

**Genetic Variation and Genetic Population Structure of Bull trout in the Lemhi River Drainage,  
Idaho.  
Final Report**



**Christine C. Kozfkay, Sr. Fisheries Research Biologist  
Matthew R. Campbell, Genetics Lab Manager  
Eric Tretter, Fisheries Technician  
Idaho Department of Fish and Game**

**Report 08-140**

**Challenge Cost Share Project DLA070208  
U.S. Department of Interior, Bureau of Land Management  
Salmon Field Office**

**December 2008**

## **Abstract**

This report describes results from a collaborative study between the Bureau of Land Management (BLM) and the Idaho Department of Fish and Game (IDFG). The focus of this study was to examine genetic variation and genetic population structure of bull trout in the Lemhi River Watershed in east central Idaho. Many of the tributary streams in the Lemhi River are seasonally disconnected from the main-stem river. The results of this study showed high levels of genetic differentiation (high  $F_{ST}$  estimates and high allocation success for the maximum likelihood assignment test) and indicated that the majority of populations were reproductively isolated. (Genetic connectivity was only observed in parts of Hayden Creek and Big Timber Creek. The populations with the highest levels of potential gene flow also exhibited the highest levels of genetic variation. These results confirm the importance of connectivity in maintaining genetic diversity. Currently, there are management activities underway that should allow for increased connectivity among populations over the next 30 years. This report not only serves as a baseline for monitoring purposes for these habitat and conservation improvements but also outlines ways that genetic data can help monitor the success of these projects.

## **Introduction**

Bull trout (*Salvelinus confluentus*) are a char native to the northwestern United States and Canada. The historical range of bull trout extends from northern California to southern Alaska (Spruell et al. 2003). Bull trout have the most specific habitat requirements of any of the salmonid species; they require the coldest water for spawning, the cleanest substrate for spawning and rearing, and complex habitats with woody debris and undercut banks (Watson and Hillman 1997, Dunham et al. 2003). Bull trout also exhibit different life-histories (resident, migratory forms) and connected habitats are vital to maintain annual spawning and feeding movements for migratory fish. These requirements make bull trout sensitive to changes in temperature regimes (Selong et al. 2001) and habitat alterations (Dunham and Rieman 1999) and there is a risk that small, isolated populations of bull trout may become susceptible to local extinctions (Rieman et al. 2007).

Concerns regarding the long-term viability of bull trout led to their original listing as threatened under the Endangered Species Act (ESA) in 1998 as five distinct population segments. Subsequently, in 1999, they were reclassified as threatened as a single distinct population segment in the coterminous United States (DPS; USFWS 1999). In 2004, the USFWS initiated a

5-year status review to determine whether the level of protection was still appropriate. The status review determined that the species should retain its threatened status for the time-being in the coterminous United States but recommended re-evaluating the designation of multiple DPS's. ([http://www.fws.gov/pacific/bulltrout/5-yr%20Review/BTFINAL\\_42508.pdf](http://www.fws.gov/pacific/bulltrout/5-yr%20Review/BTFINAL_42508.pdf)). Currently, efforts are underway to designate multiple DPS's and assess the status of bull trout within each of the potential DPS's. For this study, we will refer to the terminology used in the bull trout recovery plan to describe population units: 1) recovery units 2) core areas and 3) local populations (USFWS 2002). In total, 27 recovery units were originally delineated across the species' range based upon genetic characteristics and management jurisdictions. The plan considered core areas to be local populations of bull trout that were partially isolated but shared some degree of gene flow while local populations were characterized by those occupying individual streams.

The recovery unit of interest for this study was the Salmon River recovery unit and the core area of interest is the Lemhi River core area. The Lemhi River drainage is a low-gradient, spring-fed system located in eastern Idaho. It encompasses over 800,000 acres with 80% of the area managed as public lands by the U.S. Forest Service, Bureau of Land Management, and Idaho Department of Fish and Game. Within the Lemhi River drainage, there are 26 major tributaries. Surveys suggest that bull trout are widely distributed throughout the drainage, however, there are issues concerning connectivity within this drainage and large, migratory fish are not as prevalent as in other areas in the Upper Salmon River (Schoby and Curet 2007). Most tributaries have been seasonally disconnected from the main-stem river due to irrigation water withdrawals and unscreened irrigation ditches (Lamperth et al. 2007). There are numerous water conservation projects in the Lemhi River drainage with short and long-term goals to improve habitat and re-connect 10-16 tributaries to the main-stem river over the next 30 years. Recent efforts to identify distribution, habitat use, and movement patterns for fluvial populations of bull trout in the upper Salmon River basin have provided important information to guide management decisions and the planning of these habitat conservation and improvement projects (Schoby and Curet 2007, Lamperth et al. 2007).

Genetic analyses can provide relevant information to help guide management of bull trout within the Lemhi River drainage. Genetic assignment tests can potentially replace tagging data and identify the population of origin for migrating fluvial bull trout captured in the main-stem Lemhi River. This information can be used to evaluate the response of bull trout to improvement projects as well as determine migration rates among local populations. However, a pre-requisite to using this methodology requires that each tributary can be uniquely characterized (Hansen et al. 2001). Genetic diversity estimates can also be monitored before and after re-connection

efforts to test the temporal stability of baseline allele frequencies and to gain a better understanding of the interplay between meta-population structure and genetic diversity. The objectives of this study are 1) describe population genetic structure and baseline levels of genetic diversity 2) compare levels of diversity within the isolated tributaries to the fluvial population of bull trout in Hayden Creek 3) genetically characterize each tributary and determine the power of assigning fish back to each tributary. Collectively, results from this study will provide important baseline data for bull trout within the Lemhi River drainage and should assist with the evaluation of future habitat improvement projects.

## **Methods**

### *Sampling and DNA Extraction*

During 2003-2004, 442 samples were collected from 25 sample sites by Idaho Department of Fish and Game (IDFG) and Bureau of Land Management (BLM) personnel as part of a larger study monitoring bull trout populations in the Lemhi River drainage using redd counts and snorkel counts. Temporal samples were taken from both Stroud Creek and Hayden Creek; and in some cases, multiple sites were sampled from the same tributary (Bear Valley Creek, Big Timber Creek, E.F. Hayden Creek, Kadletz Creek, Stroud Creek). These collections were analyzed (following the methods below) to determine if they could be pooled by tributary. Sample sizes and pooled locations for genetic analyses are presented in Table 1 and Figure 1. Samples were stored in 100%, non-denatured ethanol until DNA extraction. DNA was extracted using a Nexttec extraction method (XpressBio).

### *Microsatellite Amplification*

All of the samples were amplified with fifteen microsatellite loci: *Smm22* (Crane et al. 2004), *Sco102*, *Sco105*, *Sco107*, *Sco109*, *Sco110* (WDFW unpublished), *Omm1128*, *Omm1130* (Rexroad et al. 2001), *Sfo18* (Angers et al. 1995), *Sco200*, *Sco202*, *Sco212*, *Sco215*, *Sco216*, *Sco220* (DeHaan and Ardren 2005). Of these 15 microsatellite loci, 12 are considered to be core loci (Kozfkay 2008). All of the core loci were included in the analyses except for locus *Sco212*. This locus was excluded because we found alleles larger than our size standard (>500 bp) which could not be accurately sized. PCR reaction conditions were multiplexed into 4 panels. PCR reactions for Panel A were conducted in 5 µl reactions containing 1X Qiagen multiplex PCR master mix (Qiagen Co.), 0.30 µM *Sco200* primer, 0.20 µM *Sco215* primer, 0.20 µM *Sco102* primer, 0.20 µM *Sfo18* primer, 0.20 µM *Sco220* primer and 0.20 µM *Sco110* primer. Cycling was performed with a PTC-100 thermocycler (MJ Research) with the following profile: 95°C for

15 min, 25 cycles of 94°C for 30 sec, 60°C for 1.5 min, 72°C for 1 min and a final extension at 60°C for 30 min. PCR reactions for Panel B were conducted in 5 µl reactions containing 1X Qiagen multiplex PCR master mix (Qiagen Co.), 0.13 µM Sco202 primer, 0.20 µM Omm1128 primer, 0.20 µM Smm22 primer, 0.20 µM Sco105 primer. Cycling was performed with a PTC-100 thermocycler (MJ Research) with the following profile: 95°C for 15 min, 25 cycles of 94°C for 30 sec, 57°C for 1.5 min, 72°C for 1 min and a final extension at 60°C for 30 min. PCR reactions for Panel C were conducted in 5 µl reactions containing 1X Qiagen multiplex PCR master mix (Qiagen Co.), 0.35 µM Omm1130 primer, 0.30 µM Sco107 primer, 0.20 µM Sco106 primer, 0.13 µM Sco218 primer. Cycling was performed with a PTC-100 thermocycler (MJ Research) with the following profile: 95°C for 15 min, 35 cycles of 94°C for 30 sec, 57°C for 1.5 min, 72°C for 1 min and a final extension at 60°C for 30 min. PCR reactions for Panel D were conducted in 15 µl reactions containing 1X Taq Buffer (Applied Biosystems), 1.5 mM MgCl<sub>2</sub>, 0.20 µM DNTPs, 0.50 µM Sco109 primer and 0.5 units Taq Polymerase (Applied Biosystems). Cycling was performed with a PTC-100 thermocycler (MJ Research) with the following profile: 94°C for 3 min, 38 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec and a final extension at 72°C for 7 min. All PCR products were electrophoresed using an ABI 3100 automated sequencer (Applied Biosystems) platform. PCR product was added to 0.35 µl Liz Size Standard and 30 µl of Formamide. Fragments were sized against GS500 LIZ size standard (Applied Biosystems) using GENESCAN version 3.1 and GENEMAPPER v. 3.5.1 software (Applied Biosystems).

### *Microsatellite Data Analyses*

In the locations where sampling was conducted in multiple years or across short distances, an AMOVA (Analysis of Molecular Variance Analysis) was performed using ARLEQUIN version 2.0 to evaluate the amount of genetic variation attributable to differences within and between locations (Excoffier et al. 1992). Overall, the amount of genetic variation between sites on the same stream was low (less than 4%) so these collections were pooled for subsequent analyses. Each population was tested for Hardy-Weinberg equilibrium and linkage disequilibrium using Genepop on the web (Raymond and Rousset 1995). A sequential Bonferroni correction was used to adjust significance for multiple comparisons (Rice 1989). An alpha value of 0.05 was chosen for statistical significance for all analyses.

Genetic diversity was measured by the number of alleles per locus (*A*) and expected heterozygosity (*H<sub>e</sub>*) using FSTAT version 2.9.3 (Goudet 2001). Pairwise *F<sub>ST</sub>* estimates (Weir and Cockerham 1984) were generated using Arlequin 2.0 with significance based upon a permutation

process. A regression analysis of average heterozygosity per stream vs. average pairwise  $F_{ST}$  per stream and average number of alleles per locus vs. average pairwise  $F_{ST}$  estimates per stream was also performed to evaluate the relationship between gene flow and genetic diversity.

An assignment test, GENECLASS 2.0 (Piry et al. 2004), was also used to assign fish back to their population of origin and test the feasibility of using genetic data for the future allocation of fluvial fish in the main-stem Lemhi River back to their natal stream. Fish were assigned to a stream with greater than 90% confidence. An unrooted neighbor-joining (NJ) tree using Cavalli-Sforza and Edward's (1967) chord distance ( $D_{ce}$ ) was used to display the population relationships using the software POPULATIONS 1.2.14 (Langella 2001) and TREEVIEW (Page 1996). One thousand bootstrap replicates were performed to evaluate tree topology.

## Results

A total of 210 tests for Hardy-Weinberg equilibrium were performed and 17 of the tests were rejected at  $\alpha = 0.05$ , which was slightly higher than expected by chance (10.5 tests expected from Type I error of 0.05). The highest number of rejected tests per locus was 3 out of 14 tests for *Sco200* and *Sco220* and the highest number of rejected tests per population was 4 out of 15 tests for Cabin Creek. When a Bonferroni correction was applied to all p-values (p-value < 0.0002), only two of the tests were rejected. This data indicated that there was no association between the rejected tests and a locus or population and our assumptions of Hardy-Weinberg equilibrium could be met.

A total of 3,150 tests for linkage disequilibrium were performed and 153 of the tests were rejected at  $\alpha = 0.05$ , which was approximately what is expected by chance (157 tests expected from Type I error of 0.05). None of these tests clustered around a particular locus pair, indicating no association among loci. None of the tests clustered around a particular sample location either. Therefore, our assumptions of linkage equilibrium could also be met.

### *Genetic Diversity*

The total number of alleles per locus observed ranged from one allele at *Sfo18* to 29 alleles at *Smm22*. Levels of genetic diversity ranged from 2.87 alleles and 40% heterozygosity in N.F. Little Timber Creek to 10.47 alleles and 74% heterozygosity in Hayden Creek (Table 1, Figure 2). No samples were identified as brook/bull trout hybrids based on alleles observed at five diagnostic loci.

### *Genetic Differentiation*

The level of genetic differentiation, as measured by  $F_{ST}$  estimates, ranged from 0.00 for Hayden Creek and Bear Valley Creek to 0.39 for Rough Canyon Creek and North Fork Little Timber Creek (Table 2). All of the  $F_{ST}$  estimates were highly significant indicating significant genetic differentiation among the majority of the tributary streams in the Lemhi River drainage. Average  $F_{ST}$  estimates ranged from 0.08 for Hayden Creek to 0.27 for Little Timber Creek. Both allelic diversity and expected heterozygosity were highly correlated with average  $F_{ST}$  estimates;  $R^2 = 0.82$  and  $0.94$ , respectively.

#### *Assignment Tests*

The assignment test yielded high levels of self-assignment indicating that isolated or resident fish reside within the majority of sample locations (Table 3). There were a few locations where migration and gene flow is occurring among tributaries: 1) Bear Valley Creek and Hayden Creek and 2) Big Timber Creek and Rocky Creek and Cabin Creek. Fish from Bear Valley Creek were assigned to Hayden Creek and fish from Hayden Creek were assigned to Bear Valley Creek. There were also many fish that could not be assigned with greater than 90% confidence to either Hayden Creek or Bear Valley Creek and were given unknown classifications because either location was likely. Fish from Big Timber Creek were assigned to both Rocky Creek and Cabin Creek (and vice versa) while Rocky Creek and Cabin Creek did not have fish assigned to one another. This suggests that while some migration is occurring among these sites; random mating is not occurring. There were also many fish from these sites that could not be assigned with greater than 90% confidence and were given unknown classifications. Unknown classifications were rare for all other populations in this study.

#### **Discussion**

Migration barriers within this drainage appear to be the principle factor affecting population genetic structure. Connectivity was only seen in parts of the Big Timber Creek and Hayden Creek tributaries. Bear Valley Creek and Hayden Creek experienced the highest amounts of cross-assignments and were not genetically differentiated from one another suggesting high gene flow. However, East Fork Hayden Creek, a tributary to Hayden Creek, was highly differentiated from Hayden Creek and Bear Valley Creek. This is most likely because a large, resident component exists within this tributary (Lamperth et al. 2007). In the Big Timber Creek tributary, fish appeared to be moving between Rocky Creek and Big Timber Creek and Cabin Creek and Big Timber Creek but not between Rocky Creek and Cabin Creek. This could be due to intermittent water levels, low densities of fish, or predominately resident life-histories within

Rocky Creek and Cabin Creek. The rest of the populations displayed high pairwise genetic distance estimates and high levels of self-assignment indicating reproductive isolation.

Genetic diversity is maintained by large population sizes and/or sufficient gene flow (Ray 2001, Consuegra et al. 2005). Populations that are isolated and lower in population size can lose genetic diversity through genetic drift and inbreeding (Allendorf and Ryman 2002). Generally allelic diversity is lost faster than heterozygosity and is a better indicator of population status (Nei et al. 1975, Norris et al. 1999, Spencer et al. 2000). The standardized set of microsatellite loci allows for a direct comparison of genetic diversity across the species range, enabling us to put our results into a larger perspective. Levels of genetic diversity were within the range observed for 75 bull trout populations throughout the species' range (W. Ardren, USFWS, unpublished data); Hayden Creek displayed the third highest levels of genetic diversity while N.F. Little Timber Creek displayed the second lowest levels of genetic diversity across the range. In the Hayden Creek watershed, the only long-term functionally connected tributary evaluated in this study, relatively large populations of fluvial bull trout exist with connectivity to other populations within the watershed and the main-stem Lemhi River (Lamperth et al. 2007, Schoby and Curet 2007). Although Big Timber Creek is functionally disconnected from the main-stem Lemhi River there is over 80 km of main-stem and tributary habitat. The results of this study suggest that the amount of available habitat in the Big Timber watershed provide the proper environment to support a genetically diverse bull trout population. The factors of drainage size and connectivity have apparently sustained high levels of genetic diversity within the Big Timber and Hayden creek bull trout populations.

East Fork Hayden Creek, Stroud Creek and Kenney Creek also had high levels of genetic diversity. The diversity observed in East Fork Hayden and Kenney creeks can likely be explained due to partial main-stem connectivity that allows some migration to occur during high-water years or large population sizes within these tributaries. Stroud Creek however, is a tributary to Lee Creek which is perpetually isolated from the main-stem Lemhi River. The genetic diversity in this watershed is most likely explained by the presence of complex unscreened irrigation systems that allow bull trout to leave and enter this watershed from adjacent occupied bull trout watersheds. Big Eight Mile Creek south and east of Lee Creek and Mill Creek north and east of Lee Creek both support robust bull trout populations and via the irrigation systems have the potential to recruit bull trout into the Lee Creek watershed. An inclusion of samples from Big Eight Mile Creek and Mill Creek in future analyses could look at gene flow between these watersheds and Stroud Creek. Stroud Creek is isolated from populations outside of Lee Creek, its parent stream, so the high levels of diversity are surprising.

Lower levels of diversity within the other populations in the Lemhi River drainage is likely a reflection of sampling smaller, resident populations with limited to no access to other populations or the main-stem river. The regression analysis supported this result where the populations with the lowest average pairwise  $F_{ST}$  estimates had the highest levels of genetic diversity. These results emphasize the importance of connectivity to maintain levels of genetic diversity within the Lemhi River drainage and this is especially important in N.F. Little Timber Creek, Little Mill Creek and Rough Canyon Creek where allelic diversity estimates are at the low end of the range.

Re-establishing connectivity within the Lemhi River drainage is a high priority for fish managers. Plans are in place to remove fish barriers, re-establish riparian vegetation along corridors, increase stability of stream banks, increase the number and quality of rearing and resting pools, make improvements to irrigation diversions (e.g. screening), and install cattle crossings in many areas (Idaho Soil Conservation Commission 1995). This genetic data suggests that habitat improvement projects in N.F. Little Timber Creek, Little Mill Creek and Rough Canyon Creek might provide the maximal level of benefit in terms of preserving genetic diversity. Genetic assignment tests can also be useful in evaluating the response of bull trout to improvement projects. Fish sampled in the main-stem Lemhi River could be assigned to their natal population and information such as migration rates, colonization rates, and time to establish a fluvial population could be determined. However, sufficient statistical power is needed to confirm that an individual is indeed a migrant and not just assigned by chance. Our results indicated that fish could be assigned to individual tributaries with high confidence (>90%) except in the tributaries where gene flow was occurring: Hayden Creek and Big Timber Creek. Fish going to either Bear Valley Creek or Hayden Creek and fish going to Big Timber Creek, Rocky Creek, or Cabin Creek could not be distinguished from one another (as seen by the large number of ambiguous assignments). These locations would have to be pooled into a Hayden Creek and Big Timber Creek reporting group for genetic monitoring purposes. This would result in 9 major reporting groups for the 13 sampled locations.

The power of assignment tests is determined by the level of differentiation among sampled populations, number of loci examined, levels of diversity at the examined loci, and baseline representation (Corneut et al. 1999). If there are other populations in the Lemhi River drainage that haven't been sampled but can migrate among the sampled populations, these fish will erroneously be assigned to the most similar population in the baseline. Therefore, it is important to include all existing populations within the baseline. Currently, high levels of differentiation allow for high assignment success to the reporting groups. As connectivity

increases in the Lemhi River drainage, reporting groups may change (and include more locations) as more populations exchange gene flow. This will depend upon whether the population is migratory or resident and the degree of straying among locations. Bull trout populations have been found to have a high degree of reproductive isolation over short distances (Kanda and Allendorf 2001), so the amount and scale of genetic exchange following re-connection will be of interest to monitor. Changes in genetic diversity following re-connection will also be important to monitor and may lead to important insights regarding meta-population structure.

Table 1. Genetic diversity estimates and sample sizes for 14 bull trout sampling locations. Abbreviations are as follows:  $H_e$  = average expected heterozygosity and  $A$  = average number of alleles per locus,  $H_{e(Core)}$  and  $A_{(Core)}$  refers to diversity at the 12 USFWS standardized core loci used to compare diversity across the range of bull trout (W. Ardren, USFWS, unpublished data).

Site Number	Stream Name	Sample Size	Sample Year	$H_e$	$A$	$A_{(Core)}$	$H_{e(Core)}$
1*	Bear Valley Creek	40	2004	0.73	9.73	9.42	0.76
2*	Big Timber Creek	29	2003	0.74	9.13	8.58	0.75
3	Deer Creek	30	2005	0.54	6.20	5.83	0.53
4*	EF Hayden Creek	60	2004	0.70	8.40	8.00	0.72
5	Hayden Creek	50	2006	0.74	10.47	9.92	0.77
6*	Kadletz Creek	35	2004	0.58	6.20	5.92	0.59
7*	Little Mill Creek	25	2005	0.57	4.47	4.17	0.58
8	NF Little Timber Creek	15	2003	0.40	2.87	2.92	0.40
9	MF Little Timber Creek	11	2003	0.66	5.73	5.58	0.65
10	Rocky Creek	21	2003	0.71	7.93	7.75	0.73
11	Rough Canyon Creek	18	2005	0.51	3.53	3.67	0.54
12*	Stroud Creek	48	2004	0.70	8.00	7.67	0.73
13	Cabin Creek	30	2003	0.70	7.53	6.62	0.64
14	Kenney Creek	30	2003	0.71	7.73	7.42	0.71

\*Indicates pooled sample locations

Table 2: Levels of genetic differentiation among bull trout populations as measured by pairwise  $F_{ST}$  values.

	Bear Valley Creek	Stroud Creek	Big Timber Creek	Rocky Creek	Hayden Creek	Deer Creek	NF Little Timber Creek	MF Little Timber Creek	Little Mill Creek	Rough Canyon Creek	EF Hayden Creek	Kadletz Creek	Cabin Creek	Kenney Creek
Stroud Creek	0.06													
Big Timber Creek	0.03	0.05												
Rocky Creek	0.05	0.06	0.02											
Hayden Creek	0.00	0.05	0.02	0.04										
Deer Creek	0.16	0.16	0.16	0.16	0.15									
NF Little Timber Creek	0.24	0.22	0.19	0.20	0.22	0.33								
MF Little Timber Creek	0.06	0.06	0.05	0.05	0.04	0.18	0.24							
Little Mill Creek	0.15	0.18	0.15	0.17	0.14	0.28	0.32	0.20						
Rough Canyon Creek	0.20	0.18	0.16	0.17	0.17	0.30	0.39	0.20	0.32					
EF Hayden Creek	0.04	0.08	0.05	0.06	0.04	0.20	0.24	0.07	0.16	0.19				
Kadletz Creek	0.09	0.14	0.11	0.14	0.09	0.25	0.32	0.13	0.27	0.28	0.12			
Cabin Creek	0.06	0.08	0.04	0.05	0.06	0.17	0.25	0.08	0.17	0.21	0.07	0.16		
Kenney Creek	0.07	0.11	0.08	0.11	0.06	0.23	0.30	0.13	0.19	0.19	0.09	0.17	0.11	
<b>Average</b>	<b>0.09</b>	<b>0.11</b>	<b>0.09</b>	<b>0.09</b>	<b>0.08</b>	<b>0.21</b>	<b>0.27</b>	<b>0.12</b>	<b>0.21</b>	<b>0.23</b>	<b>0.11</b>	<b>0.17</b>	<b>0.12</b>	<b>0.14</b>

Table 3: Assignment Test Results using 14 microsatellite loci. Each column represents the population of sampling origin and each row lists the assigned populations. For example, Of the 40 fish sampled in Bear Valley Creek; 18 fish were assigned to Bear Valley Creek, 8 were assigned to Hayden Creek, and 14 could not be assigned with high certainty. Populations in bold represent areas of gene flow.

	Bear Valley Creek	Hayden Creek	Stroud Creek	Big Timber Creek	Rocky Creek	Cabin Creek	EF Hayden Creek	NF Little Timber Creek	MF Little Timber Creek	Kadletz Creek	Deer Creek	Little Mill Creek	Rough Canyon Creek	Kenny Creek
Bear Valley Creek	<b>18</b>	<b>10</b>												
Hayden Creek	<b>8</b>	<b>20</b>							1	1				1
Stroud Creek			57											
Big Timber Creek				<b>14</b>	<b>3</b>	<b>3</b>			1					
Rocky Creek				<b>3</b>	<b>10</b>									
Cabin Creek				<b>1</b>		<b>23</b>								
EF Hayden Creek		1					59							
NF Little Timber Creek		1						15						
MF Little Timber Creek									7					
Kadletz Creek										33				
Deer Creek											30			
Little Mill Creek												22		
Rough Canyon Creek													18	
Kenny Creek														25
Unknown Classification	14	18	1	11	7	4	1		2	2		1		4
<b>Total</b>	<b>40</b>	<b>50</b>	<b>58</b>	<b>29</b>	<b>20</b>	<b>30</b>	<b>60</b>	<b>0</b>	<b>11</b>	<b>36</b>	<b>0</b>	<b>23</b>	<b>18</b>	<b>30</b>

Figure 1. Sampling locations of bull trout populations in the Lemhi River drainage, Idaho. Numbers refer to the sample names in Table 1.

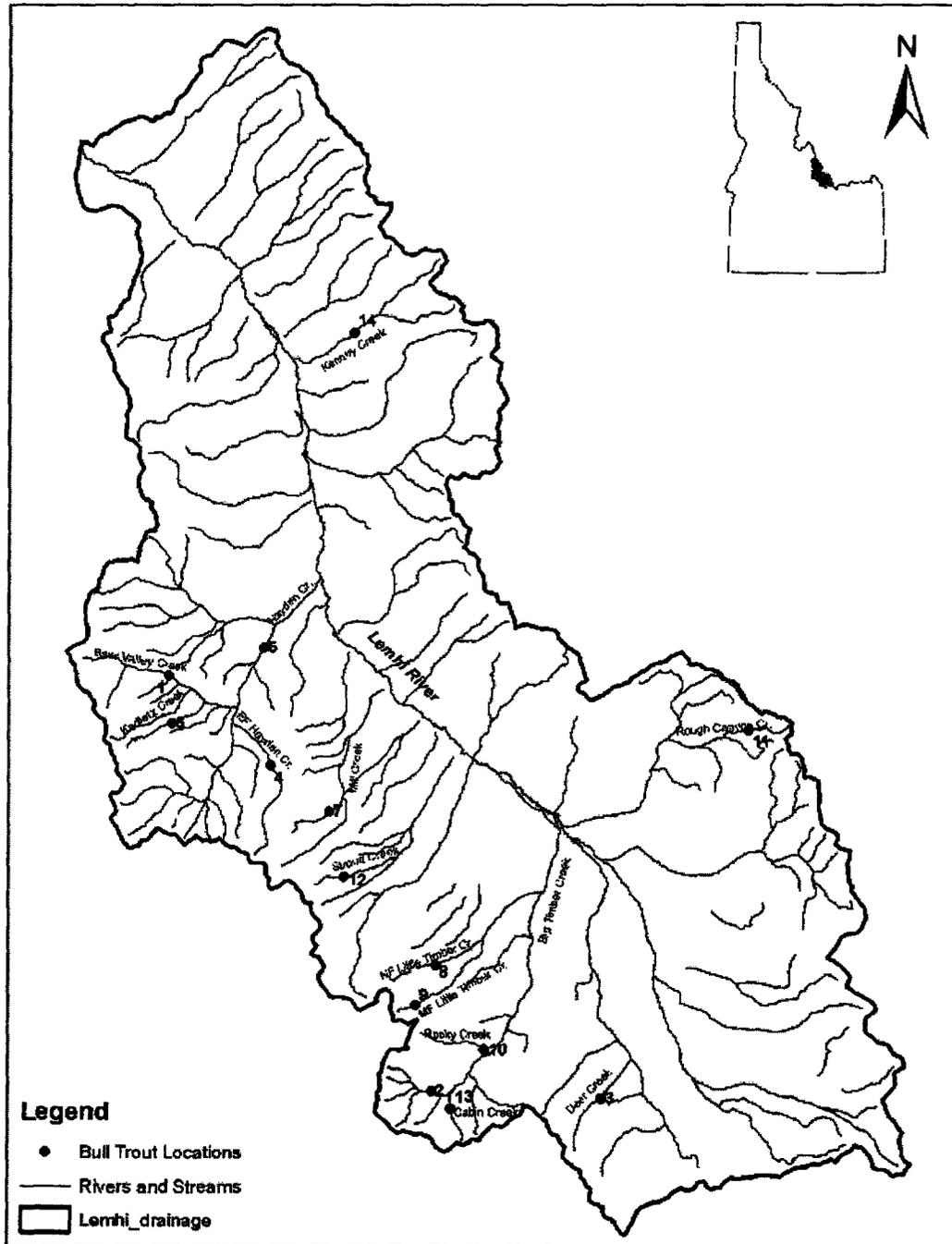


Figure 2 Regression analysis of average pairwise Fst estimates against genetic diversity estimates  
a) heterozygosity b) allelic diversity

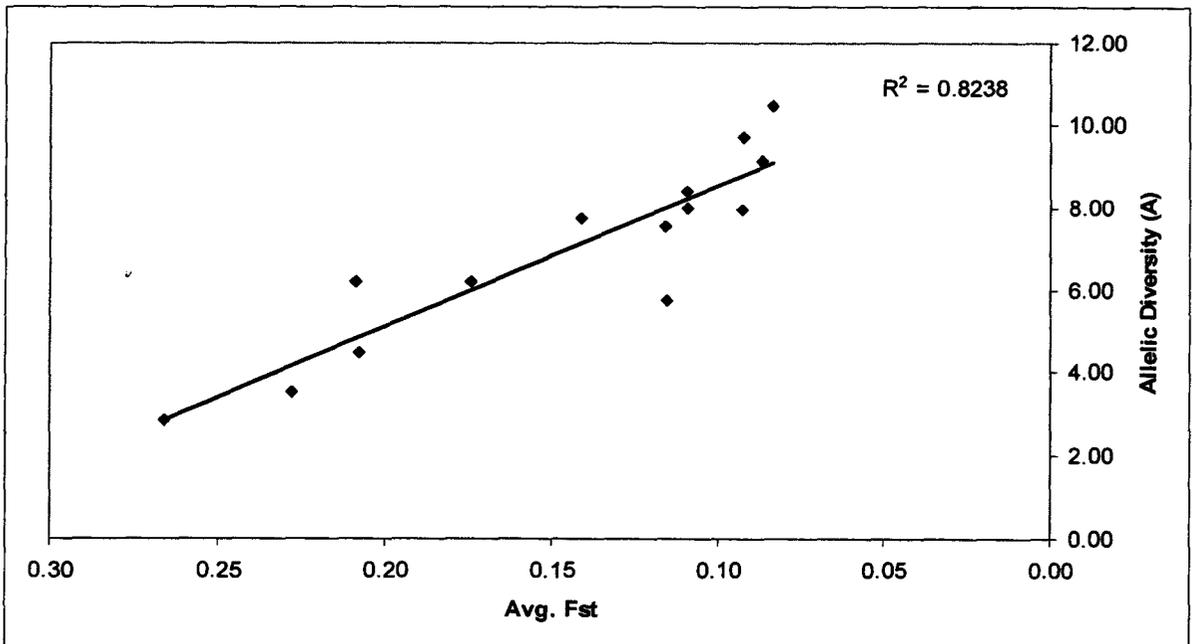
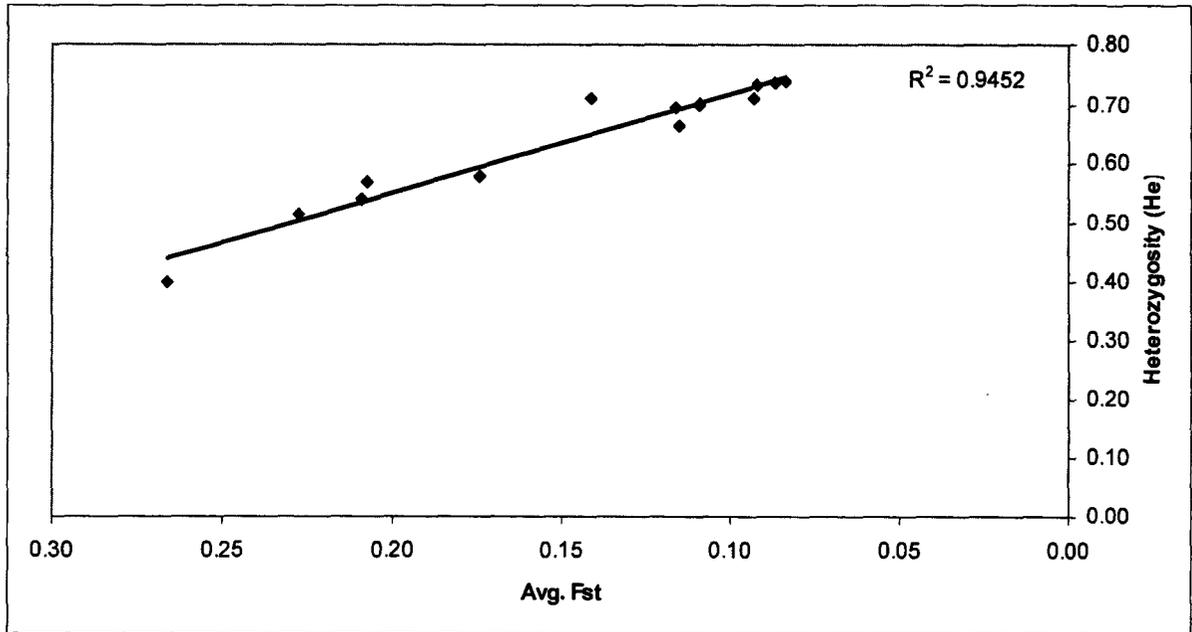
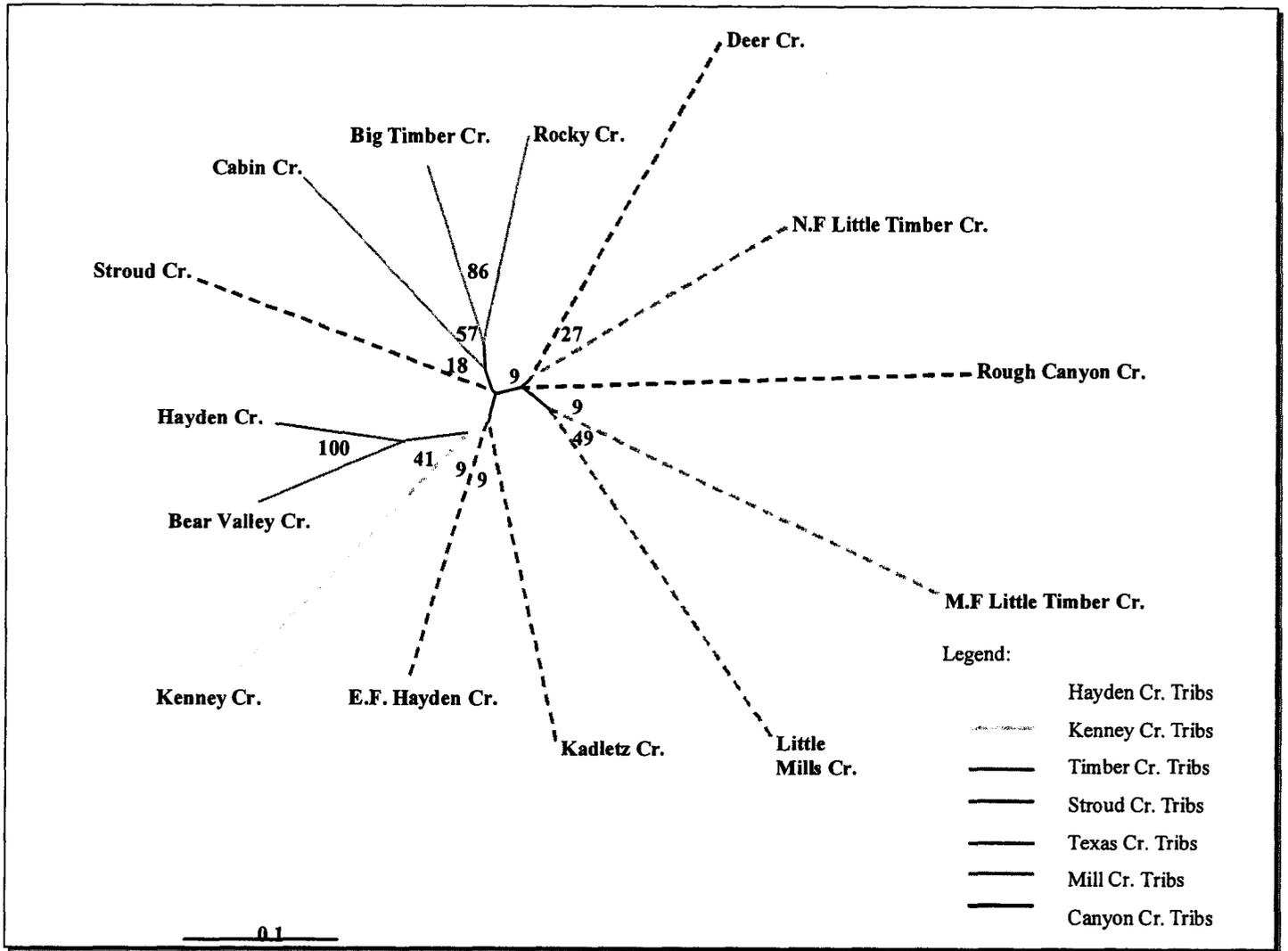


Figure 3: UPGMA dendrogram using  $D_{ce}$  for bull trout populations in the upper Salmon River basin. Each population is color-coded according to tributary location and dashed lines indicate seasonally disconnected streams.



## Literature Cited

- Allendorf, F. W., Ryman, N. 2002. The role of genetics in population viability analysis. In D.R. McCullough and S.R. Beissinger, editors. Population viability analysis. University of Chicago Press, Chicago.
- Angers, B., L. Bernatchez, A. Angers, and Desgroseillers, L.. 1995. Specific microsatellite loci for brook char reveal strong population subdivision on a microgeographic scale. *Journal of Fish Biology* 47(supplement A):177-185.
- Cavalli-Sforza, L. L., and Edwards, A.W.F. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 32:550-570.
- Consuegra, S., Vespoor, E., Knox, D., and de Leaniz, C.G. 2005. Assymmetric gene flow and the evolutionary maintenance of genetic diversity in small, peripheral Atlantic salmon populations. *Conservation Genetics* 6: 823-842.
- Corneut, J.-M., Piry, S., Luikart, G., Estoup, A., and Solignac M. 1999. Comparison of methods to select or exclude populations as origins of individuals. *Genetics* 153: 1989-2000.
- Crane, P.A., C.J. Lewis, E.J. Kretschmer, S.J. Miller, W.J. Spearman, A.L. DeCicco, M.J. Lisac, M.J. and Wenberg, J.K.. 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 Ardren, W.R. . 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.
- Dunham, J. and Rieman, B. 1999. Metapopulation structure in bull trout: influences of physical, biotic, and geometrical landscape characteristics. *Ecological Applications* 9: 642-655.
- Dunham, J. Rieman, B., and Chandler, G. 2003. Influences of temperature and environmental

variables on the distribution of bull trout within streams at the southern margin of its range. *North American Journal of Fisheries Management* 23: 894-904.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.

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/software/fstat.html>.

Hansen, M. M., Kenchington, E., and Nielsen, E.E.. 2001. Assigning individual fish to populations using microsatellite DNA markers. *Fish and Fisheries* 9:93-112.

Idaho Soil Conservation Commission. 1995. Model Watershed Plan – Lemhi, Pahsimeroi, and East Fork of the Salmon River. Report to Bonneville Power Administration..

Kanda, N., and Allendorf, F.W. 2001. Genetic population structure of bull trout from the Flathead River basin as shown by microsatellites and mitochondrial DNA markers. *Transactions of the American Fisheries Society* 130: 92-106.

Kozfkay, C. C., 2008. Assessment of bull trout genetic diversity and population structure in the upper Salmon River and Little Lost River basins. In Campbell and Kozfkay, *Native Species Investigations*, IDFG Report 08-01.

Lamperth, J., Claire, C.W., and Lutch, J. 2007. Fluvial bull trout *Salvelinus confluentus* migratory dynamics and life history in the Lemhi River sub-basin, Idaho. IDFG Report 07-11.

Langella, O. 2001. Populations 1.2.24. Population genetic structure (individuals or populations distances, phylogenetic trees).  
<http://www.pge.cnrs.gif.fr/bioinfo/populations/> (March 2005)

Nei, M., T. Maruyama, and Chakraborty, R.. 1975. The bottleneck effect and genetic variability in populations. *Evolution* 29:1–10.

- Norris A.T., Bradley D.G., Cunningham E.P. (1999). Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture* 180: 247–264.
- Page, R. D. M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12:357-358.
- Piry S., Alapetite A., Cornuet, J.-M., Paetkau D., Baudouin, L., Estoup, A. (2004) GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection. *Journal of Heredity* 95:536-539.
- Ray, C. 2001. Maintaining genetic diversity despite local extinctions: a spatial scaling problem. *Biological Conservation* 100: 3-14.
- Raymond, M., and F. Rousset. 1995. GENEPOP (v.1.2). A population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248-249.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.
- Rieman, B.E., Isaak, D., Adams, S., Horan, D., Nagel, D., Luce, C, and Myers, D. 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.
- Schoby, G.P., and Curet, T. 2007. Seasonal migrations of bull trout, westslope cutthroat trout, and rainbow trout in the upper Salmon River Basin, Idaho. IDFG Report 07-12.
- Selong, J.H., McMahon, T.E., Zale, A.V., and Barrows, F.T. 2001. Effect of temperature on growth and survival of bull trout ,with application of an improved method for determining thermal tolerance in fishes. *Transactions of the American Fisheries Society* 130: 1026-1037.

- Spencer C.C., Neigel J.E., Leberg P.L. (2000). Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology* 9: 1517-1528.
- Spruell, P., A. R. Hemmingsen, P. J. Howell, N. Kanda, and Allendorf, F.W. 2003. Conservation genetics of bull trout: geographic distribution of variation at microsatellite loci. *Conservation Genetics* 4: 17-29.
- U. S. Fish and Wildlife Service (USFWS). 1998. Final rule to list Columbia River and Klamath River population segments of the bull trout as a threatened species. *Federal Register* 63:31647-31674.
- U.S. Fish and Wildlife Service (USFWS). 2002. U. S. Fish and Wildlife Service. 2002. Chapter 17, Salmon River Recovery Unit, Idaho. 194 p. *In*: U.S. Fish and Wildlife Service. Bull Trout (*Salvelinus confluentus*) Draft Recovery Plan. Portland, Oregon.
- Watson, G. and Hillman, T.W. 1997. Factors affecting the distribution and abundance of bull trout: an investigation at hierarchical scales. *North American Journal of Fisheries Management* 17: 237-252.
- Weir, B. S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.

Prepared by:

Christine C. Kozick  
Sr. Fisheries Research Biologist

Matthew R. Campbell  
Genetics Lab Manager

Eric Trexler  
Fisheries Technician

Approved by:

IDAHO DEPARTMENT OF FISH AND GAME

  
Robert E. Schreyer, Chief  
Fisheries Bureau

  
William D. Horton  
State Fishery Manager