



NATIVE SPECIES INVESTIGATIONS

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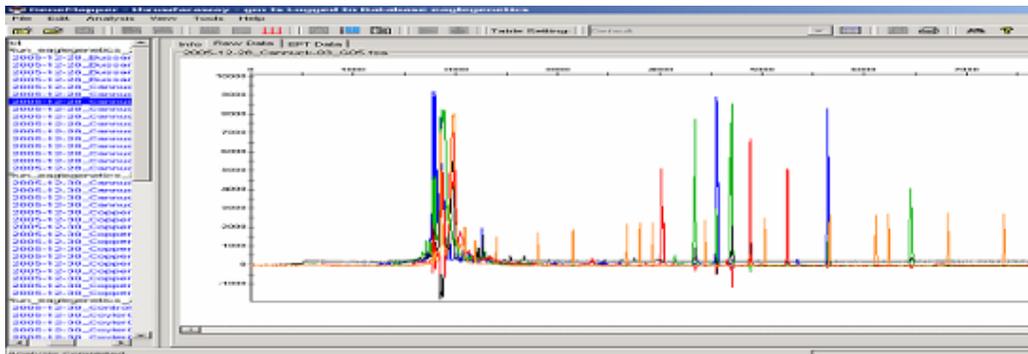


Figure 2. Example of multiplex electrophoresis run on 3100. Peaks in orange are size standards. Peaks in red, green, and blue are alleles at 8 different loci diagnostic between rainbow trout and cutthroat trout.

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Project 2: Native Species Investigations

- Subproject 1: Assessment Of Bull Trout Genetic Diversity And Population Structure in the Upper Salmon River and Little Lost River Basins**
- Subproject 2: Genetic Purity of Westslope Cutthroat Trout in the Moyie River, Idaho**
- Subproject 3: Origin and Purity of Yellowstone Cutthroat Trout Populations in the Henrys Fork Drainage**

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EXECUTIVE SUMMARY

Since the spring of 2002, Idaho Department of Fish and Game (IDFG) has operated a fish genetics laboratory to provide an efficient, cost-effective means of generating detailed genetic information necessary for the improved management and conservation of Idaho's native fish species. This report describes three research projects completed by the lab during the July 1, 2005 to June 30, 2006 contract period. The first project describes collaborative research with the USDA Forest Service examining bull trout in the upper Salmon River and Little Lost River. The project was successful in optimizing and standardizing a set of 12 new microsatellite loci with four other northwest genetics labs. These standardized loci were subsequently used to assess hybridization, genetic diversity, and genetic differentiation among bull trout populations in the study area. Hybridization from brook trout was not observed in any population. Genetic diversity of study populations was generally higher than what has been previously reported for bull trout using a different set of microsatellite loci and appeared uniform throughout upper Salmon River and Little Lost River basins. Genetic differentiation was high among all populations indicating little gene flow, likely a result of habitat fragmentation as well as specific life history characteristics of the species (strong homing fidelity when spawning). Results from this study should assist managers in prioritizing habitat restoration and stream reconnection projects.

The second project describes research assessing rainbow trout hybridization and introgression in westslope cutthroat trout populations in the Moyie River and Kootenai River drainages. This research is timely given that the Endangered Species Act (ESA) status of Westslope cutthroat trout is still in litigation. In total, 741 samples of *Oncorhynchus sp.* sampled from 11 tributaries that flow into the mainstem Moyie River, two tributaries to the mainstem Kootenai River, and a 15-mile stretch of the mainstem Kootenai River were genetically analyzed. A screen of eight codominant nuclear DNA (nDNA) markers and a mitochondrial DNA marker diagnostic between rainbow trout and cutthroat trout identified hybrids in the majority of tributaries sampled throughout the Moyie River drainage in Idaho. However, introgression levels (% rainbow trout [RBT] alleles detected out of total) were low. Substantially higher levels of hybridization and introgression were observed at two of the sample locations in the Kootenai River drainage. More research is needed to understand why hybridization/introgression levels differed between drainages, but these results should assist managers with the difficult task of determining what conservation and management status westslope cutthroat trout populations in these areas should receive.

The final project describes collaborative research with the Henrys Fork Foundation designed to assess the origin and genetic purity of seven Yellowstone cutthroat trout populations found above natural waterfalls in the Henrys Fork drainage. A total of 256 samples were screened with both diagnostic hybridization markers as well as with a Restriction Fragment Length Polymorphism (RFLP) mitochondrial DNA (mtDNA) marker. Genetic screening did not find any evidence of rainbow trout or westslope cutthroat trout hybridization/introgression in any of the sample locations examined. All samples exhibited genotypes/haplotypes indicative of pure Yellowstone cutthroat trout. Results of the mtDNA/RFLP analyses suggested that the most likely origin of these isolated Yellowstone cutthroat trout populations were from past hatchery introductions of Yellowstone Lake strain/origin Yellowstone cutthroat trout.

Several additional research projects that were worked on during the July 1, 2005 to June 30, 2006 contract period, but not detailed in this report, need to be mentioned. A collaborative project with Trout Unlimited was completed examining the genetic diversity and origin of rainbow trout within the Boise River. Results supported the utility of a mtDNA/RFLP assay for

assessing intraspecific *O. mykiss* hybridization (Campbell and Cegelski 2005) and indicated that the two populations analyzed were of mixed ancestry from multiple hatchery strains as well as native *O. mykiss* (<http://tedtruebloodtu.org/tuprojts.htm>). An additional project completed this year involved a genetic investigation of the two existing rainbow trout strains (Kamloops and R9s) raised at the IDFG Hayspur Hatchery. A screen of 13 microsatellite loci indicated that the two strains have remained genetically differentiated and that the R9 strain exhibited greater genetic diversity (heterozygosity and number of alleles per locus) than the Kamloops strain. These results should assist with future broodstock management decisions. Finally, a collaborative project with the Idaho Department of Environmental Quality was completed, examining the purity of *O. mykiss* in Threemile Creek, a tributary to the Clearwater River. Results indicated that samples collected above a waterfall on Threemile Creek (considered a likely migration barrier) exhibited an mtDNA haplotype common to native, interior *O. mykiss* populations. This haplotype was also the dominant haplotype observed in samples collected below the waterfall. However, the presence of three additional haplotypes, one of which has been observed previously in other nonnative hatchery populations we have screened, suggests that the population below the lowest barrier is likely of mixed ancestry, containing both native as well as nonnative hatchery haplotypes. Results of this study should be useful in the development of management plans for the Threemile Creek watershed.

JOB PERFORMANCE REPORT
SUBPROJECT #1: ASSESSMENT OF BULL TROUT GENETIC DIVERSITY AND
POPULATION STRUCTURE IN THE UPPER SALMON RIVER AND LITTLE LOST
RIVER BASINS

State of: Idaho Grant No.: F-73-R-25, Fishery Research
Project No.: 2 Title: Native Species Investigations
Subproject #1: Assessment of Bull Trout Genetic Diversity and Population Structure in the Upper Salmon River and Little Lost River Basins

Contract Period: July 1, 2005 to June 30, 2006

ABSTRACT

This report describes results from a collaborative study between the Bureau of Land Management and the Idaho Department of Fish and Game. There were three main objectives to this study. The first objective was to assist in a multilaboratory standardization effort to develop a new suite of microsatellite loci for bull trout. A second objective was to use this new suite of microsatellite loci to assess current levels of genetic diversity and genetic differentiation of bull trout in the upper Salmon River and Little Lost River recovery units. The last objective was to assess genetic relationships between bull trout in the Pahsimeroi River drainage and Little Lost River drainages and determine the colonization source and time of Little Lost River bull trout. Twelve microsatellite loci were chosen, with a wide range of characteristics useful for hybridization (between brook trout and bull trout and Dolly Varden and bull trout), phylogenetic, and population genetic structure studies. These loci also displayed higher levels of polymorphism than the previous set of loci. An investigation of bull trout genetic diversity and population structure with these loci indicated that genetic diversity levels were uniform throughout the upper Salmon River and Little Lost River basins, with the exception of three populations. Genetic differentiation was high among all of the sampled populations, indicating little gene flow among populations. This is likely due to both habitat fragmentation as well as specific life history characteristics of the species (strong homing fidelity when spawning). Because gene flow is needed to sustain core populations within these recovery units, efforts may be needed to reconnect fragmented tributaries. This study indicates that this genetic data coupled with assignment test methodologies will be useful for evaluating migration and success of reconnection efforts in the future. This study was not able to determine the source and colonization time for Little Lost River bull trout due to little resolution with both microsatellites and mitochondrial DNA. Therefore, future investigations may need to focus on additional genetic markers to address this objective.

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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 populations have declined throughout much of their range in the conterminous United States due to habitat degradation and fragmentation (Rieman and McIntyre 1993), blockage of migratory corridors (Neraas and Spruell 2001), and introductions of nonnative fishes such as brown trout *Salmo trutta*, lake trout *S. namaycush*, and brook trout *S. fontinalis* (Gunckel et al. 2002; Epifanio et al. 2003). While bull trout currently reside across a broad geographic range, many local populations of bull trout are small and isolated and may be susceptible to local extinctions. These concerns led to the listing of bull trout as threatened under the Endangered Species Act in 1999 (USFWS 1998).

In the 2002 draft recovery plan for bull trout, the USFWS indicates that the goal of recovery is to “ensure the long-term persistence of self-sustaining, complex interacting groups of bull trout distributed across the species’ native range so that the species can be de-listed” (USFWS 2002). To accomplish this over-arching goal, a hierarchy of population units was identified to cover different spatial scales: 1) recovery units, 2) core areas, and 3) local populations. In total, 27 recovery units were 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. Recovery units contained as few as one local population to multiple core areas and local populations.

The recovery unit of interest for this study was the Salmon River recovery unit. The Salmon River basin extends across central Idaho from the Snake River to the Montana border. Within this recovery unit, 10 core areas and 125 local populations were identified. The Salmon River basin is characterized by numerous fluvial disruptions due to irrigation withdrawals (Colvin 2005). Irrigation canals and water withdrawal can potentially disrupt natural stream flows and impact the migratory capabilities of fish during spawning. The degree of disruption and impact on native bull trout populations is not currently known for many stream networks and is likely dependent upon peak flow conditions.

Genetic analyses can aid in the management and recovery of bull trout. Two essential elements for recovery are the maintenance of genetic diversity and genetic exchange among local populations (USFWS 2002). Baseline levels of genetic diversity are thus needed in order to assess temporal changes in diversity. Genetic exchange among local populations can also be assessed using traditional genetic distance measures and newer assignment test methodologies. This information can be used to redefine core areas (local populations with partial migration) as well as evaluate the success of reconnection efforts. This may be especially important in the Pahsimeroi River drainage where most streams are seasonally disconnected from the mainstem river. Genetic assignment tests can potentially replace tagging data and help identify the population of origin (natal stream) for migrating fluvial bull trout captured in the mainstem Pahsimeroi River. However, newer assignment test methodologies require that each population be uniquely characterized (Hansen et al. 2001). The amount of genetic differentiation among populations and ability to assign migrants back to the population of origin are needed so that inferences can be made regarding migration.

Previous microsatellite analyses have provided limited resolution for bull trout population structure due to limited levels of genetic variation (Neraas and Spruell 2001; Spruell et al. 2003).

These microsatellite loci were not developed for bull trout, and polymorphism (variation) at a locus tends to decrease as a function of evolutionary distance from the source species (FitzSimmons et al. 1995). Therefore, the loci may be highly variable in the species they originated within but not as variable in bull trout. This could lead to incorrect inferences regarding genetic diversity and population relationships. With this knowledge, the first objective of this research was to assist in a multilaboratory standardization (Idaho Department of Fish and Game [IDFG]), United States Fish and Wildlife Service [USFWS], University of British Columbia [UBC], University of Montana [UM], Washington Department of Fish and Wildlife [WDFW]) effort to develop a new suite of microsatellite loci from a bull trout library with increased resolving power (increased number of alleles and heterozygosity). A second objective of this research was to use the new suite of microsatellite loci to assess current levels of genetic diversity and genetic differentiation of bull trout in the upper Salmon River and Little Lost River recovery units. The last objective was to assess genetic relationships between bull trout in the Pahsimeroi River drainage and Little Lost River drainages. It is not currently known whether bull trout were transplanted into the Little Lost River drainage from a population in Big Gulch Creek or if they historically colonized the Little Lost River drainage from the Salmon River drainage. Collectively, results from this study provide important baseline data and a better understanding of historic and current population relationships.

OBJECTIVES

1. Assist in a multi-laboratory standardization effort to develop a new suite of microsatellite loci from a bull trout library with increased resolving power (increased number of alleles and heterozygosity).
2. Assess current levels of genetic diversity and genetic differentiation of bull trout in the upper Salmon River and Little Lost River recovery units
3. Assess genetic relationships between bull trout in the Pahsimeroi River drainage and Little Lost River drainages

METHODS

Microsatellite Standardization Effort

In the fall of 2003, representatives from five laboratories met to discuss the selection of a core set of microsatellite loci that would be commonly used across laboratories. Both the USFWS and WDFW laboratories subcontracted with Genetic Identification Services (GIS) to build a microsatellite library specific for bull trout. A set of 33 microsatellite loci was obtained from these collaborations (DeHaan and Ardren 2005; C. Bettles, WDFW, unpublished data). A set of 22 microsatellite markers was selected for initial amplification based upon the following characteristics obtained from preliminary data generated by USFWS and WDFW: 1) robustness of the Polymerase Chain Reaction (PCR) amplification and ease and reliability of scoring, 2) polymorphism levels (number of alleles per locus and heterozygosity), 3) ability to differentiate bull trout from brook trout, 4) ability to differentiate bull trout from Dolly Varden, and 5) ability to distinguish interior bull trout populations from coastal bull trout populations and define conservation units such as Evolutionarily Significant Units (ESUs).

The next step of the standardization process involved distributing a set of 96 reference samples to all of the participating laboratories and subsequent genotyping of these samples with the 22 identified microsatellite loci. Data was submitted to the USFWS and aligned to determine the percent accuracy for each laboratory and identify any potential errors or problem loci. Problems such as null alleles (differential amplification and identification of a homozygous genotype that should be heterozygous), imperfect repeats, and nonspecific bands could arise and decrease the ease and reliability of scoring. Robustness of the PCR amplification was also a consideration, and a few laboratories were unable to successfully amplify certain loci. Overall, there was 90% concordance across laboratories for each individual locus.

The last step of the standardization process involved selecting a subset of the 22 microsatellite loci that would be the final core set of loci. A questionnaire was developed and sent to each laboratory that addressed how many loci should be included in the final set, how many should be diagnostic for bull trout and brook trout, how many should be diagnostic for bull trout and Dolly Varden, and how many should identify the interior/coastal break. Each laboratory was also asked to recommend and justify the microsatellite loci that they recommended. Results were summarized across laboratories and each of the above five characteristics were taken into consideration.

A core set of 12 microsatellite loci were chosen, five of which were diagnostic between bull trout and brook trout, two of which were diagnostic between bull trout and Dolly Varden, and five of which were also diagnostic for the inland/coastal split. The size ranges of the loci were also considered so that multiple loci could be electrophoresed together on the ABI 3100 DNA Analyzer (Applied Biosystems, Foster City, California.) (Table 1).

Table 1. Microsatellite panel and dye color, locus name, number of alleles observed per locus (A_o), observed heterozygosity (H_o), range observed in allele sizes and ability to differentiate bull trout from brook trout and Dolly Varden, and interior and coastal populations for each of the twelve microsatellite loci.

Panel (color):	Locus	A_o	H_o	range	Brook	Dolly	Int. vs. coastal
A (B)	Sco110	9	0.58	145-300	X		X
A (R)	Sco200	13	0.60	110-225			
A (Y)	Sfo18	1	0.00	120-190	X		X
B (G)	Omm1128	20	0.70	160-399	X	X	
B (B)	Sco109	26	0.81	180-500	X		
B (B)	Sco202	5	0.42	100-157			
B (R)	Sco215	3	0.34	250-350	X		
B (Y)	Sco220	16	0.74	200-400			
C (G)	Sco102	5	0.39	120-208			
C (B)	Sco212	12	n/a	210-340			X
C (R)	Smm22	12	0.85	135-350			
D (Y)	Sco105	9	0.64	118-230			X

Sampling and DNA Extraction

In 2004, 344 tissue samples were collected from 13 sample sites by IDFG and United States Forest Service (USFS) personnel as part of a larger study investigating native salmonid

abundance in the upper Salmon River basin. Samples were stored in 100% nondenatured ethanol until DNA extraction. DNA was extracted using a salt-chloroform method described by Paragamian et al. (1999).

Sample sizes and locations of the selected sample sites (n = 13) for genetic analyses are presented in Table 2 and Figure 1.

Table 2. Study site number, sampling location, and corresponding basin (core area), year sampled, sample size (N), average expected heterozygosity (H_e), and average number of alleles per locus (A).

Site #	Sampling Location	Basin (Core Area)	Year Sampled	N	H_e	A
1	Big Timber Creek	Lemhi River	2003	30	0.64	6.36
2	Kenny Creek	Lemhi River	2003	30	0.67	7.27
3	Badger Creek	Little Lost River	2003	30	0.58	3.73
4	Little Lost River	Little Lost River	2003	30	0.57	5.00
5	Big Gulch Creek	Pahsimeroi River	2003	20	0.31	2.36
6	Burnt Creek	Pahsimeroi River	2003	30	0.52	5.27
7	North Fork Big Creek	Pahsimeroi River	2003	30	0.69	7.27
8	Patterson Creek	Pahsimeroi River	2003	29	0.65	6.27
9	West Fork Pahsimeroi	Pahsimeroi River	2003	4	0.68	3.91
10	Germania Creek	EF Salmon River	2004	30	0.42	3.73
11	Opal Lake	Opal Lake	2004	30	0.31	3.55
12	upper Lick Creek	Salmon	2004	21	0.55	4.91
13	West Pass Creek	EF Salmon River	2004	30	0.58	5.45

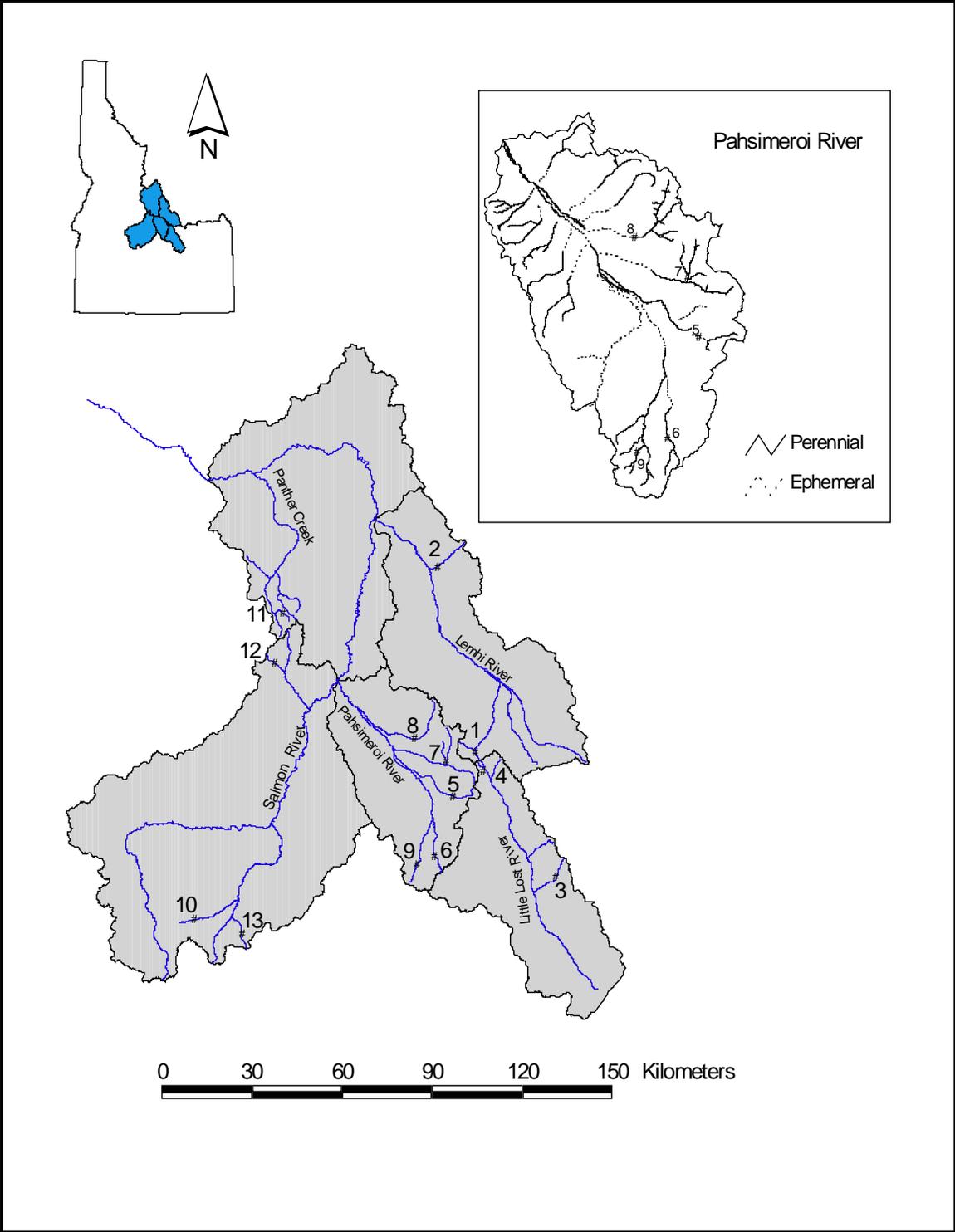


Figure 1. Sampling locations for the 13 bull trout populations analyzed. The zoomed in figure shows current connectivity in the Pahrump River drainage (Colvin 2005).

Microsatellite Amplification

All of the samples were amplified with the core set of 12 microsatellite loci. A 15 μ l PCR reaction was conducted for each locus using 2 μ l of DNA extract (unknown concentration). PCR reaction conditions are presented in Table 3 and thermal cycler profiles are presented in Table 4.

Table 3. PCR reaction conditions for the 12 microsatellite loci. Locus name, reaction volume of PCR, amount of extracted DNA (extract), taq buffer, MgCl₂, dNPT's, primers, taq polymerase, and H₂O in each reaction.

Locus	Reaction Volume	Extract	10X Taq buffer	MgCl ₂	dNPT's	Primers	Taq Polymerase	H ₂ O
Sco110	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sco200	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sfo18	15 μ L	2 μ L	1.0 X	2.00mM	0.20mM	0.50mM	0.03U/ μ L	8.40 μ L
Omm1128	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sco109	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sco202	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sco215	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sco220	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sco102	15 μ L	1 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	9.70 μ L
Sco212	15 μ L	2 μ L	1.0 X	2.50mM	0.20mM	0.50mM	0.03U/ μ L	8.10 μ L
Smm22	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L
Sco105	15 μ L	2 μ L	1.0 X	1.50mM	0.20mM	0.50mM	0.03U/ μ L	8.70 μ L

Table 4. Thermocycler profiles for the 12 microsatellite loci. Locus name, temperature, and time for each step: initial denaturation, denaturation, annealing, and extension. Number of total amplification cycles. Temperature and time for final extension step and cool down step.

Locus	Initial denaturation		Denaturation		Annealing		Extension		# of cycles	Extension		Cool Down	
	Temp	Time	Temp	Time	Temp	Time	Temp	Time		Temp	Time	Temp	Time
Sco110	94°C	3:00 min	94°C	0:30 min	55°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco200	94°C	3:00 min	94°C	0:30 min	60°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sfo18	94°C	3:00 min	94°C	0:30 min	55°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Omm1128	94°C	3:00 min	94°C	0:30 min	50°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco109	94°C	3:00 min	94°C	0:30 min	55°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco202	94°C	3:00 min	94°C	0:30 min	60°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco215	94°C	3:00 min	94°C	0:30 min	55°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco220	94°C	3:00 min	94°C	0:30 min	60°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco102	94°C	3:00 min	94°C	0:30 min	59°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco212	94°C	3:00 min	94°C	0:30 min	60°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Smm22	94°C	3:00 min	94°C	0:30 min	55°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min
Sco105	94°C	3:00 min	94°C	0:30 min	55°C	0:30 min	72°C	0:30 min	37	72°C	7:00 min	4°C	10:00 min

All PCR products were electrophoresed using an ABI 3100 automated sequencer (Applied Biosystems) platform. PCR products from Sco110, Sco200, and Sfo18 were electrophoresed together, PCR products from Omm1128, Sco109, Sco202, and Sco220 were electrophoresed together, PCR products from Sco102 and Smm22 were electrophoresed together, and Sco105 was electrophoresed alone. One μl of each PCR product was added to 0.35 μl Liz Size Standard and 30 μl of Formamide. Fragments were sized against GS500 ROX size standard (Applied Biosystems) using GENESCAN version 3.1 and GENEMAPPER v. 3.5.1 software (Applied Biosystems).

Microsatellite Data 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), observed heterozygosity (H_o), and expected heterozygosity (H_e) using FSTAT version 2.9.3 (Goudet 2001). Pairwise F_{ST} estimates (Weir and Cockerham 1984) were generated using Arlequin 2.0 with significance based upon a permutation process. 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.

The jackknife procedure of WHICHRUN 3.2 (Banks and Eichert 2000) was used to assign fish back to their population of origin. This procedure empirically evaluates allocation success and whether there will be enough resolution to assign potential migrants back to their population of origin. All fish with greater than six loci were included in this analysis and the proportion of fish assigned correctly to the population of origin was summarized for each population. The West Fork Pahsimeroi location was excluded from this analysis since only four samples were analyzed, and they do not provide an adequate baseline for genetic diversity.

Mitochondrial DNA Analysis

A subset of the samples was used within the mitochondrial DNA (mtDNA) analysis (Badger Creek, $N = 5$; Big Gulch Creek, $N = 5$; Little Lost River, $N = 5$; NF Big Creek, $N = 5$; Patterson Creek, $N = 5$; and WF Pahsimeroi River, $N = 4$). The cytochrome b (cyt-b) mitochondrial DNA gene region was amplified following procedures described by Mays (2001). Primers flanking the cyt-b gene region, (cyt b-765) 5'- GAA AAA CCA YCG TTG TWA TTC AAC T -3' and (cyt b-766) 5'- GTT TAA TTA GAA TYT YAG CTT TGG G -3' were purchased from Integrated DNA Technology (Coralville, Iowa). Amplifications were performed in a 40 μl reaction consisting of 0.5-3.0 μl DNA extract, 4.0 μl 10X buffer (Perkin Elmer), 4.0 μl MgCl_2 , 3.2 μl BSA, 1.0 μl DMSO, 4.0 μl of each primer, 3.2 μl 10.0 mM dNTPs (10mM each of dATP, dCTP, dGTP, and dTTP), 0.15 μl Taq polymerase (Perkin-Elmer). Polymerase chain reaction conditions consisted of an initial denaturing cycle of 95°C for 3 minutes, followed by 39 cycles of denaturation at 95°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 70°C for 2 minutes 30 seconds, with a final extension at 70°C for 3 minutes.

Approximately 1300 bp of the *cyt-b* gene region were sequenced. Sequencing reactions were performed with a BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems) using the forward (765) and reverse (766) primers. Sequenced products were cleaned using gel filtration plates (Edge Biosystems) and were run out on a Prism 3730 DNA sequencer (Applied Biosystems). Sequences were edited and aligned using Sequencher (version 4.1.2, Gene Codes Corporation).

RESULTS

Tests for Hardy-Weinberg equilibrium revealed that genotypes were in expected proportions except for 22 of the 156 tests. Seven of the 22 rejected tests clustered by locus Sco212, suggesting significant departures from Hardy-Weinberg equilibrium. This locus was also hard to amplify (58% success rate); therefore, it is likely that there are problems with the amplification and null alleles at this locus. This locus was dropped from further analyses. There was no association between the other rejected tests and a locus or population. A total of 858 tests for linkage disequilibrium were performed and 85 of the tests were rejected at $\alpha = 0.05$, which was slightly higher than expected by chance (42.9 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.

Genetic Diversity

The total number of alleles per locus observed ranged from one allele for Sfo18 to 26 alleles for Sco109. Expected heterozygosity ranged from 0.00 for locus Sfo18 to 81% for locus Sco109.

The average number of alleles per sample location ranged from 2.36 in Big Gulch Creek to 12.18 in West Pass Creek, and heterozygosity levels ranged from 31% in Big Gulch Creek to 69% in NF Big Creek (Table 2). Genetic diversity was relatively uniform across sample locations with the exceptions of Opal Lake, Germania Creek, and Big Gulch Creek. These populations had lower levels of genetic diversity and heterozygosity. In the West Fork Pahsimeroi population, only four samples were collected, so genetic diversity may not be accurate. Surprisingly, this population still had higher levels of genetic diversity than the three populations listed above.

Genetic Differentiation

Differences in allele frequencies indicate that there are significant genetic differences among populations. F_{ST} estimates ranged from 0.01 to 0.55 (Table 5). A UPGMA tree based on D_{ce} was used to visualize the genetic relationships among populations (Figure 2). The dendrogram suggests that all of the populations are highly differentiated from one another. Populations in the Lemhi drainage clustered with one another, but not all of the Pahsimeroi populations clustered together. The Big Gulch Creek population did not cluster with the other Pahsimeroi populations. However, there was not strong statistical support in that bootstrap levels were less than 50% for all branches.

The program WHICHRUN was used to assess assignment success for each study population (WF Pahsimeroi River was excluded due to insufficient sample sizes). Assignment probabilities ranged from 79% to 100%. The ability to correctly assign bull trout to the population

of origin was over 95% for all of the populations, except for Big Timber Creek, NF Big Creek, and Patterson Creek (Table 6).

Hybridization

No brook trout alleles were observed at any of the five diagnostic loci.

Mitochondrial DNA Analysis

Only one haplotype was observed within all of the samples analyzed.

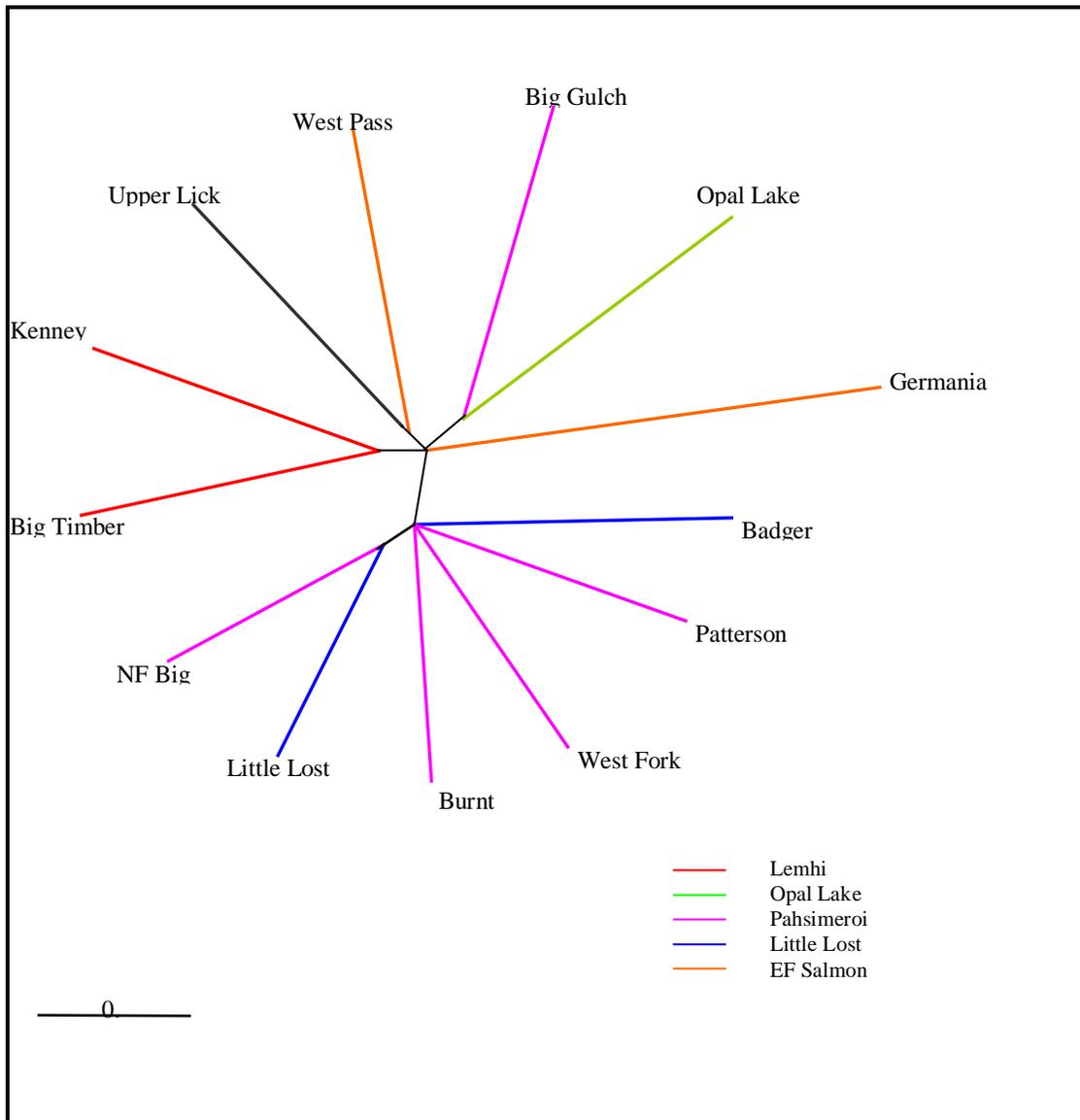


Figure 2. Unrooted UPGMA tree of genetic relationships among 13 Bull trout populations from the Upper Salmon River and Little Lost River Basins, based on Cavalli-Sforza and Edwards (1967) chord distance.

Table 5. Pairwise F_{ST} estimates for the 13 sampled bull trout populations.

	Big Timber Cr.	Kenny Cr.	Badger Cr.	Little Lost R.	Big Gulch Cr.	Burnt Cr.	NF Big Cr.	Patterson Cr.	WF Pahsimeroi R.	Germania Cr.	Opal Lake	Upper Lick Cr.
Kenny Cr.	0.11											
Badger Cr.	0.15	0.18										
Little Lost River	0.14	0.15	0.15									
Big Gulch Cr.	0.34	0.28	0.34	0.35								
Burnt Cr.	0.18	0.17	0.15	0.15	0.31							
NF Big Cr.	0.10	0.08	0.10	0.07	0.28	0.10						
Patterson Cr.	0.09	0.11	0.11	0.14	0.29	0.11	0.06					
WF Pahsimeroi River	0.14	0.09	0.12	0.09	0.41	0.10	0.01	0.08				
Germania Cr.	0.28	0.24	0.33	0.23	0.56	0.35	0.19	0.29	0.26			
Opal Lake	0.36	0.30	0.35	0.41	0.44	0.42	0.31	0.34	0.42	0.52		
Upper Lick Cr.	0.20	0.13	0.26	0.23	0.36	0.23	0.15	0.17	0.15	0.32	0.37	
West Pass Cr.	0.19	0.18	0.19	0.17	0.40	0.22	0.08	0.16	0.10	0.22	0.33	0.19

Table 6. Assignment allocation results for the “jackknife” procedure in WHICHRUN. The assignments are presented in each row for each location. The bolded values on the diagonal indicate the proportion of individuals that were assigned back to the population of origin.

	Big Timber Cr.	Kenny Cr.	Badger Cr.	Little Lost R.	Big Gulch Cr.	Burnt Cr.	NF Big Cr.	Patterson Cr.	Germania Cr.	Opal Lake	Upper Lick Cr.	West Pass Cr.
Big Timber Cr.	0.86	~	~	~	~	~	~	0.10	~	~	~	0.03
Kenny Cr.	~	1.00	~	~	~	~	~	~	~	~	~	~
Badger Cr.	~	~	1.00	~	~	~	~	~	~	~	~	~
Little Lost R.	~	~	~	1.00	~	~	~	~	~	~	~	~
Big Gulch Cr.	~	~	~	~	1.00	~	~	~	~	~	~	~
Burnt Cr.	~	~	0.03	~	0.03	0.93	~	~	~	~	~	~
NF Big Cr.	~	~	~	0.11	~	~	0.79	0.04	0.03	0.03	~	~
Patterson Cr.	~	~	~	~	~	0.13	0.04	0.83	~	~	~	~
Germania Cr.	~	~	~	~	~	~	~	~	1.00	~	~	~
Opal Lake Cr.	~	~	~	~	~	~	~	~	~	1.00	~	~
Upper Lick Cr.	~	~	~	~	~	~	~	~	~	~	0.95	0.05
West Pass Cr.	~	~	~	~	~	~	~	~	~	~	~	1.00

DISCUSSION

Previous genetic studies have identified bull trout as a species with low levels of genetic variation (Neraas and Spruell 2001; Spruell et al. 2003). Bull trout may still have lower levels of diversity compared to other salmonids (Bettles et al. 2005); however, it is not as low as previously thought. For example, the average heterozygosity observed by Spruell et al. (2003) in 65 bull trout populations from the northwestern United States using four microsatellite loci was 0.186. Heterozygosity values in 21 bull trout populations from the Boise basin ranged from 0.019 to 0.335 (Whiteley et al. 2003). The average heterozygosity of the 13 populations examined in this study was 0.552. Therefore, it appears as if the new suite of microsatellite loci reveal increased levels of genetic variation compared to the former loci. 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. The levels of genetic variation observed in this study were similar to those recently reported by other researchers using a subset of the same loci (Bettles et al. 2005; P. DeHaan, USFWS, unpublished data).

While all of the populations examined in this study generally had higher levels of genetic diversity than previously reported for bull trout using a different set of microsatellite loci, diversity was not uniform among sites. Lower levels of genetic variation were detected in Opal Lake, Big Gulch Creek, and Germania Creek. This is most likely due to random genetic drift. Random genetic drift can reduce levels of diversity in small, isolated populations. Opal Lake is a naturally isolated population (formed by a landslide) with no overland outlet (USFWS 2002). Currently, the Opal Lake population is the only local population in the entire Opal Lake core area, and abundance estimates indicate that less than 500 fish support this population (USFWS 2002). Therefore, reduced levels of genetic diversity in this population are likely a function of random genetic drift. Big Gulch Creek and Germania Creek also appear to be isolated. Germania Creek is isolated above a natural barrier (USFWS 2002), and Big Gulch Creek has been isolated due to anthropogenic habitat alterations for approximately 100 years or more (Bart Gamett, USFS, and Kate Forster, BLM, personal communication).

Even in connected habitats, bull trout have been characterized as having low levels of genetic diversity within streams and high levels of genetic divergence between streams. These results indicate that bull trout have a high fidelity to natal streams and that life history is an important factor shaping population genetic structure. The patterns of genetic structure observed in this study were consistent with previous findings. Pairwise F_{ST} estimates were high among all comparisons, suggesting very low levels of gene flow among sample locations. Low gene flow is probably due to a combination of natural life history characteristics and anthropogenic causes. Numerous irrigation diversions and dewatered areas throughout the upper Salmon River basin have likely contributed to the reduction and fragmentation of populations (Colvin 2005). Ongoing efforts to reconnect tributary streams to the mainstem Pahsimeroi River may ultimately assist in increasing gene flow among populations and restoring migratory populations in this core recovery area.

In the Lemhi River, Little Lost River, and EF Salmon River drainages, only two populations were analyzed from each of the drainages and our initial results indicate that the populations within each of these drainages also exhibit high levels of genetic differentiation from one another, indicating low gene flow. We plan to analyze additional samples from the Lemhi River in 2008 (with the same set of core microsatellite loci), which should allow a more thorough description of the genetic structure of bull trout populations in this drainage.

Assignment tests can be used to assign adfluvial bull trout to natal streams and further understand spawning migrations. However, sufficient statistical power is needed to confirm that an individual is indeed a migrant and not just assigned by chance. Statistical power is determined by the amount of genetic differentiation among populations, number of sampled populations, degree of polymorphism at the loci, number of sampled loci, and sample size (Hansen et al. 2001). In this study, we were able to assign fish back to the population of origin >93% for the majority of the populations. This was largely based upon the high levels of genetic differentiation among populations ($F_{ST} \sim 0.10$ to 0.55). Therefore, this method shows promise in addressing migration. Three of the populations had lower self-assignment success. For these populations, it may be necessary to increase the number of loci or incorporate a new sampling design. For example, the low assignment power of NF Big Creek (Pahsimeroi River) was due to some assignment of these fish to the Little Lost River (Little Lost River). However, if the fish were sampled within the Pahsimeroi basin, it may not be necessary to include Little Lost River sampling locations in the baseline (since it is unlikely that they came from these locations). In this analysis, fish will also be assigned to the most likely population even if the true population of origin is missing from the baseline. Therefore, an increased baseline will be needed in the future to increase assignment accuracy and precision. Temporal samples may also be important to assess the stability of allele frequencies over time and utility of this baseline for future work.

Genetic drift can exaggerate historical levels of genetic variation and alter population relationships. This may be reflected within the neighbor-joining tree. The Big Gulch Creek populations did not cluster according to drainage location. An examination of allele frequency distributions indicated that this population was fixed or nearly fixed for one allele at many of the loci. The impacts of genetic drift within this population likely confound its historical relationship. Previously, it has been suggested that the origin of bull trout in the Little Lost River may have been from Big Gulch Creek, either from an historic canal connecting the drainages or from an earlier transplantation (Bart Gamett, personal communication). At this point, we are unable to use microsatellites to address this question since the Big Gulch Creek population had such low levels of diversity. Additional loci may increase statistical confidence (bootstrap support) and provide guidance as to other likely source populations.

Mitochondrial DNA analyses were also used in an attempt to assess historical relationships between bull trout in Big Gulch Creek and the Little Lost River. However, since only one haplotype was detected in all of the samples examined, we were unable to identify a likely source population for bull trout in the Little Lost River. A lack of mitochondrial DNA diversity observed among all of these populations is consistent with recent colonization of the upper Salmon River basin from a single Wisconsinan glacial refuge, possibly located in the lower Columbia River (Haas 1988). However, a possible lower mutation rate for the Cytochrome b gene region or strong genetic drift may also have contributed to the fixation of mtDNA variation at this locus. Analyses with additional mtDNA gene regions (and samples) may help determine which of these factors have influenced present patterns of genetic diversity, and may also help to resolve the question of origin of bull trout in the Little Lost River.

RECOMMENDATIONS

1. Coordinate sampling efforts in the upper Salmon River to increase populations and samples per population in the baseline.

2. Consider screening the samples described in this report with additional mtDNA gene regions to help determine what factors have influenced present patterns of genetic diversity, and attempt to resolve the question of origin of bull trout in the Little Lost River.
3. Continue collaborative, multi-laboratory efforts to describe genetic variation and structure of bull trout across their native range with the standardized set of microsatellite loci.

JOB PERFORMANCE REPORT
SUBPROJECT #2: GENETIC PURITY OF WESTSLOPE CUTTHROAT TROUT IN THE
MOYIE RIVER, IDAHO

State of: Idaho Grant No.: F-73-R-25, Fishery Research
Project No.: 2 Title: Native Species Investigations
Subproject #2: Genetic purity of westslope cutthroat
trout in the Moyie River, Idaho
Contract Period: July 1, 2005 to June 30, 2006

ABSTRACT

This project describes research assessing rainbow trout hybridization and introgression in westslope cutthroat trout populations in the Moyie River and Kootenai River drainages. This research is timely given that the Endangered Species Act (ESA) status of westslope cutthroat trout is still in litigation. In total, 741 samples of *Oncorhynchus* sp. sampled from 11 tributaries that flow into the mainstem Moyie River, two tributaries to the mainstem Kootenai River, and a 15-mile stretch of the mainstem Kootenai River were genetically analyzed. A screen of eight codominant nuclear DNA (nDNA) markers and a mitochondrial DNA (mtDNA) marker diagnostic between rainbow trout and cutthroat trout identified hybrids in the majority of tributaries sampled throughout the Moyie River drainage in Idaho. However, introgression levels (% RBT alleles detected out of total) were low (no sites with >2.34%). Substantially higher levels of hybridization and introgression were observed at two of the sample locations in the Kootenai River drainage. More research is needed to understand why hybridization/introgression levels differed between drainages, but these results should assist managers with the difficult task of determining what conservation and management status westslope cutthroat trout populations in these areas should receive.

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INTRODUCTION

Although the U.S. Fish and Wildlife Service (USFWS) determined on August 7, 2003 that listing of the westslope cutthroat trout (WCT) *Oncorhynchus clarkii lewisi* as a threatened or endangered species under the Act was not warranted (68 FR 68152), the finding remains under litigation. The primary argument in appeals to the ruling has been that the USFWS inappropriately included hybridized populations as westslope cutthroat trout in the unit considered for listing. Much of the current legal and scientific debate is focused on how hybridization/introgression levels should be assessed (morphologically versus genetically) and whether any level of hybridization/introgression should preclude a population from ESA consideration.

In response to this ongoing litigation, genetically characterizing populations across the species' range remains a high priority for both State and Federal agencies that oversee management and conservation of the species. This study focuses on describing the extent of hybridization/introgression in 11 tributaries that flow into the mainstem Moyie River, two tributaries to the mainstem Kootenai River, and a 15 mile stretch of the mainstem Kootenai River between river miles 244 and 257 (Figure 3). These sample locations are important because many have not been previously screened for rainbow trout (RBT) *O. mykiss* hybridization/introgression or have not been screened recently. In addition, recent research in the upper Kootenay River Basin in Canada suggests that hybridization is increasing and that hybrid swarms are likely to develop (Rubidge et al. 2004).

METHODS

All samples were screened with eight codominant nuclear DNA (nDNA) markers (Occ16, Occ34, Occ35, Occ36, Occ37, Occ38, Occ42, and OM55) diagnostic between rainbow trout and cutthroat trout (Ostberg and Rodriguez 2002; 2004). Recently, we have developed procedures that allow us to amplify all eight nDNA loci in one Polymerase Chain Reaction (PCR) amplification and electrophorese all eight loci in one run on a 3100 DNA fragment analyzer/sequencer (Figure 4). This has dramatically improved the speed and efficiency in screening populations for rainbow trout hybridization and introgression. In addition to being diagnostic between rainbow trout and cutthroat trout, one of the nDNA markers (OM55) is also diagnostic between westslope cutthroat trout and Yellowstone cutthroat trout (YCT) *O. clarkii bouvierii*, allowing assessment of intraspecific hybridization between these two subspecies. All samples were also screened with a mitochondrial DNA (mtDNA) marker diagnostic between all three taxa (D-loop digested with the restriction enzyme *Rsa-I*).

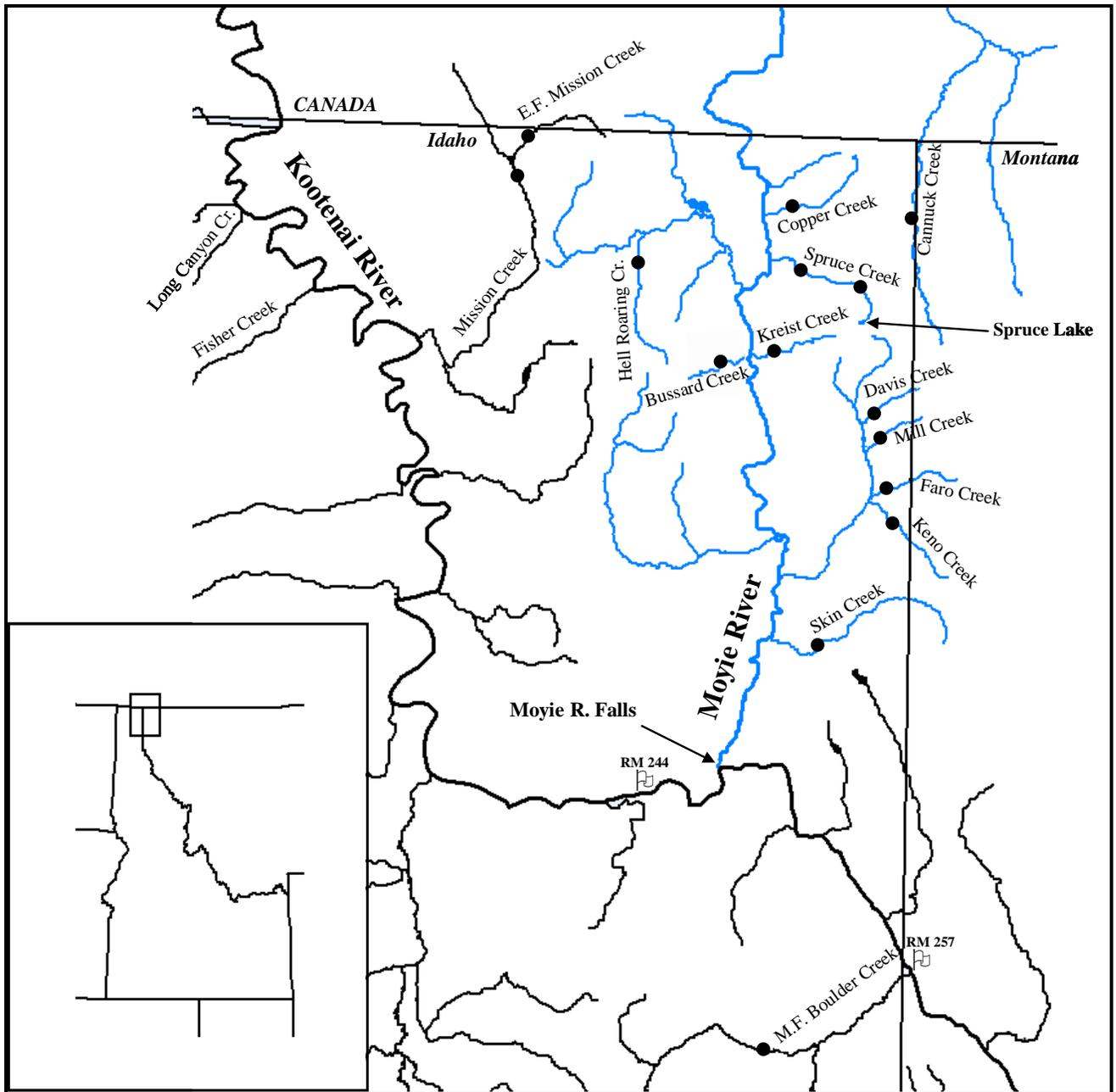


Figure 3. Sample locations from 11 tributaries that flow into the mainstem Moyie River (in blue), two tributaries to the mainstem Kootenai River, and a 15 mile stretch of the mainstem Kootenai River between River mile 244 and 257.

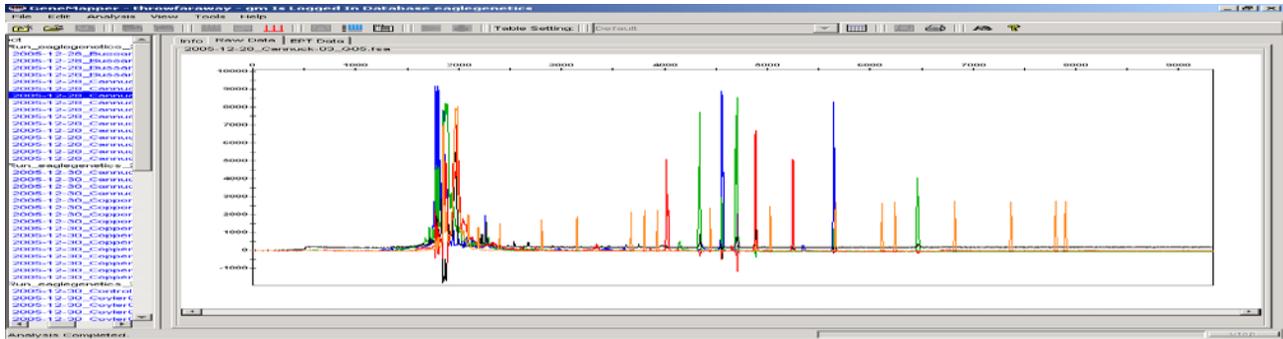


Figure 4. Example of multiplex electrophoresis run on 3100. Peaks in orange are size standards. Peaks in red, green, and blue are alleles at eight different loci.

Individual hybridization sample classification was based on composite nDNA and mtDNA genotypes following procedures outlined by Ostberg and Rodriguez (2006) and Kozfkay et al. (2007). Samples were classified as “cutthroat trout-like” if they were homozygous for cutthroat alleles at all loci, “rainbow trout-like” if they were homozygous for rainbow trout at all loci, and “hybrid” if they possessed a mixture of alleles from the two parental species. Hybrids were further classified into two categories: first-generation hybrids (F_1) if they were heterozygous at all loci, and later-generation hybrids ($>F_1$) if they possessed a mix of heterozygous and homozygous loci. With eight codominant nDNA loci, our probability of mistaking a more advanced backcross hybrid ($>F_1$) as an F_1 hybrid is less than 1% (Boecklen and Howard 1997). Introgression levels at each site was reported as the number of rainbow trout alleles observed in fish classified as cutthroat trout-like and $>F_1$ hybrids out of the total alleles examined.

RESULTS

We identified hybrids in seven of the 11 Moyie River tributaries examined (Table 7). Two of these creeks (Keno and Spruce) had previously been identified as having pure WCT (USFWS 1999). Three hybrids were identified in Keno Creek (all $>F_1$). Of the three, two had RBT mtDNA and one had WCT mtDNA. All three were identified in reach 1, the lowest elevation site (~950 meters). Only fish with genotypes indicative of WCT were found in the two reaches sampled higher up in the drainage.

Table 7. Sample location, sample size, # of genotypes indicative of westslope cutthroat trout (cutthroat trout-like), rainbow trout (rainbow trout-like), >F₁ hybrids, and F₁ hybrids, and percent rainbow trout introgression, detected among the 15 sampled creeks in the Moyie River and Kootenai River drainages, Idaho.

Sample Location	Sample Size	Cutthroat Trout-like	Rainbow Trout-like	>F ₁ Hybrid	F ₁ Hybrid	% RBT Introgression
Bussard Creek	36	31	0	5	0	0.77%
Cannuck Creek	50	50	0	0	0	None
Copper Creek	50	50	0	0	0	None
Davis Creek	50	50	0	0	0	None
Faro Creek	41	37	0	4	0	0.85%
Keno Creek	50	47	0	3	0	1.25%
Kreist Creek	35	31	0	4	0	0.63%
Mill Creek	50	50	0	0	0	None
Hell Roaring Cr.	50	46	0	4	0	0.79%
Skin Creek	50	49	0	1	0	None*
Spruce Creek	50	38	0	12**	0	2.34%
Mission Creek	50	50	0	0	0	None
EF Mission Creek	50	49	0	1	0	0.14%
MF Boulder Creek	50	2	11	35	2	N/A
Mainstem Kootenai R.	79	5	54	16	4	N/A

* 1 individual was identified as a hybrid with mtDNA of RBT.

** Of the 12 >F₁ hybrids identified, 3 were identified with both WCT and YCT alleles, 1 was identified with WCT, YCT, and RBT alleles, and the remaining 8 individuals were identified with WCT and RBT alleles.

Spruce Lake, at the headwaters of Spruce Creek, has been stocked with both rainbow trout and Yellowstone cutthroat trout, and concerns have been previously expressed about the potential of hybridization from downstream migration (USFWS 1999). We identified 12 >F₁ hybrids in Spruce Creek. This was the highest number of hybrids identified at any site within the Moyie River drainage. Three were identified with both WCT and YCT alleles, one was identified with WCT, YCT, and RBT alleles, and the remaining eight were identified with WCT and RBT alleles. Eight of the hybrids (and all of the individuals with YCT alleles) were identified above a culvert, just below the lake. Interestingly, one fish sampled above the culvert was identified with extra large posterior spots, a pattern common in Yellowstone cutthroat trout. Fish sampled in Roaring Creek, a tributary to the Middle Fork of the Salmon River, were also identified as having especially large posterior spots, and were also later confirmed as YCT X WCT hybrids (Campbell and Cegelski 2002).

Of the two sites in which hybrids had been identified in previous allozyme studies, we confirmed hybridization in one (Skin Creek). However, we did not identify RBT nDNA introgression. The single hybrid identified was homozygous for WCT alleles at all loci, but contained RBT mtDNA, indicative of a multigenerational backcross hybrid. Alleles characteristic of both westslope cutthroat trout and Yellowstone cutthroat trout were previously identified in 25 samples from Copper Creek (USFWS 1999). We did not detect any hybrids among the 50 fish sampled from Copper Creek. All samples were collected below Copper Falls (26 meters high) within about 1.6 km from the mouth. No fish were identified in the stream reach sampled above the falls.

Of the remaining seven Moyie River tributaries screened in this study, four were identified with hybrids (Bussard Creek, Faro Creek, Kriest Creek, and Hell Roaring Creek). We found no reports indicating that any of these four creeks had ever been genetically screened for hybridization. All of the hybrids identified were $>F_1$ and all contained WCT mtDNA. No more than two rainbow trout alleles were observed in any individual's genotype (16 total alleles examined), and no fish exhibited any loci homozygous for RBT alleles. This suggests that all of these fish are likely multiple generational backcrosses (F_3 or greater). As expected, corresponding population introgression levels were low (0.14%-0.79%). No hybrids were found in Canuck Creek, Davis Creek, or Mill Creek. Only one of these tributaries appears to have been previously genetically screened for hybridization. Samples from Canuck Creek were analyzed with allozymes in 1994 and were also found to be pure WCT (USFWS 1999).

Of the four sample locations examined outside the Moyie River drainage, the two from the Mission Creek drainage also exhibited low levels of hybridization/introgression, with only one hybrid ($>F_1$) identified in EF Mission Creek (possessing WCT mtDNA). In contrast, the other two sites exhibited very different patterns and much higher levels of hybridization. Of the 79 samples from the mainstem Kootenai River, 16 possessed genotypes indicative of $>F_1$ hybrids, 4 of F_1 hybrids, 5 of WCT and 54 of RBT. Hybrids exhibited both RBT and WCT mtDNA, although the frequency with RBT mtDNA was higher in both F_1 hybrids (3 versus 1) and $>F_1$ hybrids (12 versus 4). Observed hybridization levels in the MF Boulder Creek were even higher than those observed in the mainstem Kootenai River. Of the 50 samples screened, 35 possessed genotypes indicative of $>F_1$ hybrids, 2 of F_1 hybrids, 2 of WCT, and 11 of RBT. Of the 35 $>F_1$ hybrids, 30 exhibited RBT mtDNA and 5 exhibited WCT mtDNA. One F_1 hybrid exhibited WCT mtDNA and the other exhibited RBT mtDNA. Tests on both populations for genotypic linkage disequilibrium (GENEPOP; Raymond and Rousset 1995) were rejected.

DISCUSSION

Westslope cutthroat trout are the only *Oncorhynchus* species native to Moyie River drainage. Moyie River Falls likely prevented natural invasion of rainbow trout similar to other barrier falls found on the Spokane, Pend Oreille, Snake, and Upper Kootenai rivers (Behnke 1992). However, also similar to these other river drainages, the Moyie River drainage has been stocked extensively with nonnative trout (primarily rainbow trout, but also Yellowstone cutthroat trout and out-of-basin westslope cutthroat trout). The British Columbia Ministry of Fisheries has stocked Moyie Lake since the early 1920s (Fisheries Inventory Data Queries, <http://srmapps.gov.bc.ca/apps/fidq/>). The Idaho Department of Fish and Game stocked the Moyie River from the early 1970s through the mid 1990s (<http://fishandgame.idaho.gov/apps/stocking/>), and apparently there are now self-reproducing rainbow trout populations in parts of the drainage (Jody Walters, IDFG, personal communication).

Previously, it has been suggested that allopatric westslope cutthroat trout populations are especially vulnerable to hybridization with rainbow trout because they have not developed the same reproductive isolating mechanisms that maintain species' integrity in sympatric populations (Behnke 1992). Additionally, research has indicated that hybridization is increasing in magnitude and distribution in the upper Kootenay River drainage in Canada (Rubidge and Taylor 2005). Given the long history of nonnative trout stocking, and the presence of some naturally reproducing rainbow trout populations, it seems surprising that higher levels of rainbow trout introgression were not observed among the sample locations in the Moyie River drainage examined as part of this study. While hybrids were found in the majority (~63%) of tributaries

sampled throughout the Moyie River drainage in Idaho, introgression levels (% RBT alleles detected out of total) were low. No sites had observed RBT introgression levels greater than 2.34% and no individual fish with genotypes indicative of RBT or F₁ hybrids were detected.

The results observed in the Moyie River drainage were in sharp contrast to what was observed in sites from MF Boulder Creek and the mainstem Kootenai River. Although tests indicated that samples from both of these sites were not drawn from randomly mating populations (hybrid swarms), it is clear that there has been a substantial breakdown of reproductive isolating mechanisms between rainbow trout and westslope cutthroat trout in these areas. These results are unexpected given that the two species in these areas are naturally sympatric and presumably should demonstrate well developed isolating mechanisms. One possible explanation for these results is that these areas have also received stocking of nonnative RBT (primarily of coastal origin), which may not exhibit the same asynchrony in spawn timing and distribution with WCT as native RBT. Although we were unable to find substantial evidence of hatchery stocking in Boulder Creek or the mainstem Kootenai River (IDFG stocking database), hatchery rainbow trout have been stocked in tributaries to the Kootenai River. In fact, Williams and Jaworski (1995) reported that redband trout populations in two creeks in the Kootenai River drainage (Long Canyon Creek and Fisher Creek) were completely replaced by coastal rainbow trout as a result of hatchery RBT outplantings.

The development of intraspecific genetic markers to distinguish native redband trout from hatchery rainbow trout may assist in identifying sources of hybridization in sympatric westslope cutthroat trout populations in the future. Recently, the two allozyme loci that have traditionally been used to assess intraspecific hybridization were converted to diagnostic nuclear DNA single nucleotide polymorphisms (SNPs) assays (Brunelli et al. In Press). Additionally, our lab is currently investigating the utility of an additional 10 SNP assays in distinguishing native redband trout from multiple hatchery rainbow trout strains (IDFG, unpublished data).

With regards to the interspecific hybridization issues as part of this study, there are several relevant conservation/management questions that IDFG will have to consider in light of this studies findings. Specifically:

1. What conservation status should the introgressed populations identified in this study receive? Should they be included as cutthroat trout in ESA status reviews? How should they be managed?
2. What is the probability that RBT hybridization and introgression observed within WCT populations in the Moyie River drainage and in the Mission Creek drainage will increase over time?
3. What management strategies could potentially reduce the spread and increase of hybridization and introgression in these areas?

Currently, all of the populations examined thus far in the Moyie River drainage and in the Mission Creek drainage would be meet criteria for management as either “core conservation” (>99% pure) or “conservation” populations (>90% pure), as proposed by state agencies (Anonymous 2000). In addition, all populations, except Spruce Creek, would meet the criteria proposed by the USFWS for inclusion as WCT under the ESA. All populations exhibit less than 20% nonnative RBT or YCT alleles (USFWS 2003). Westslope cutthroat trout in Spruce Creek would probably be ineligible for protection because not all of the members of the population “express a range of morphological variation that conforms to the scientific taxonomic description

of WCT" (USFWS 2003). However, a case could be made in Spruce Creek that we have actually sampled multiple populations (hybrids were clearly more frequent in the upper sample site, above a culvert, than in lower sites). Therefore, it is somewhat unclear whether all fish in Spruce Creek would be ineligible for protection. This situation highlights one of the difficulties the USFWS will have to consider when implementing their 20% ruling/recommendation.

Much of the current debate regarding ESA status reviews for WCT appears focused on deciding what particular introgression level should be used to define a WCT population under the Act (i.e. 0% versus 20%; Campton and Kaeding 2005). We have previously proposed that perhaps a more important part of evaluating the status of WCT would be to provide information about the relative probability that hybridization and introgression in a particular population or area will increase over time (Shepard et al 2003). We have evidence in the case of Spruce Creek that hybridization and introgression have increased over time. No hybrids were identified in 1995 (USFWS 1999). Ten years later, RBT and YCT introgression was detected. It would appear that the concerns expressed previously about the presence of YCT and RBT above Spruce Creek were warranted, and managers would have been correct if they had listed in a status review that the probability of Spruce Creek remaining pure was "low." This type of specific information not only provides information relevant to the current status of WCT in this area, but it also highlights areas where specific management actions might improve the viability of WCT (i.e. removal of nonnative YCT and RBT from Spruce Lake).

Predicting the probability of hybridization/introgression increase and spread throughout the rest of the Moyie River drainage requires a range of information including whether observed introgression is a result of past versus ongoing processes. We observed no fish with genotypes indicative of RBT or F₁ hybrids in any of the tributaries. Instead, we observed multiple generation backcross hybrids. If no naturally reproducing RBT populations were present in the Moyie River drainage and no stocking of fertile RBT was occurring, then present hybridization/introgression levels would be interpreted as the result of past RBT stocking. Under this scenario, the fate of RBT introgression (whether it will increase, decrease, or remain the same) would be dependent on the relative fitness of hybrid genotypes/RBT in the population and genetic drift. While it is unknown what the long-term fitness effects are from low levels of introgression, it is likely that ongoing hybridization and introgression will have a far greater influence on population viability than past introgression events (one reason is that a WCT population that is actively undergoing hybridization is competing with RBT for resources and reproduction).

Although the pattern of hybridization/introgression within most of these tributaries is consistent with older hybridization events, it is still possible that introgression in these areas is an ongoing phenomenon. Several studies have recently suggested that hybrid straying is the mechanism by which introgression is spread throughout drainages, resulting over time in increases in the number of hybridized populations in an area and the amount of introgression at the population level (Hitt et al. 2003; Rubidge and Taylor 2005). Identifying all possible sources of hybrids throughout the Moyie River drainage (from naturally reproducing RBT populations) will be necessary to properly evaluate future threats and provide management direction. Stocked RBT are likely less of a threat at this point since it appears that the British Columbia Ministry of Fisheries follows a similar management strategy as Idaho in using sterile RBT for many of its stocking programs. Only Gerrard strain rainbow, that have been treated to induce triploidy, have been released into Moyie Lake since 2003 (Fisheries Inventory Data Queries, <http://srmapps.gov.bc.ca/apps/fidq/>).

It is unclear what management actions (if any) could be implemented in areas like the mainstem Kootenai River and MF Boulder Creek, which have historically supported naturally sympatric RBT and WCT populations but now exhibit substantial hybridization and introgression. Obviously, these areas represent a possible source of straying hybrids for tributaries like Mission Creek. If migration barriers do not exist on Mission Creek (e.g., waterfalls, culverts), then the low levels of hybridization/introgression observed in this drainage indicate that some other factor or combination of factors has limited the upstream dispersal and/or successful reproduction of RBT and hybrids. Identifying these factors will also be useful in predicting the spread of hybridization and introgression in these drainages in the future.

RECOMMENDATIONS

1. Analyze an additional 1263 *Oncorhynchus sp.* samples collected recently from ~20 tributaries to the mainstem Kootenai River with the same set of diagnostic interspecific hybridization markers.
2. Screen *O. mykiss* populations in these areas with genetic markers diagnostic between hatchery rainbow trout and native redband trout as they become available.

JOB PERFORMANCE REPORT
SUBPROJECT #3: ORIGIN AND PURITY OF YELLOWSTONE CUTTHROAT TROUT
POPULATIONS IN THE HENRYS FORK DRAINAGE

State of: Idaho Grant No.: F-73-R-25, Fishery Research

Project No.: 2 Title: Native Species Investigations

Subproject #3: Origin and purity of Yellowstone cutthroat trout populations in the Henrys Fork drainage

Contract Period: July 1, 2005 to June 30, 2006

ABSTRACT

This project describes collaborative research with the Henrys Fork Foundation designed to assess the origin and genetic purity of seven Yellowstone cutthroat trout populations found above natural waterfalls in the Henrys Fork drainage. A total of 256 samples were screened with both diagnostic hybridization markers as well as with a Restriction Fragment Length Polymorphism (RFLP) mitochondrial DNA (mtDNA) marker. Genetic screening did not find any evidence of rainbow trout or westslope cutthroat trout hybridization/introgression in any of the sample locations examined. All samples exhibited genotypes/haplotypes indicative of pure Yellowstone cutthroat trout. Results of the mtDNA/RFLP analyses suggest that the most likely origin of these isolated Yellowstone cutthroat trout populations were from past hatchery introductions of Yellowstone Lake strain/origin Yellowstone cutthroat trout.

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INTRODUCTION

Few Yellowstone cutthroat trout *Oncorhynchus clarkii bouvierii* populations remain in their historic range in the Henrys Fork drainage (Van Kirk and Gamblin 2000). Overexploitation, habitat degradation and fragmentation, chemical treatments to remove nongame fish, and subsequent nonnative trout stocking have all contributed to the extirpation of Yellowstone cutthroat trout throughout the drainage. Most populations have now been replaced by nonnative rainbow trout *O. mykiss*, which have produced an extremely important recreational fishery in the area. Possible exceptions are tributaries in the drainage that are isolated above barriers (waterfalls), although in some cases it is unclear whether these represent complete migration barriers. In addition, many of these areas were historically fishless and have also been stocked with nonnative rainbow trout and both native and nonnative cutthroat trout. The origin and purity of Yellowstone cutthroat trout found in many of these areas is unknown.

The primary objective of this study was to assess the purity of 256 Yellowstone cutthroat trout samples collected from seven sample locations in the Henrys Fork drainage (Figure 5 and Table 8). A secondary objective was to attempt to determine the origin of Yellowstone cutthroat trout in the Fall and Bechler rivers. Both of these rivers were historically fishless, isolated above natural waterfalls, and the existing populations may be of Yellowstone Lake origin (Yellowstone Lake Hatchery, Wyoming) or Henrys Fork drainage origin (Ashton and Warm River hatcheries, Idaho). Yellowstone National Park personnel believe that westslope cutthroat trout *O. clarkii lewisi* may have also been stocked in these areas in addition to rainbow trout (Jim DeRito, Henrys Fork Foundation, personal communication). Confirmation of pure, local origin populations would potentially identify populations that would be useful in future translocation and supplementation efforts throughout the drainage.

METHODS

In order to address the first objective (purity) we screened samples with a mitochondrial DNA (mtDNA) marker diagnostic between Yellowstone cutthroat trout, westslope cutthroat trout, and rainbow trout, and seven diagnostic nuclear DNA (nDNA) markers (Occ16, Occ34, Occ35, Occ36, Occ37, Occ38, and OM55). The nDNA markers are codominant Simple Sequence Repeat (SSR) markers which are diagnostic based on size differences in the Polymerase Chain Reaction (PCR) products between rainbow trout and cutthroat trout (Ostberg and Rodriquez 2002). One locus (OMM55) is also diagnostic between all three taxa (Yellowstone cutthroat trout, westslope cutthroat trout, and rainbow trout).

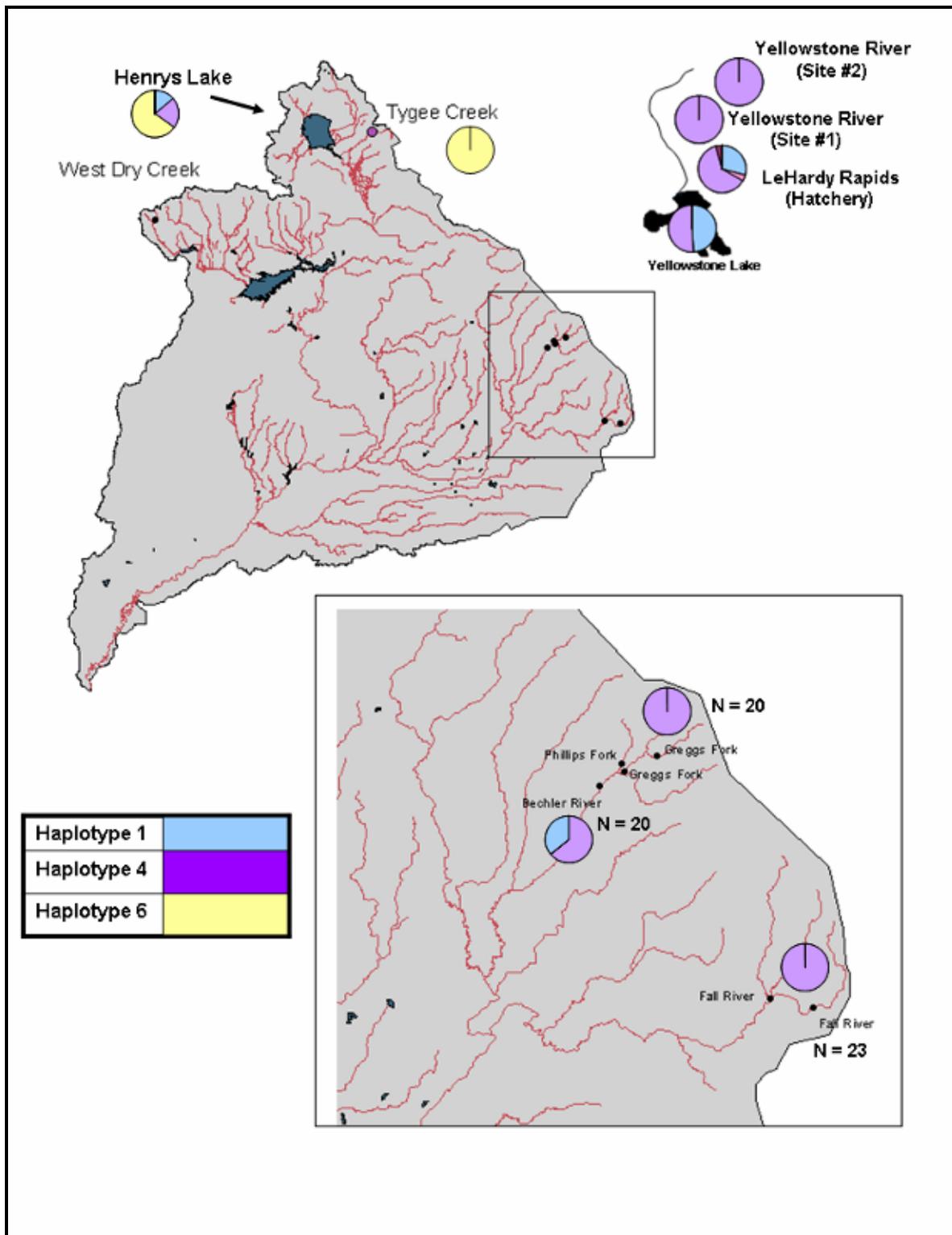


Figure 5. Sampling locations in Henrys Fork drainage and haplotype frequencies (represented by pies). Phillips Fork samples (N = 2; Haplotype 1) not shown.

To address the second objective, concerning the origin of Yellowstone cutthroat trout in the Fall and Bechler Rivers (Henrys Lake strain or Yellowstone Lake strain), we screened a subset of samples from these areas with a mtDNA Restriction Fragment Length Polymorphism (RFLP) marker that has previously yielded haplotype differences between Henrys Lake and Yellowstone Lake cutthroat trout (Campbell et al. 2002).

Table 8. Stream, location description, UTM zone and coordinates, length of sampling in meters (m), date of sampling, number (#) of samples collected, and phenotypic species identification of genetic samples collected in the Henrys Fork drainage in 2005.

Stream	Location Description	UTM Zone (NAD27)		Length of sampling (m)	Date of sampling	# of samples	Phenotypic species identification	
		Easting	Northing					
Fall River	1.7 km above Beula Lake	12	520065	4889121	100	9-21-05	50	All YCT, except fish #11 may be hybrid
Fall River	1.2 km below Beula Lake (and below Bradley Falls)	12	517432	4889733	200	9-22-05	56	All YCT
Bechler River	1.5 downstream from Three Rivers Junction	12	507526	4903036	400	9-16-05	52	All YCT
Greggs Fork	0.45 km upstream from confluence with Bechler River, above Forlorn Falls and below Twister Falls	12	508999	4903849	425	9-13-05 and 9-14-05	25	All YCT, except fish #s 1,2, and 11 may be hybrids
Greggs Fork	3.2 km upstream from Bechler River confluence, above Twister Falls	12	511030	4904679	100	9-14-05	23	All YCT, note #19 and #32 are whole fry
Phillips Fork	0.6 km upstream from Bechler River confluence, above Phillips Fork Falls	12	508879	4904328	450	9-15-05	37	All YCT
West Dry tributary	1.1 km upstream from West Dry Creek	12	438681	4928406	100	8-8-05	13	All YCT, except #5

RESULTS

Purity

We did not find any evidence of rainbow trout or westslope cutthroat trout hybridization/introgression in any of the sample locations examined. All samples exhibited genotypes/haplotypes indicative of pure Yellowstone cutthroat trout. The probability of detecting introgression within a population is dependent on the number of samples examined and the number of diagnostic loci/alleles examined. Sample sizes for all of the sample locations in this study (except for West Dry tributary, N = 13) were sufficient to have >95% probability of detecting as little as 1% rainbow trout introgression.

Origin

Two haplotypes (Haplotype 1 and Haplotype 4) were observed among a subset of 65 samples screened (Figure 5). Haplotype 4 was the only haplotype observed among 23 samples from the Fall River and 20 samples from the Greggs Fork. Both haplotypes were observed in 20 samples from the Bechler River (Haplotype 1, N = 8; Haplotype 4, N = 12). Only two samples

were screened from the Phillips Fork and both samples exhibited Haplotype 1. Haplotypes 1 and 4 are the most common haplotypes observed in Yellowstone Lake and in samples from the Clark Fork Fish Hatchery (LeHardy Rapids strain). Haplotype 4 is fixed in two other populations we have examined from the Yellowstone River. Haplotype 1 and Haplotype 4 are also found in Henrys Lake (~10% and ~23%, respectively). However, based on historical stocking records and the frequency pattern of these two haplotypes in Henrys Lake tributaries, we have previously proposed that their presence in the lake may be the result of introductions of cutthroat trout from Yellowstone Lake (Campbell et al. 2002).

The most common haplotype observed in Henrys Lake (~67%) and fixed in samples from Tyghee Creek (a tributary just below the lake) is Haplotype 6. This haplotype is not present in samples from Yellowstone Lake or the Yellowstone River and has not been observed in any drainage outside of Idaho. The fact that Haplotype 6 is not observed among the 65 samples examined in this study suggests that these pure Yellowstone cutthroat trout populations are most likely not of Henrys Lake strain/origin and are more likely the product of past introductions from Yellowstone Lake strain/origin Yellowstone cutthroat trout.

DISCUSSION

Although the Yellowstone cutthroat trout populations from the Fall and Bechler rivers screened as part of this study exhibited no rainbow trout introgression, the fact that they likely originated from outside the upper Snake River basin indicates that they would not be ideal sources of genetic diversity for within-basin conservation purposes. Expanded research efforts are planned to sample additional Yellowstone cutthroat trout populations in the Henrys Fork and Sinks drainages (De Rito and Emery-Miller 2006). Genetic evaluation of these populations to determine purity and genetic structure may identify sources of fish for future translocation or supplementation efforts in the Henrys Fork drainage.

RECOMMENDATIONS

1. Continue genetic screening in the Henrys Fork and Sinks drainages to identify sources of fish for future translocation or supplementation efforts in the Henrys Fork drainage.

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