering a channel with a natural streambed.

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.

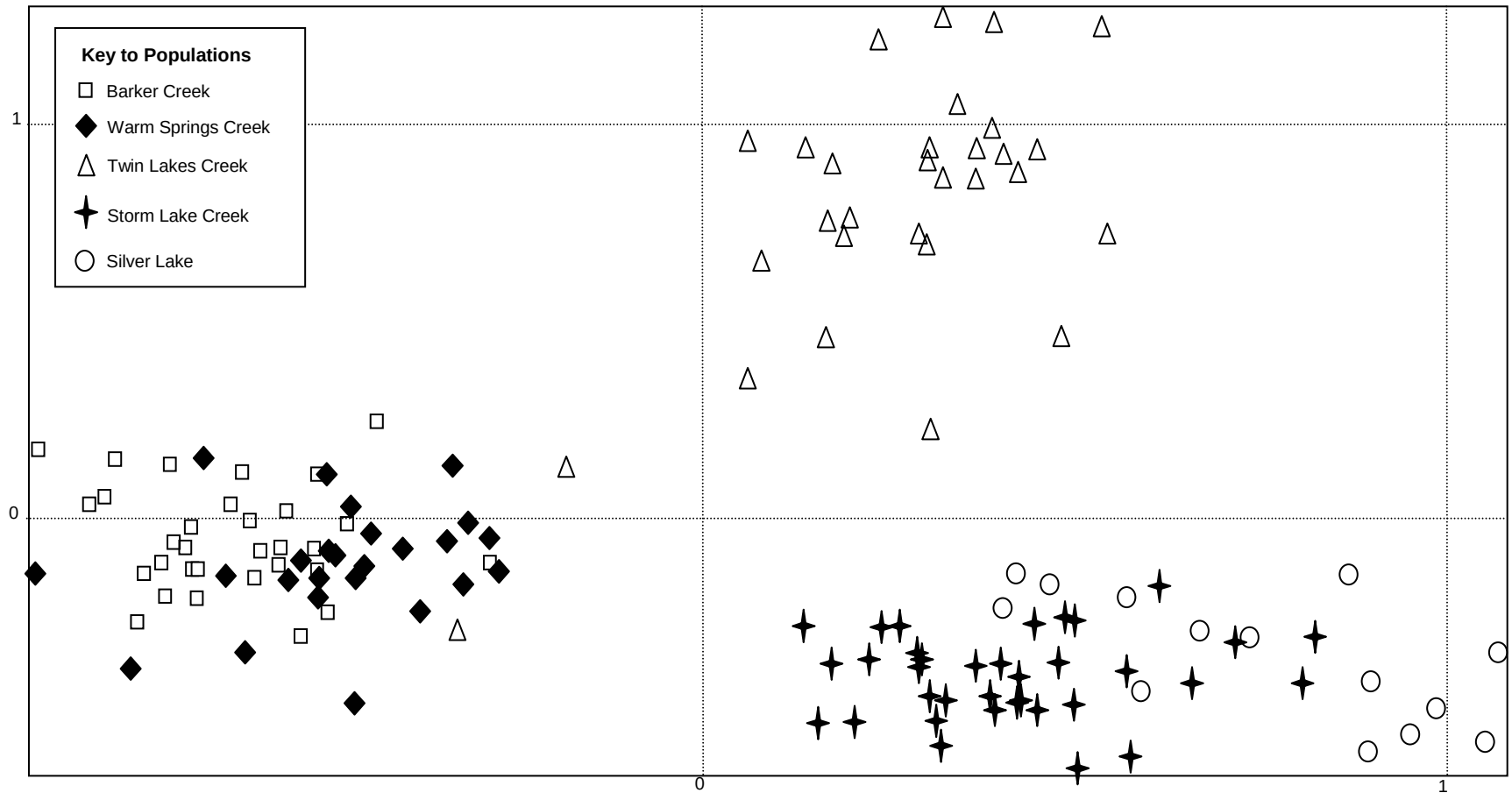

Figure 7. Factorial correspondence analysis (FCA) of bull trout collected in Warm Springs Creek, MT. Each point on the graph represents an individual bull trout in the analysis. Points that cluster closer together are more genetically similar.

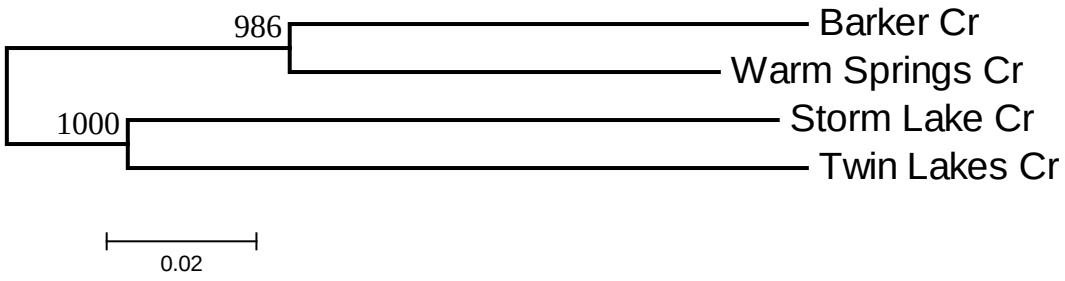

Figure 8. Neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance. Values at the nodes represent the number of bootstrap replicates (out of 1000) that showed the displayed topology.

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

Multiplex Set 1 **T_A= 54˚C**

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

Multiplex Set 2 **T_A= 59˚C**

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

Multiplex Set 3 **T_A=56˚C**

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

T_A= Annealing temperature

Appendix 2. Allele frequencies at 16 microsatellite loci for the bull trout collections analyzed in this study. Hybrid individuals have been removed.

Locus	Allele	Barker Creek	Storm Lake Creek	Twin Lakes Creek	Silver Lake	Warm Springs Creek
Omm1128	273	0.152	0.000	0.017	0.000	0.000
	277	0.000	0.314	0.150	0.214	0.200
	302	0.030	0.000	0.000	0.000	0.000
	306	0.394	0.029	0.383	0.143	0.360
	310	0.000	0.000	0.000	0.143	0.000
	319	0.136	0.000	0.050	0.000	0.000
	335	0.288	0.143	0.083	0.071	0.440
	339	0.000	0.514	0.317	0.429	0.000
Omm1130	302	0.106	0.000	0.035	0.000	0.000
	306	0.076	0.043	0.000	0.000	0.478
	312	0.000	0.143	0.069	0.321	0.000
	316	0.333	0.371	0.259	0.036	0.152
	320	0.000	0.314	0.000	0.143	0.000
	328	0.000	0.000	0.121	0.000	0.000
	332	0.076	0.029	0.483	0.214	0.196
	336	0.409	0.100	0.035	0.286	0.152
	340	0.000	0.000	0.000	0.000	0.022
Sco102	166	0.273	0.071	0.667	0.036	0.260
	169	0.727	0.929	0.333	0.964	0.740
Sco105	170	0.000	0.071	0.000	0.000	0.000
	178	0.576	0.071	0.683	0.214	0.240
	182	0.000	0.200	0.250	0.500	0.000
	186	0.152	0.657	0.033	0.286	0.620
	190	0.000	0.000	0.017	0.000	0.140
	194	0.273	0.000	0.017	0.000	0.000
Sco106	196	0.242	0.543	0.017	0.429	0.220
	200	0.000	0.000	0.000	0.000	0.100
	212	0.061	0.300	0.033	0.536	0.120
	216	0.349	0.000	0.233	0.000	0.120
	220	0.182	0.000	0.033	0.000	0.020
	224	0.061	0.000	0.033	0.000	0.000
	228	0.106	0.157	0.650	0.036	0.420
Sco107	269	0.303	0.157	0.050	0.036	0.380
	273	0.227	0.371	0.433	0.250	0.500
	277	0.394	0.300	0.433	0.143	0.020
	281	0.000	0.157	0.017	0.536	0.000
	297	0.076	0.000	0.000	0.000	0.100
	301	0.000	0.014	0.067	0.036	0.000

Appendix 2. Continued

Locus	Allele	Barker Creek	Storm Lake Creek	Twin Lakes Creek	Silver Lake	Warm Springs Creek
Sco109	250	0.033	0.000	0.018	0.000	0.040
	258	0.000	0.103	0.018	0.786	0.000
	262	0.000	0.000	0.000	0.000	0.060
	266	0.400	0.293	0.304	0.071	0.460
	270	0.000	0.017	0.625	0.107	0.200
	274	0.000	0.000	0.036	0.000	0.000
	296	0.017	0.000	0.000	0.000	0.000
	312	0.150	0.017	0.000	0.000	0.000
	320	0.000	0.121	0.000	0.000	0.000
	326	0.000	0.414	0.000	0.036	0.000
	350	0.000	0.035	0.000	0.000	0.000
	408	0.283	0.000	0.000	0.000	0.120
	412	0.117	0.000	0.000	0.000	0.120
Sco200	126	0.091	0.057	0.250	0.179	0.200
	130	0.000	0.257	0.650	0.643	0.100
	134	0.076	0.371	0.000	0.143	0.020
	167	0.833	0.314	0.100	0.036	0.680
Sco202	130	0.500	0.471	0.550	0.571	0.380
	134	0.500	0.529	0.450	0.429	0.620
Sco212	241	0.000	0.000	0.183	0.000	0.000
	249	0.167	0.043	0.133	0.107	0.040
	269	0.046	0.000	0.017	0.000	0.240
	295	0.788	0.957	0.667	0.893	0.720
Sco215	289	1.000	1.000	1.000	1.000	1.000
Sco216	225	0.076	0.000	0.293	0.000	0.040
	237	0.561	0.600	0.397	0.500	0.000
	241	0.000	0.043	0.000	0.107	0.000
	245	0.152	0.071	0.000	0.321	0.260
	257	0.212	0.286	0.310	0.071	0.700
Sco218	221	0.000	0.000	0.033	0.000	0.000
	225	0.000	0.000	0.117	0.000	0.000
	233	0.015	0.071	0.000	0.286	0.000
	237	0.000	0.029	0.000	0.000	0.000
	245	0.515	0.014	0.033	0.107	0.280
	249	0.242	0.300	0.567	0.250	0.200
	253	0.136	0.471	0.200	0.321	0.200
	257	0.000	0.014	0.000	0.036	0.000
	273	0.076	0.100	0.050	0.000	0.000

Appendix 2. Continued

Locus	Allele	Barker Creek	Storm Lake Creek	Twin Lakes Creek	Silver Lake	Warm Springs Creek
Sco218	277	0.015	0.000	0.000	0.000	0.000
	281	0.000	0.000	0.000	0.000	0.320
Sco220	310	0.273	0.071	0.267	0.071	0.000
	318	0.000	0.014	0.033	0.107	0.000
	322	0.076	0.643	0.183	0.214	0.460
	342	0.015	0.000	0.000	0.000	0.000
	347	0.636	0.129	0.133	0.429	0.520
	351	0.000	0.143	0.383	0.179	0.020
Sfo18	145	0.167	0.886	0.917	1.000	0.320
	151	0.833	0.114	0.083	0.000	0.680
Smm22	198	0.015	0.000	0.000	0.000	0.020
	202	0.000	0.014	0.000	0.000	0.180
	206	0.773	0.400	0.133	0.179	0.500
	210	0.015	0.000	0.017	0.000	0.000
	218	0.000	0.000	0.017	0.000	0.000
	230	0.000	0.000	0.017	0.000	0.000
	234	0.000	0.143	0.033	0.393	0.000
	238	0.000	0.000	0.383	0.000	0.020
	242	0.167	0.000	0.017	0.000	0.140
	246	0.030	0.000	0.067	0.000	0.000
	250	0.000	0.114	0.017	0.000	0.140
	254	0.000	0.329	0.267	0.429	0.000
	258	0.000	0.000	0.033	0.000	0.000