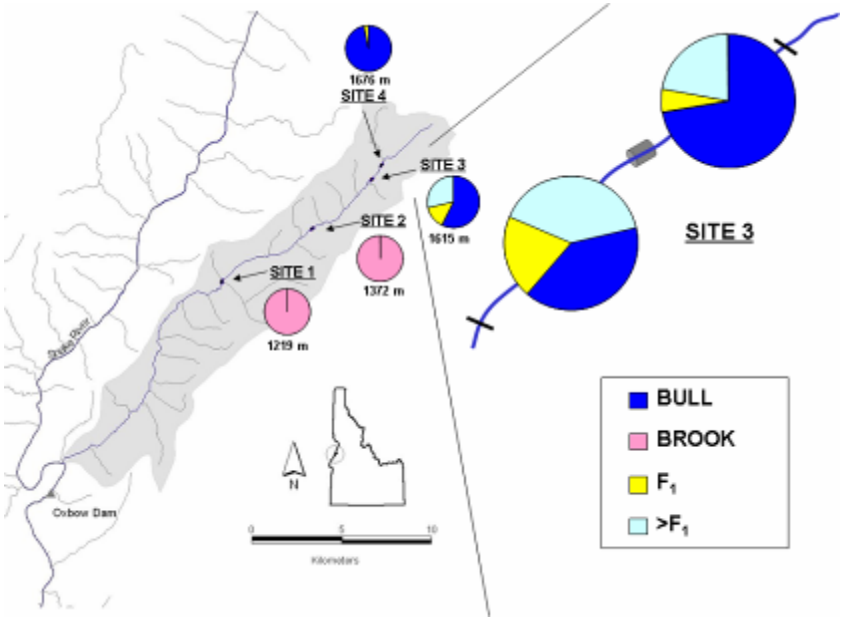
FISHERY RESEARCH

NATIVE SPECIES INVESTIGATIONS

Grant # F-73-R-25

July 1, 2004 to June 30, 2005



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Annual Performance Report

July 1, 2004 to June 30, 2005

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Project 2: Native Species Investigations

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PREFACE

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, 2004 to June 30, 2005 contract period. The first project describes collaborative research with the USDA Forest Service examining the origin and divergence of mountain whitefish in the Big Lost River Basin. Mitochondrial DNA results from this study should assist managers with the development of conservation management units for the species and in addressing Endangered Species Act (ESA) concerns.

The second research project describes a collaborative project with the Idaho Power Company examining hybridization between brook trout and bull trout in Indian Creek, Idaho, a tributary to the Snake River. Bull trout were listed as Threatened under the ESA in 1998. One of the specific tasks outlined in the bull trout recovery plan is to provide an assessment of the threat from hybridization with brook trout throughout the species' range. A genetic screen of samples from Indian Creek with mitochondrial and nuclear DNA markers diagnostic between brook trout and bull trout revealed a narrow hybrid zone distributed along an elevational gradient. The screen also identified both F_1 and $>F_1$ hybrids and demonstrated high concordance between genetic and phenotypic identifications. Results from this study should assist managers in assessing hybridization risks from brook trout populations and in the development of brook trout and hybrid removal strategies.

The final research project described in this report involves a collaborative project with Trout Unlimited examining translocation efforts for Bonneville cutthroat trout. Bonneville cutthroat trout populations have been extirpated throughout their historic range due to a number of factors including the stocking of nonnative rainbow trout, which have hybridized with or replaced populations in many areas. As part of efforts to restore pure Bonneville cutthroat trout populations, Trout Unlimited, the Confederated Tribes of the Goshute Reservation, and a number of State (Utah, Nevada, and Idaho) and Federal agencies are working together to reintroduce pure populations into several areas throughout their historic range. The purpose of this study was to evaluate an experimental reintroduction effort in Utah, with the primary goal of assessing whether renovation efforts (removal of rainbow trout and hybrids) were successful. Results identified juvenile hybrids in the test stream following reintroduction efforts, indicating that efforts to remove all hybrids prior to translocation of pure Bonneville cutthroat trout were ineffective. However, the identification of juveniles with genotypes indicative of pure cutthroat and a mtDNA haplotype matching that found in the donor population, suggests that at least a portion of the transplanted adults were reproductively successful. Fisheries managers will have to consider these results carefully prior to translocation efforts in Idaho.

Several additional research projects that were either worked on or completed during the July 1, 2003 to June 30, 2004 contract period but not included in this report need to be mentioned. A paper titled *Multi-Scale Genetic Structure of Yellowstone Cutthroat Trout in the Upper Snake River Basin* was submitted and accepted for publication by the journal *Transactions of the American Fisheries Society*. This paper describes the genetic population structure of Yellowstone cutthroat trout in Idaho using microsatellite DNA analyses (Snake River Native Salmonid Assessment, BPA project #199800200). A progress report to the Bureau of Land Management was completed on the genetic population structure of redband trout in southwest Idaho (*Redband trout beneficial use assessment: Genetic investigation of population*

structure, IDFG Report Number 05-46). Two microsatellite DNA optimization/standardization efforts (one for redband trout and one for bull trout) were completed during the last contract period. These efforts involve the selection of a core set of microsatellite loci that will be used by all participating laboratories and standardization procedures to ensure that all laboratories identify and document the same alleles at each locus. The advantage of microsatellite standardization is that data generated in one laboratory for a specific species (e.g., redband trout) can be directly compared to data generated by another laboratory. Additionally, in the case of redband trout and bull trout, the collaborative process of choosing particular loci resulted in a core set of loci whose characteristics allow a greater number of questions to be addressed. For instance, the core set of loci that were chosen for bull trout allow for assessments of hybridization from both Dolly Varden as well as brook trout. Also, the final bull trout core set contains loci that are substantially more variable than the loci used previously, which ultimately may result in improved understanding of the species' rangewide genetic diversity and population structure. The final research project worked on during the last contract period but not summarized in this report involves a continuation of a project described in last year's annual report involving hybridization issues between native Westslope cutthroat trout and introduced rainbow trout in the Coeur d'Alene River basin, Idaho. During the past year, samples sizes were increased from 68 to 106, and a suite of phenotypic characteristics were examined for their usefulness in distinguishing Westslope cutthroat trout from rainbow trout and hybrids. Results indicate that there are no phenotypic characteristics to reliably distinguish all hybrids from Westslope cutthroat trout. However, certain characteristics (spot shape, number, and distribution) appear to be good at determining when an individual fish is not a pure Westslope cutthroat trout. These results should help managers in providing guidelines to anglers in instances when fishing regulations have been modified to allow harvest of hybrids.

JOB PERFORMANCE REPORT
SUBPROJECT #1: MITOCHONDRIAL DNA PHYLOGEOGRAPHY OF MOUNTAIN WHITEFISH *PROSOPIUM WILLIAMSONI* IN IDAHO, UTAH, AND MONTANA: EMPHASIS ON THE ORIGIN AND DIVERGENCE OF MOUNTAIN WHITEFISH IN THE BIG LOST RIVER, IDAHO

State of: Idaho

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

Project No.: 2

Title: Native Species Investigations

Subproject #1: Mitochondrial DNA phylogeography of mountain whitefish *Prosopium williamsoni* in Idaho, Utah, and Montana: Emphasis on the origin and divergence of mountain whitefish in the Big Lost River, Idaho

Contract Period: July 1, 2004 to June 30, 2005

ABSTRACT

Nucleotide sequences of the cytochrome b gene region (~615 bp) of mitochondrial DNA (mtDNA) were used to assess the phylogeography of mountain whitefish in Idaho, Montana, and Utah. Initial results support previous allozyme and microsatellite studies that found evidence for three well-defined genetic assemblages corresponding geographically to the 1) Upper Snake River drainage (upstream of Shoshone Falls) and the Bonneville basin, 2) the Snake River drainage (downstream of Shoshone Falls) including the Pahsimeroi and Salmon Rivers, and 3) the upper Missouri River. Levels of sequence divergence between these three genetic assemblages were equal to or greater than published levels of divergence found in other salmonid subspecies occupying these drainages. These initial results, along with previous research indicating that mountain whitefish in the Big Lost appear to be genetically and morphologically distinct from other mountain whitefish populations (Whiteley and Gamett 2003), justify managing them as a distinct management unit, independent from other populations in the larger genetic assemblage to which they belong.

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INTRODUCTION

Mountain whitefish *Prosopium Williamsoni* are a widely distributed member of the Salmonidae family that are found throughout Western North America from the Lahontan Basin in California and Nevada, north to the Yukon-British Columbia Border in Canada (Wydoski and Whitney 1979). Recent microsatellite and allozyme investigations have focused on understanding rangewide patterns of genetic structure and phylogeography of the species, with one goal being to identify and conserve historically isolated, genetically and evolutionarily independent, groups of populations (Whiteley 2005). In Idaho, populations of mountain whitefish in the Big Lost River basin are of particular conservation interest because: 1) previous research has suggested that they are morphologically and genetically distinct (Whiteley and Gamett 2003); 2) they are geographically isolated in part of a series of closed surface drainage basins known as the "Sinks Drainages" (Whiteley and Gamett 2003); and 3) they have experienced extensive declines in distribution and abundance (Idaho Department of Fish and Game [IDFG] 2005).

OBJECTIVES

1. Assess whether mitochondrial DNA sequencing analyses provide concordant results to previous allozyme and microsatellite results regarding the origin and divergence of mountain whitefish in the Big Lost River basin.

2. Provide additional genetic information regarding the species' population structure and evolutionary history from which future conservation efforts in Idaho can be developed and effectively evaluated.

METHODS

Approximately 615 bp of the Cytochrome b mtDNA gene region were sequenced on 60 samples from 11 populations (Figure 1, Table 1). Sample locations and number of samples collected at each site are listed below (Table 1).

Total genomic DNA was extracted from a 1 mm piece of fin clip following methods described by Paragamian et al. (1999), adapted from protocols by Sambrook et al. (1989) and Hillis et al. (1996). DNA was resuspended in 100 µl TE. The Cytochrome B (CytB) gene region was amplified in a 40 µl reaction consisting of 0.5-3.0 µl DNA extract (approx. 2.5 ng/ µl), 4.0 µl 10X buffer (Perkin Elmer), 4.0 µl MgCl2, 3.2 µl BSA, 1.0 µl DMSO, 4.0 µl of each primer (2uM): (765) 5'- GAA AAA CCA CCG TTG TTA TTC AAC T -3' and (766) 5'- GTT TAA TTA GAA TAT CAG CTT TGG G -3', 3.2 µl 10.0 mM dNTPs (10mM each of dATP, dCTP, dGTP, and dTTP), 0.15 µl Perkin-Elmer Taq polymerase, and 13.45-15.95 µl dH2O. Amplification was carried out in a Biorad/MJ PTC-100 thermal cycler as follows: preheating at 95°C for 3 min, followed by 39 cycles of denaturing at 95°C for 45 seconds, annealing at 48°C for 45 seconds, and elongation at 70°C for 2.5 minutes. Amplification was completed with a final extension at 70°C for 3 minutes.

Sequencing reactions were performed at the Nevada Genomics Center (University of Nevada, Reno, Nevada) with a BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1

(Applied Biosystems) using the forward primer (765). Sequenced products were cleaned using Edge Biosystems gel filtration plates (Edge Biosystems, Gaithersburg, Maryland) and were run out on a Prism 3730 DNA sequencer (Applied Biosystems).

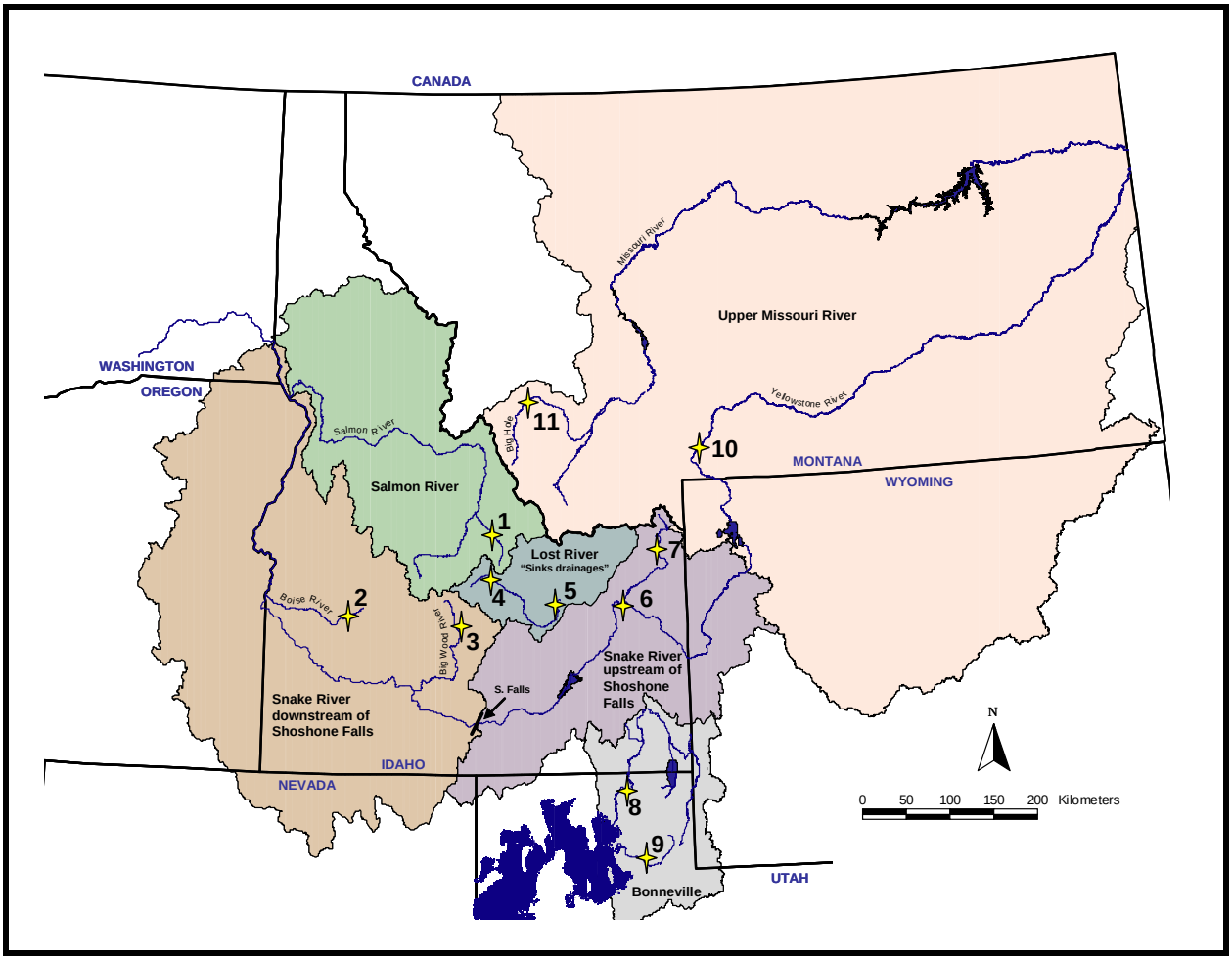

Figure 1. Mountain whitefish sampling sites from six drainage basins (Salmon River, Lost River, Snake River-below Shoshone Falls, Snake River-above Shoshone Falls, Bonneville, and Upper Missouri River). Numbers correspond to populations on Table 1.

Table 1. Mountain whitefish sampling sites from six drainage basins (Salmon River, Lost River, Snake River-below Shoshone Falls, Snake River-above Shoshone Falls, Bonneville, and Upper Missouri River). Numbers correspond to populations on Figure 1. Counts in columns under each haplotype category identify the number of individuals from each population with a particular haplotype; empty cells indicate zeros.

			Haplotypes																		
Pop. #	**Population**	**Basin**	**Hap 1**	**Hap 2**	**Hap 3**	**Hap 4**	**Hap 5**	**Hap 6**	**Hap 7**	**Hap 8**	**Hap 9**	**Hap 10**	**Hap 11**	**Hap 12**	**Hap 13**	**Hap 14**	**Hap 15**	**Hap 16**	**Hap 17**	**Hap 18**	**N**
1	Pahsimeroi R.	Salmon River	2	3																	5
2	Boise R.	Snake River (below Shoshone Falls)				5	1	1													7
3	Big Wood R.	Snake River (below Shoshone Falls			4																4
4	Big Lost R. (upper)	Big Lost River									6										6
5	Big Lost R. (lower)	Big Lost River									6										6
6	Upper Snake R. (Menan)	Snake River (above Shoshone Falls)										1	2								3
7	Henrys Fork	Snake River (above Shoshone Falls)							6	6											1 2
8	Bear River	Bonneville													2						2
9	Weber R.	Bonneville											1	2							3
10	Yellowstone R.	Upper Missouri River																4		1	5
11	Big Hole R.	Upper Missouri River														2	3	1	1		7

Sequences were edited using Sequencher™ (version 4.1.2, Gene Codes Corporation, Ann Arbor, Michigan) and the consensus sequences were aligned using the Clustal X program (version 1.81; Thompson et al. 1997) on the SDSC workbench. The software program MEGA (version 3.0; Kumar et al. 2003) was used to estimate pairwise sequence divergence between haplotypes and to construct a bootstrapped (10000 replicates) UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and neighbor joining tree (both with the Kimura-2 parameter model).

RESULTS

Eighteen haplotypes were identified from the 60 samples sequenced (Table 1, Figure 2). Few haplotypes were shared by multiple populations. The exceptions were Haplotype 11 identified within the Upper Snake and Weber rivers and Haplotype 16 identified within the Big Hole and Yellowstone rivers (Table 1, Figure 2). A UPGMA distance (Kimura two-parameter) bootstrap analysis (Figure 2) identified three distinct genetic assemblages corresponding geographically to the 1) Snake River (upstream from Shoshone Falls) and the Bonneville Basin, 2) the upper Missouri River Basin, and 3) the Snake River (downstream from Shoshone Falls) and the Salmon River Basin. The neighbor joining dendogram yielded the same topology (not shown). One haplotype (Haplotype 9) was observed in samples from the Upper and Lower Big Lost River (populations 4 and 5). Haplotype 9 was most similar (0.33%) to Haplotype 8 from the Henrys Fork (population 7) and strongly clustered (98% bootstrap) with samples from the Snake River (upstream from Shoshone Falls) and the Bonneville basin.

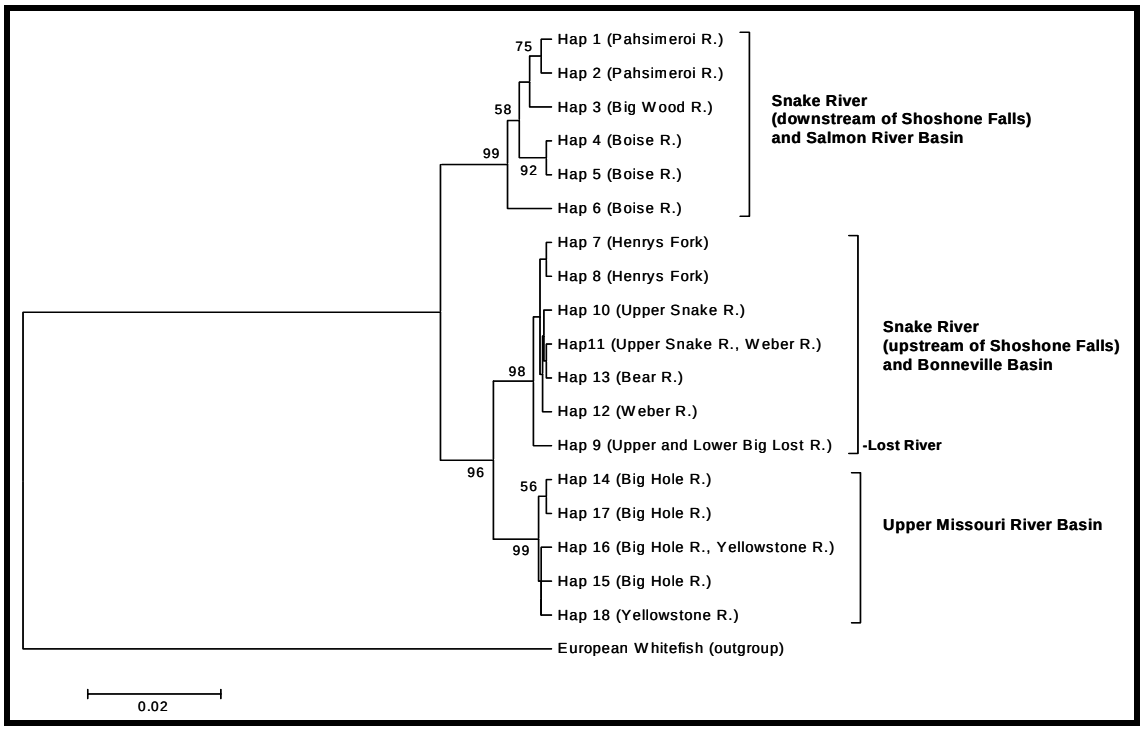

Figure 2. UPGMA dendogram based on the Kimura-2 distance for the 18 haplotypes identified through sequencing. Bootstrap values (all were >50) were based on 10000 replications. European whitefish *Coregonus lavaretus* was used as an out-group.

Pairwise divergence estimates within each of the three major genetic assemblages were variable, ranging from 0.16-0.49% in the Upper Missouri River assemblage to 0.16-1.65% in the Snake River (downstream from Shoshone Falls) and the Salmon River basin (Figure 3). Pairwise divergence estimates between the three genetic assemblages were extremely high, ranging from 1.31 to 4.56%. For comparison purposes, estimates of mtDNA sequence divergence among Westslope cutthroat trout *Oncorhynchus clarkii lewisi* and Yellowstone cutthroat trout *O. clarkii bouvieri* range from 1.5 to 1.9% (Gyllensten and Wilson 1987; IDFG unpublished data). Sequence divergence between rainbow trout *O. mykiss* and cutthroat trout has been estimated at between 4.0 to 4.5% (Gyllensten and Wilson 1987; IDFG unpublished data).

	Hap	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
#Pahsimeroi1	1																		
#Pahsimeroi8	2	0.33																	
#BigWood3	3	0.66	0.66																
#Boise6	4	1.15	1.15	0.82															
#Boise4	5	0.99	0.99	0.66	0.16														
#Boise13	6	1.15	1.15	1.15	1.65	1.49													
#HenrysFork3	7	2.83	2.83	2.83	3.34	3.17	3.00												
#HenrysFork2	8	2.66	2.66	2.66	3.17	3.00	2.83	0.16											
#UpperBigLost19	9	2.32	2.32	2.32	2.83	2.66	2.49	0.49	0.33										
#UpperSnakeMenan46	10	3.00	3.00	3.00	3.52	3.34	3.17	0.49	0.33	0.66									
#UpperSnakeMenan43	11	2.83	2.83	2.83	3.34	3.17	3.00	0.33	0.16	0.49	0.16								
#Weber19	12	3.00	3.00	3.00	3.52	3.34	3.17	0.49	0.33	0.66	0.33	0.16							
#Bear32	13	3.00	3.00	3.00	3.51	3.34	3.17	0.49	0.33	0.65	0.33	0.16	0.33						
#BigHole12	14	3.69	3.69	3.86	4.39	4.21	3.69	1.98	1.81	1.98	1.81	1.65	1.81	1.81					
#BigHole3	15	3.69	3.69	3.86	4.39	4.21	3.69	1.98	1.81	1.98	1.81	1.65	1.81	1.81	0.33				
#BigHole11	16	3.69	3.69	3.51	4.03	3.86	3.69	1.65	1.48	1.65	1.48	1.31	1.48	1.48	0.33	0.33			
#BigHole6	17	3.86	3.86	4.03	4.56	4.39	3.86	2.15	1.98	2.15	1.98	1.81	1.98	1.98	0.16	0.49	0.49		
#Yellowstone26	18	3.52	3.52	3.52	4.04	3.86	3.34	1.82	1.65	1.82	1.65	1.48	1.65	1.65	0.33	0.33	0.33	0.49	

Snake River (downstream of Shoshone Falls and Salmon River Basin)

Snake River (upstream of Shoshone Falls and Bonneville Basin)

Upper Missouri River Basin

Figure 3. Percent sequence divergence among haplotypes (haplotype reference sample number listed after population name). Grayed areas show pairwise divergence estimates within each of the three major genetic assemblages.

DISCUSSION

Results thus far of mtDNA sequencing analyses are in complete concordance with previous allozyme and microsatellite investigations that indicate that mountain whitefish across the study area are comprised of three distinct genetic assemblages (Whiteley 2005). Management of this species should focus on conserving diversity within and maintaining divergence among each of these assemblages. Mitochondrial DNA sequencing results also support the theory that the origin of mountain whitefish in the Big Lost River is from the Upper Snake River basin. Using a molecular clock estimate for mtDNA of 1 to 2% sequence divergence per million years suggests that mountain whitefish could have been isolated in the Big Lost River basin approximately 165,000-330,000 years ago. However, this should only be considered an approximate estimate. Increasing sample sizes from the Big Lost River and Upper Snake River basin locations, as well as sequencing multiple mtDNA gene regions, would allow better precision in estimating when populations in these two basins diverged. These initial results, along with previous research indicating that mountain whitefish in the Big Lost appear to be genetically and morphologically distinct from other mountain whitefish populations (Whiteley 2005; Whiteley and Gamett 2003), justify conserving them as a distinct management unit, independent from other populations in the larger genetic assemblage to which they belong.

FUTURE PROJECT NEEDS

- Increase sample sizes to at minimum 30 individuals per sample location.
- Compare divergence estimates and tree topology derived from Cyt b sequences against an additional mtDNA gene region (D-loop).

JOB PERFORMANCE REPORT
SUBPROJECT #2: DETECTION OF BULL TROUT X BROOK TROUT HYBRIDS WITHIN INDIAN CREEK, IDAHO

State of:	Idaho	Grant No.:	F-73-R-25, Fishery Research
Project No.:	2	Title:	Native Species Investigations
Subproject #2:	Detection of Bull trout X brook trout hybrids within Indian Creek, Idaho		
Contract Period:	July 1, 2004 to June 30, 2005		

ABSTRACT

A genetic study was initiated in Indian Creek in 2002 with three main objectives: 1) determine levels of hybridization, 2) assess the ability of biologists to classify fish based upon phenotypic characteristics, and 3) determine if a culvert was a contact barrier between brook trout and bull trout. Three nuclear DNA markers and one mitochondrial DNA marker were amplified for 172 individuals at four sites. We found that of the fish sampled at site 1, 46% were hybrids, and of the fish sampled at site 4, 3% were hybrids. Only brook trout were present at sites 2 and 3. We also detected post F_1 hybrids at the two sites where hybridization had occurred. The culvert was not a complete barrier since hybrids were present at the upper sites. High concordance was found between genetic identifications and phenotypic identifications, except where backcrosses were found. Both the presence of post F_1 hybrids and misidentification of pure bull trout have important management implications. Consideration needs to be taken whether it is more prudent to remove all brook trout and hybrids, with the possible risk of removing pure bull trout, or leave hybrids in the streams, which have the potential to reproduce with pure bull trout.

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INTRODUCTION

In the Hells Canyon Complex Recovery Unit (USFWS designation), brook trout have been widely introduced and are established in many areas (Chandler and Richter 2001). Brook trout can negatively influence bull trout through competition for resources, displacement, and hybridization (Kanda et al. 2002). Failure to properly understand and manage bull trout and brook trout interactions can ultimately result in delayed recovery or local declines of bull trout populations. Therefore, both population estimates and assessments of hybridization can guide future management decisions and aid in the recovery of bull trout.

In the Idaho portion of the recovery unit, brook trout are abundant and have been observed in Indian Creek, sympatric to and downstream of a resident/fluvial bull trout population. Brook trout were documented as early as 1997 in three sites, ranging in elevation from 1220 m to 1700 m, while bull trout were only found at the higher elevation (>1600 m). While there appeared to be elevation and thermal barriers affecting the distribution of bull trout, hybrids were documented at the upper site. In 2002, a larger study was initiated to understand bull trout and brook trout dynamics within the stream.

OBJECTIVES

1. Document the extent of hybridization between bull trout and brook trout in Indian Creek.

2. Determine whether a culvert present at one of the upper sites represents a significant barrier to the movement of either species and to subsequent hybridization.

3. Determine whether biologists could accurately distinguish bull trout from brook trout and hybrids using phenotypic criteria.

METHODS

In 2002, biologists from Idaho Power Co. (IPC) collected 172 fin clips from *Salvelinus sp.* in Indian Creek (bull trout, brook trout, bull trout X brook trout hybrids) for genetic analyses. The stream was divided into four sites (site 1 lowest in the drainage, site 4 highest), all located below the confluence of Indian Creek and Camp Creek. Site 3 spanned an area both above and below a culvert (a suspected movement barrier). The four sites ranged in elevation from 1220 m to 1676 m (Figure 4). All fish were identified as either bull trout, brook trout, or hybrids based upon phenotypic characteristics.

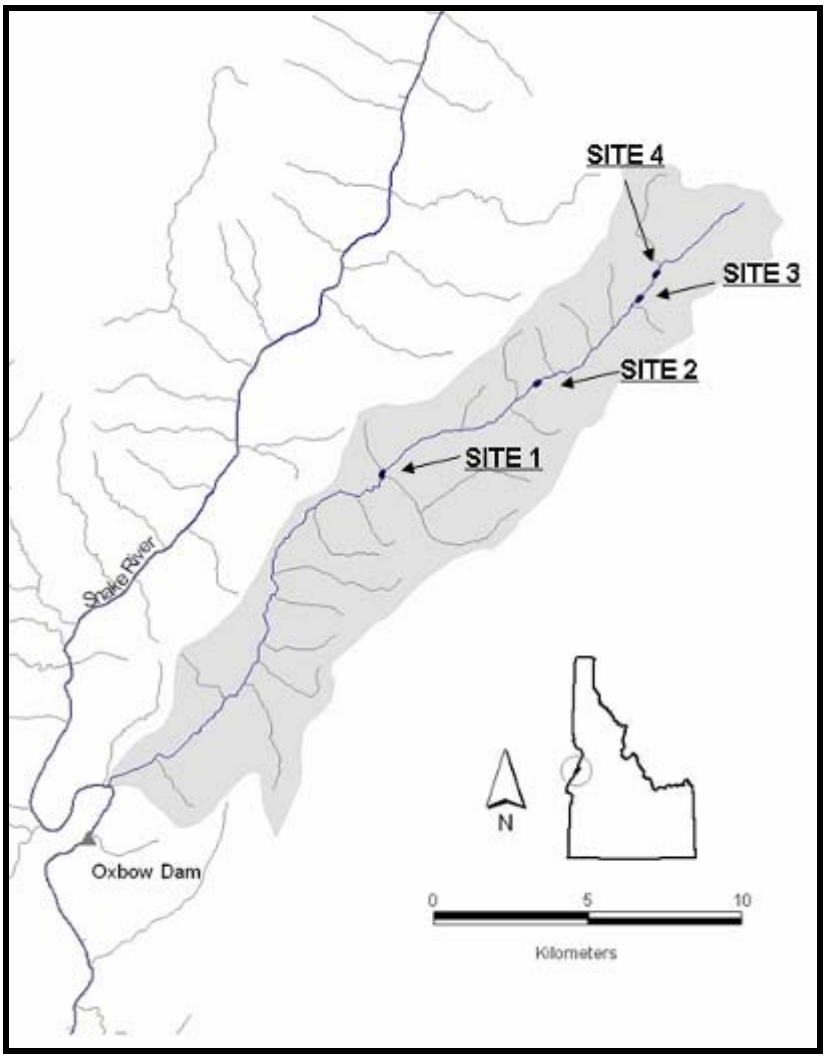

Figure 4. Sampling localities within Indian Creek, Idaho.

Three diagnostic nuclear DNA (nDNA) markers were used to differentiate bull trout from brook trout and hybrids. Two of these markers reveal size based differences in the PCR products for bull trout and brook trout. The Growth Hormone 2 (GH2) marker (Taylor et al. 2001) yields 540 bp for bull trout and 600 bp for brook trout, and the Sfo18 microsatellite marker (Angers et al. 1995) yields fragments >175 bp for bull trout and <150 bp for brook trout.

Microsatellite markers generally reveal multiple alleles, but the Sfo18 fragments >175 bp were relabeled as the “A” allele (brook trout) and fragments <150 bp were relabeled as the “B” allele (bull trout) for simplicity. The other nDNA marker (Protoncogene 53-p53) is a Restriction Fragment Length Polymorphism (RFLP), which yields diagnostic banding patterns (one uncut band at 490 bp for bull trout and two cut bands at 200 and 290 bp for brook trout) when digested with the restriction enzyme *Mse*-I (IDFG, unpublished data). An additional mitochondrial RFLP marker developed in this lab (Cyt B digested with *Rsa-I*) was used to assess the direction of hybridization.

The RFLP and GH2 PCR products were separated by horizontal gel electrophoreses through a 3% agarose-Synergel (Diversified Biotech) gel to reveal diagnostic banding patterns. PCR products of the microsatellite locus, Sfo18, were electrophoresed on an ABI 3100 capillary system. Individuals were assigned to the hybrid categories according to their composite genotypes. Table 2 lists the observed composite genotypes and the respective hybrid categories based upon Mendelian inheritance.

Table 2. Composite genotypes and associated species/hybrid categories (A = Bull trout haplotype/allele, B = Brook trout haplotype/allele).

	Cyt B/*Rsa*I	**GH2**	***Sfo*18**	**P53/*Mse*I**
Bull Trout	A	AA	AA	AA
Brook Trout	B	BB	BB	BB
F_1 Hybrid	B	AB	AB	AB
$>F_1$ hybrid	B	AB	AB	BB

A subset of samples was sent to the U.S. Fish and Wildlife Service genetics laboratory in Abernathy, Washington for verification. Fifty samples were genotyped with six microsatellite loci diagnostic between brook trout and bull trout (DeHaan and Ardren, unpublished data) and our data was updated with their results (see Appendix A for genotypes and genetic identifications).

RESULTS

There was a geographic association to the distribution of species within the drainage (Figure 5). In the lower sites (1 and 2), all samples had genotypes indicative of brook trout. In the upper sites (3 and 4), bull trout and hybrids were genetically identified but no brook trout were identified. Of the 28 samples collected in site 3, 13 were identified as hybrids (6 F_1; 7 $>F_1$), including from both above and below the culvert (Table 3; Figure 5). All 13 hybrids had bull trout mitochondrial DNA. The remaining 15 samples from site 3 were genetically identified as bull trout. Of the 65 samples collected in site 4, two samples had genotypes indicative of F_1 hybrids while the other 63 samples were genetically identified as bull trout. Levels of hybridization (number of fish identified as hybrids out of the total sampled) was 46% in site 3 and 3% in site 4.

Table 3. Distribution and percentage of bull trout, brook trout, and bull trout x brook trout hybrids at each site (above and below culvert distribution for site 3), followed by the percentage of fish that were discrepant between phenotypic and genetic identifications.

Site	**Sample Size**	**Bull**	**Brook**	**F1**	**>F1**	**% Hybrids**	**% Missed Phenotypically**
1	30	0	30	0	0	0%	3%
2	49	0	49	0	0	0%	2%
3	28	15 (12 above, 3 below)	0	6 (3 above, 3 below)	7 (3 above, 4 below)	46%	43%
4	65	63	0	2	0	3%	3%

Biologists phenotypically classified each sample as bull trout, brook trout, or bull trout x brook trout hybrid. The highest level of discrepancy in phenotypic identifications occurred in site 3 (43%) where both bull trout and hybrids were found. At this site, three samples phenotypically classified as hybrids were genetically identified as bull trout, while seven samples that were phenotypically classified as bull trout were genetically identified as hybrids. Two samples were also phenotypically identified as brook trout but were genetically identified as hybrids. In sites 1 and 2, two samples were phenotypically identified as hybrids that were genetically identified as a brook trout. In site 4, one sample phenotypically identified as a brook trout was genetically identified as a hybrid, and another sample phenotypically identified as brook trout was genetically identified as a bull trout.

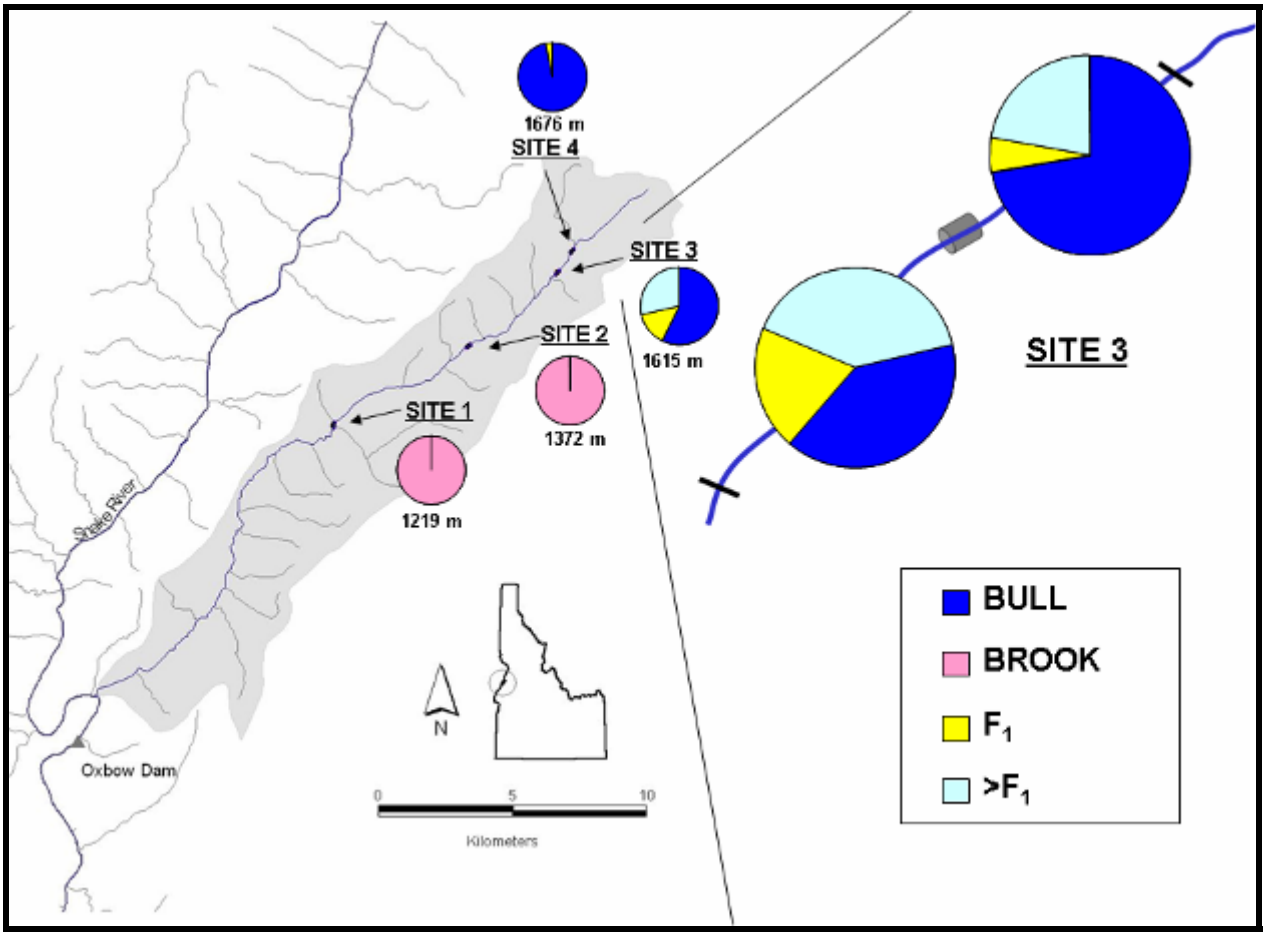

Figure 5. Distribution of bull trout, brook trout, and hybrids in Indian Creek, Idaho and extent of hybridization above and below the culvert at site 3.

DISCUSSION

This study reveals some interesting findings regarding the distribution of bull trout, brook trout, and hybrids in Indian Creek. During the time of collection, no brook trout were found in the upper sites and no bull trout were found at the lower sites. However, the presence of F_1 hybrids indicates that there had to be contact and that hybridization occurred in the last generation. This

hybrid zone appears to be between sites 3 and 4. The presence of bull trout mitochondrial DNA in all of the identified F_1 hybrids indicates that female bull trout are spawning with male brook trout. This pattern of unidirectional hybridization has been previously documented by Kanda et al. (2002) and may be due to the fact that male brook trout spawn earlier than female brook trout, mature at an earlier age than female brook trout, and often exhibit “sneaking” behavior when resident and migratory bull trout are sympatric (Kitano et al. 1994).

The mechanism for hybridization in Indian Creek is unclear but there are a number of possible explanations: 1) male brook trout or female bull trout are moving into the contact zone where hybridization is occurring and later moving out of the contact zone, 2) brook trout were present in the upper sites but not sampled, or 3) hybridization is influenced by flow regimes where high flows allowed contact in previous generations even though the two species presently do not exist in sympatry. The documentation of brook trout at the upper sites in 1997, and the fact that bull trout spawning is constrained by high temperatures (Dunham et al. 2003), suggest that male brook trout may be moving into the upper sites to spawn rather than female bull trout spawning at the lower elevations (where temperatures are presumably higher). Whatever the exact mechanism (elevation, temperature, flow regimes) affecting the distribution of bull trout, brook trout, and hybrids in Indian Creek, the culvert present at site 3 does not appear to be a complete barrier to movement, since hybrids were found above the culvert.

We observed seven $>F_1$ hybrids (backcrosses to bull trout). These results substantiate previous work by Kanda et al. (2002), which identified post F_1 hybrids and was the first study to dismiss the long held notion that all F_1 hybrids are sterile. While post-F_1 hybrids were previously reported, no hybrid swarms have been detected, which suggests that there is some reduced fertility or selection against hybrids (Kanda et al. 2002).

Overall, there was general concordance between phenotypic and genotypic identifications, except in the one site where introgressive hybridization was prominent. At site 3, hybrids were mistaken for bull trout and bull trout were mistaken for hybrids. This is not surprising given that $>F_1$ hybrids were found, which would not necessarily have phenotypic traits intermediate between the two species (Leary et al. 1995). However, this has important implications for management. Current management practices involve the removal of brook trout from key bull trout spawning areas; however, it may be prudent to remove hybrids as well since they will compete for resources and their presence may result in wasted reproductive effort for bull trout. Our results suggest that if hybrids were targeted for removal using current phenotype-based methodologies, some pure bull trout may also be removed. Consideration needs to be taken whether it is more advantageous to leave hybrids in the system that are capable of reproducing with bull trout or remove all hybrids and risk removing pure bull trout as well. Biologists for the Idaho Power Company are currently collaborating with personnel from the USDA Forest Service and Idaho Department of Fish and Game to refine current phenotypic keys, which may resolve this problem.

JOB PERFORMANCE REPORT
SUBPROJECT #3: AN ASSESSMENT OF A REINTRODUCTION EFFORT FOR BONNEVILLE CUTTHROAT TROUT *ONCORHYNCHUS CLARKII UTAH*

State of: Idaho

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

Project No.: 2

Title: Native Species Investigations

Subproject #1: An assessment of a reintroduction effort for Bonneville cutthroat trout *Oncorhynchus clarkii utah*

Contract Period: July 1, 2004 to June 30, 2005

ABSTRACT

Reintroduction efforts for Bonneville cutthroat trout are underway in several areas throughout Utah, and reintroductions have been discussed as a possible recovery strategy for Bonneville cutthroat trout in Idaho. These efforts often first involve a chemical renovation treatment to remove rainbow trout and hybrids with subsequent transplantation of fish from a pure population. The purpose of this preliminary study was to determine whether hybrids and/or rainbow trout were missed during the renovation of test stream (SF Johnson Creek) and are currently contributing to reproduction. To accomplish this objective, 37 juvenile fish were non-lethally sampled from SF Johnson Creek in August 2004 and genetically analyzed with diagnostic mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) markers to assess rainbow trout hybridization and introgression. Results indicate that hybrids were missed during the 2001 renovation. Eight of the 37 juvenile samples were identified as $>F_1$ hybrids. However, 27 of the juveniles collected had genotypes indicative of pure cutthroat and exhibited a mtDNA haplotype found in Birch Creek adults. This strongly suggests that at least a portion of the translocated adults have been reproductively successful. These results will have to be considered carefully by managers focused on restoring genetically pure Bonneville cutthroat trout throughout their historic range.

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INTRODUCTION

Numerous Bonneville cutthroat trout *Oncorhynchus clarkii utah* populations have been extirpated throughout their historic range due to a variety of natural and anthropogenic factors including drought, overfishing, habitat degradation, and the stocking of nonnative trout, which have hybridized with or replaced populations in many areas (Behnke 1992; Hepworth et al. 1997; Hepworth et al. 2002).

As part of a collaborative, regional effort to restore pure populations, Trout Unlimited, the Confederated Tribes of the Goshute Reservation, and a number of State (Utah, Nevada, and Idaho) and Federal agencies are working together to reintroduce pure Bonneville cutthroat trout in several areas throughout their historic range. One such area is the South Fork of Johnson Creek, headwaters of Deep Creek. This stream is a tributary to Deep Creek and is located in the Deep Creek Mountains on the Goshutes Indian Reservation in western Utah (Figure 6). Historically, a number of tributaries on the west slope of the Deep Creek Mountains (including SF Johnson Creek) supported pure Bonneville cutthroat trout populations. However, genetic analyses conducted in 1996 indicated that hybridization with nonnative rainbow trout *O. mykiss* was extensive in all of these streams (UDWR 1997).

In an attempt to restore genetically pure Bonneville cutthroat trout to SF Johnson Creek, the creek was chemically renovated in the fall of 2001 to remove rainbow trout and hybrids. In 2002, 60 adult Bonneville cutthroat trout were translocated into SF Johnson Creek from a genetically pure population in NF Birch Creek (Figure 6). All 60 adults were PIT-tagged, and fin clips were collected from all adults with the hope of eventually determining individual reproductive success through a possible parentage study.

In 2004, electroshocking surveys identified juvenile cutthroat trout in SF Johnson Creek, potentially providing evidence of successful reproduction of translocated adults. However, some of the fish sampled appeared to be hybrids based on phenotypic characteristics. Additionally, several of these fish sampled were out of the expected size range for juveniles that could have been produced by transplanted adults. These observations led managers to suspect that not all of the rainbow trout and hybrids were removed from SF Johnson Creek prior to translocation.

The purpose of this preliminary study was to assess whether hybrids and/or rainbow trout are contributing to current reproduction. To accomplish this objective, 37 juvenile fish were nonlethally sampled from SF Johnson Creek in August 2004. It was intended to use the original 60 fin clips from the transplanted adults for comparison purposes, but these samples were misplaced by another lab and were not available for analysis. However, 24 of the original translocated adults were recaptured during electroshocking efforts and reclipped for genetic analysis. All 61 samples (37 juveniles + 24 adults) were genetically analyzed with diagnostic mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) markers to assess rainbow trout hybridization and introgression. The identification of rainbow trout alleles within the sample of 37 juvenile fish would provide confirmation that fish missed during the original removal effort are contributing to present day production.

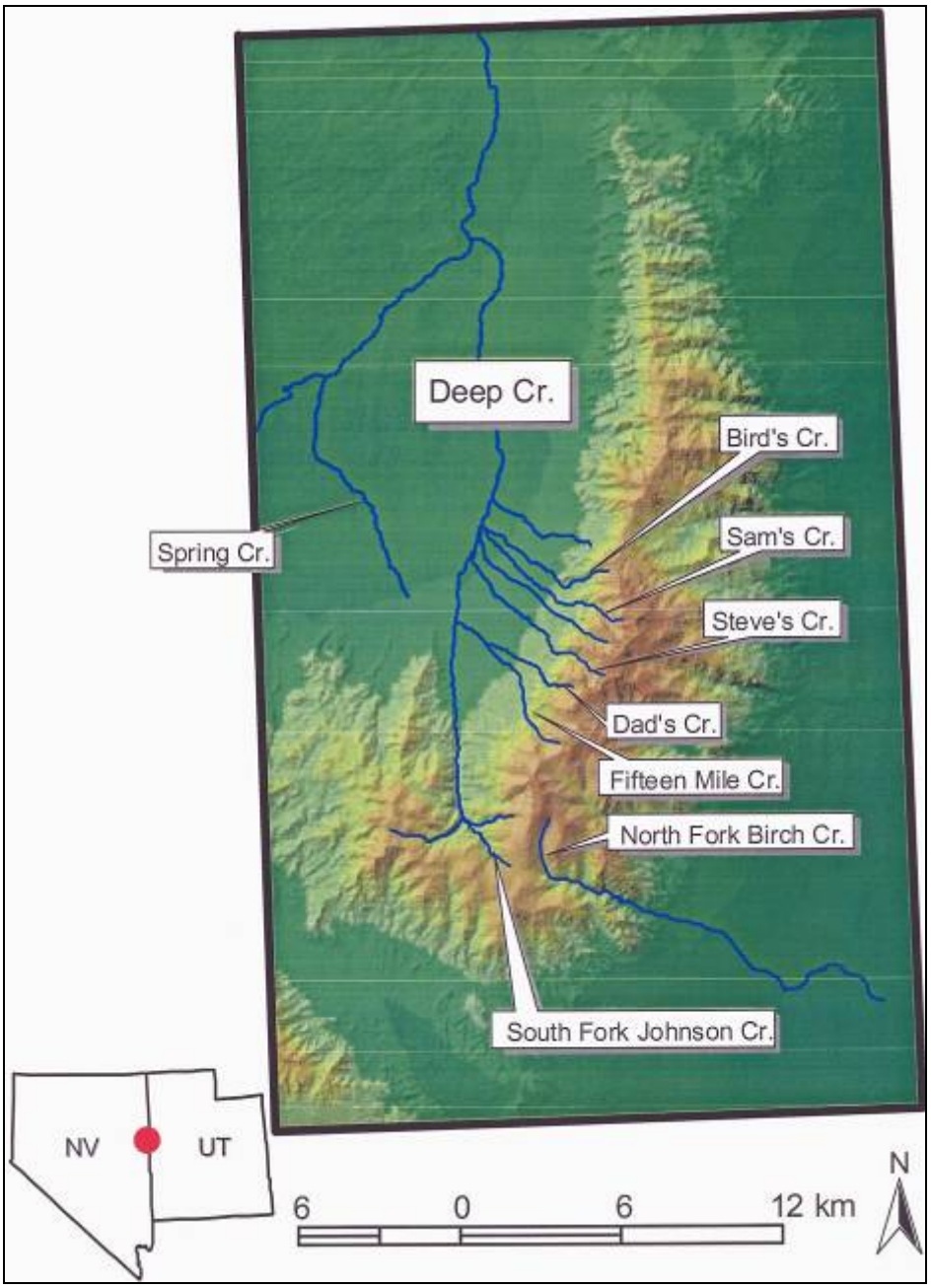

Figure 6. Tributaries on the west slope of the Deep Creek Mountains (including SF Johnson Creek) and NF Birch Creek

OBJECTIVES

1. Assess whether hybrids and/or rainbow trout are contributing to current reproduction.

METHODS

Genetic Analysis

Total genomic DNA was extracted from a 1 x 1 mm piece of fin clip using a standard salt-chloroform extraction protocol (Campbell 2000). DNA was resuspended in 100 µl TE. Five diagnostic codominant nDNA markers and one diagnostic mtDNA marker were used to assess rainbow trout hybridization and introgression within the samples. Four of the five nDNA markers are simple sequence repeat (SSR) markers (Occ 36, Occ 38, Occ 42, OM55) and are diagnostic based on size differences in the Polymerase Chain Reaction (PCR) products between rainbow trout and cutthroat trout (Ostberg and Rodriquez 2002). The other nDNA marker used in this study is a Restriction Fragment Length Polymorphism (RFLP) marker (Rag 3'/Dde-I) in which an amplified gene region (Rag3') is digested with a specific enzyme (Dde-I) to yield species specific RFLP patterns or polymorphisms (Baker et al. 2002). Products of PCR (SSR markers) and digests (RFLP marker) were electrophoresed on 3% synergels and alleles were visualized as band patterns when fluoresced under UV-light. For the markers used in this study, "A" refers to an allele unique to rainbow trout whereas "B" refers to an allele unique to cutthroat trout.

A mtDNA RFLP marker (ND12 digested with Rsa-I) was used to help identify the direction of hybridization. The ND12 mtDNA gene region was also digested with three additional restriction enzymes: Dpn-II, Hinf-I, and Msp-I. These enzymes along with Rsa-I have previously yielded mtDNA differences between and within Bonneville cutthroat trout populations (IDFG, unpublished data; Toline et al. 1999). Digests were also run on 3% agarose gels and mtDNA polymorphisms were visualized as band patterns when fluoresced under UV-light. Each unique polymorphism generated by a specific restriction enzyme was assigned a letter. The letter designations for each of the four restriction enzymes were later combined across enzymes to form a composite haplotype. Generated haplotypes were compared between samples previously analyzed from several Bonneville cutthroat trout and Yellowstone cutthroat trout collected from Utah, Nevada, Idaho, and Wyoming (IDFG, unpublished data).

RESULTS

As expected, no hybrids were observed among the 24 adults originally transplanted (Table 4). However, eight of 37 juvenile samples analyzed were identified as hybrids. All eight had genotypes indicative of >F1 hybrids, and four of the eight had genotypes indicative of either F1 X F1 or >F2 matings (Figure 7, Table 4).

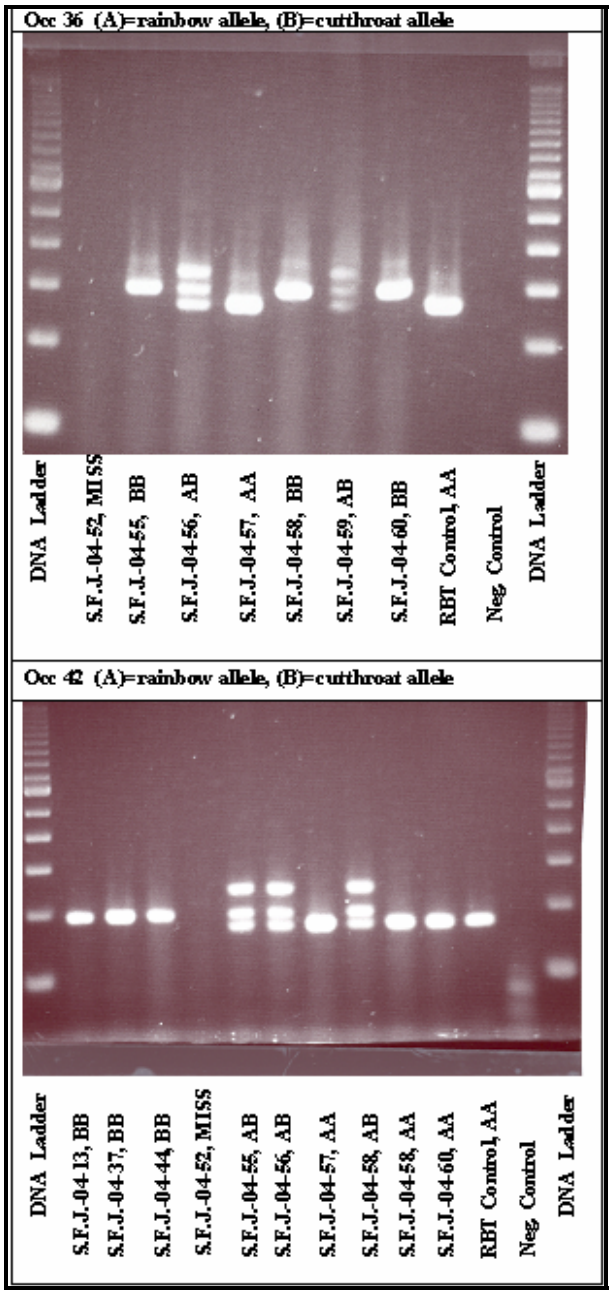

Figure 7. Photographs of two gels demonstrating hybrids. Individuals heterozygous with both a rainbow trout and cutthroat trout allele exhibit a heteroduplex band (extra upper band). Top: Occ 36 (3% agarose gel). Bottom: Occ 42 (3% agarose gel).

All samples exhibited cutthroat trout mtDNA, and all samples except for the individuals identified as hybrids exhibited the mtDNA haplotype “CBBA” previously observed in Bonneville cutthroat trout (IDFG, unpublished data; Table 4). Interestingly, all eight hybrids exhibited a different mtDNA haplotype. This haplotype “CBBG” was similar to “CBBA” except that a new polymorphism (a single restriction site difference) was observed in the Hinf-I digest (G*, Figure 8).

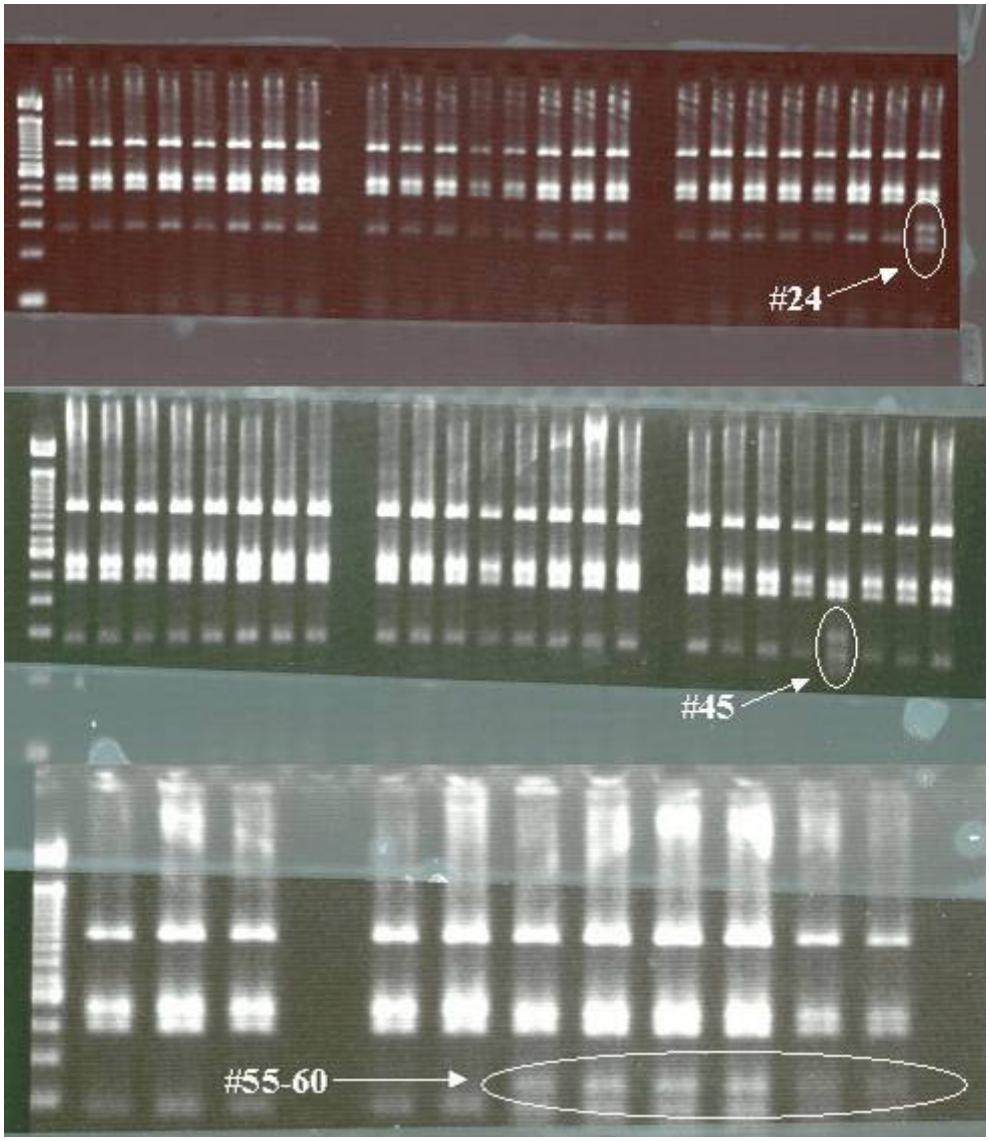

Figure 8. Photographs of three gels (ND12 digested with *Hinf-I)* showing polymorphism “G” observed in hybrids (3% agarose gels).

Table 4. Sample, origin, alleles scored for each nDNA locus, mtDNA haplotype, and genetic identification.

		Diagnostic nuclear DNA loci					mtDNA	
Sample	**Origin**	**Occ 36**	**Occ 38**	**Occ 42**	**OM 55**	**Rag3'/Dde-I**	**ND12 Haplotype**	**Genetic ID**
SF Johnson-04-1	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-2	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-3	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-4	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-5	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-6	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-7	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-8	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-9	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-10	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-11	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-12	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-13	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-14	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-15	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-16	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-17	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-18	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-19	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-20	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-21	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-22	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-23	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-24	?	AB	BB	BB	BB	BB	CBBG*	Hybrid
SF Johnson-04-25	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-26	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-27	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-28	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-29	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-30	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-31	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-32	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-33	?	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-34	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-35	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-36	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-37	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-38	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-39	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-40	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-41	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-42	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-43	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-44	Birch Cr.	BB	BB	BB	MISS	BB	CBBA	Cutthroat
SF Johnson-04-45	SF Johnson	BB	BB	AA	AB	AB	CBBG*	Hybrid
SF Johnson-04-46	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-47	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-48	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-49	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-50	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-51	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-52*	Birch Cr.	MISS	MISS	MISS	MISS	MISS	MISS	N/A
SF Johnson-04-53	SF Johnson	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-54	Birch Cr.	BB	BB	BB	BB	BB	CBBA	Cutthroat
SF Johnson-04-55	SF Johnson	BB	BB	AB	BB	BB	CBBG*	Hybrid
SF Johnson-04-56	SF Johnson	AB	BB	AB	BB	BB	CBBG*	Hybrid
SF Johnson-04-57	SF Johnson	AA	BB	AA	BB	BB	CBBG*	Hybrid
SF Johnson-04-58	SF Johnson	BB	BB	AB	BB	BB	CBBG*	Hybrid
SF Johnson-04-59	SF Johnson	AB	BB	AA	BB	AA	CBBG*	Hybrid
SF Johnson-04-60	SF Johnson	BB	BB	AA	BB	BB	CBBG*	Hybrid

* Sample SF Johnson-04-52 yielded insufficient DNA quality or quantity for PCR and RFLP analyses.

DISCUSSION

The identification of hybrids within the juvenile sample collected from SF Johnson Creek indicates that hybrids missed during initial renovation efforts are contributing to current reproduction. None of the fish sampled had genotypes indicative of pure rainbow trout (homozygous for rbt alleles at each locus examined) or of F_1 hybrids (heterozygous for both a rbt allele and cut allele at each locus examined), indicating that current hybridization is not between missed rainbow trout and introduced cutthroat from Birch Creek. Rather, all hybrids had genotypes indicative of F_1 X F_1 matings or $>F_1$ backcross matings.

All hybrids exhibited a mtDNA haplotype different ~~than~~ from that observed in the transplanted fish from Birch Creek. This is an interesting observation, but at this point sample sizes are too low to make any definitive conclusions regarding its significance. It may suggest that the two populations (SF Johnson, Birch Creek) originally did not share mtDNA haplotypes or that these haplotypes are present in very different frequencies within these populations. It also could suggest that the original hybridization crosses likely occurred between rainbow trout males and cutthroat trout females. Finally, if the hybrids currently in the creek were products of transplanted Birch Creek adults and remnant hybrids, than this would seem to suggest that the direction of hybridization is likely predominantly between hybrid females and Birch Creek males. An alternative explanation is that there are some differences in spawn timing and location between transplanted Birch Creek adults and missed SF Johnson Creek hybrids, and that current hybrid production is solely from original SF Johnson fish. Genetic analysis of any archived samples collected from SF Johnson Creek prior to renovation and increased genetic sampling of juveniles and adults currently in the Creek may help resolve this issue in the future.

While the identification of hybrids within the juvenile samples collected from SF Johnson Creek means that the goal of restoring a pure Bonneville cutthroat population to SF Johnson Creek has not been achieved, there are several positive results from these initial efforts that should be considered carefully by managers. First, if restoration efforts were successful in removing rainbow trout from SF Johnson Creek (no rainbow trout or F_1 hybrids were identified), introgression levels are unlikely to increase dramatically in the future due to the absence of new rainbow trout alleles entering the population. Secondly, six of the eight genetically confirmed hybrids were phenotypically identified by fishery biologists as either “rainbow trout,” “hybrid,” or “?” (unknown). This suggests that biologists/technicians would likely be able to reduce the number of hybrids (and RBT introgression) during future electrofishing efforts. These efforts should begin as soon as possible since a number of studies have indicated that as the percentage of rbt alleles within individual fish decreases (during successive back-crossing to cutthroat) the ability to phenotypically identify these fish decreases (Leary et al. 1996). Finally, 27 of the juveniles collected had genotypes indicative of pure cutthroat and a mtDNA haplotype matching that found in Birch Creek adults. This strongly suggests that at least a portion of the transplanted adults have been reproductively successful.

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Appendix A. Genotypes for all samples and final genetic identification.

Site	Date	Dna	Genetic #	Field ID	Length	Weight	mtDNA Cyt B Rsa*I*	nDNA p53 Mse*I*	nDNA GH2	nDNA *Sfo* 18	IDFG Genetic ID	USFWS Genetic ID
1	10/8/2002	1-1	IND-02-96	DB	238	117	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-2	IND-02-97	DBH	212	104	B	AB	AB	AB	F_1	F_1
1	10/8/2002	1-3	IND-02-98	DBH	191	58	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-4	IND-02-99	DB	130	17	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-5	IND-02-100	DBH	174	47	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-6	IND-02-101	DB	114	12	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-7	IND-02-102	DB	172	40	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-8	IND-02-103	DB	55		B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-9	IND-02-104	DB	115	12	B	AB	AB	BB	>F_1	F_1
1	10/8/2002	1-10	IND-02-105	DB	108	11	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-11	IND-02-106	DB	112	11	B	BB	AB	AB	>F_1	>F_1
1	10/8/2002	1-12	IND-02-107	DB	116	12	B	AB	BB	AB	>F_1	>F_1
1	10/8/2002	1-13	IND-02-108	DB	106	10	B	AB	BB	AB	>F_1	>F_1
1	10/8/2002	1-14	IND-02-109	EB	222	108	B	AB	BB	AB	>F_1	>F_1
1	10/8/2002	1-15	IND-02-110	DB	188	50	B	BB	BB	BB	BULL	BULL
1	10/8/2002	1-16	IND-02-111	DB	230	93	B	BB	BB	BB	BULL	BULL
1	10/8/2002	1-17	IND-02-112	DBH	168	42	B	AB	AB	AB	F_1	>F_1
1	10/8/2002	1-18	IND-02-113	DB	169	41	B	BB	BB	n/a	BULL	>F_1
1	10/8/2002	1-19	IND-02-114	DB	138	21	B	AB	AB	AB	F_1	F_1
1	10/8/2002	1-20	IND-02-115	EB	225	100	B	AB	BB	N/A	>F_1	F_1
1	10/8/2002	1-21	IND-02-116	DBH	191	68	B	AB	AB	AB	F_1	F_1
1	10/8/2002	1-22	IND-02-117	DBH	237	103	B	BB	BB	BB	BULL	BULL
1	10/8/2002	1-23	IND-02-118	DB	164	33	B	BB	BB	n/a	BULL	>F_1
1	10/8/2002	1-24	IND-02-119	DBH	157	38	B	BB	BB	n/a	BULL	F_1*
1	10/8/2002	1-25	IND-02-120	DB	190	58	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-26	IND-02-121	DB	116	12	B	BB	BB	n/a	BULL	BULL
1	10/8/2002	1-27	IND-02-122	DB	114	14	B	BB	BB	BB	BULL	BULL
1	10/8/2002	1-28	IND-02-123	DB	115	11	B	BB	BB	BB	BULL	BULL
2	10/15/2002	2-1	IND-02-1	EB	213	106	A	AA	AA	AA	Brook	Brook
2	10/15/2002	2-2	IND-02-2	EB	194	74	A	AA	AA	AA	Brook	Brook
2	10/15/2002	2-3	IND-02-3	EB	144	25	A	AA	AA	AA	Brook	Brook
2	10/15/2002	2-4	IND-02-4	EB	120	13	A	AA	AA	n/a	Brook	Brook
2	10/15/2002	2-5	IND-02-5	EB	152	30	A	AA	AA	n/a	Brook	Brook
2	10/15/2002	2-6	IND-02-6	EB	141	21	A	AA	AA	n/a	Brook	Brook
2	10/15/2002	2-7	IND-02-7	EB	178	47	A	AA	AA	n/a	Brook	Brook
2	10/15/2002	2-8	IND-02-8	EB	150	29	A	AA	AA	n/a	Brook	Brook
2	10/15/2002	2-9	IND-02-9	EB	170	53	A	AA	MISS	n/a	Brook	Brook
2	10/15/2002	2-10	IND-02-10	DBH	162	39	A	AA	MISS	n/a	Brook	Brook
2	10/15/2002	2-11	IND-02-11	EB	142	23	A	AA	MISS	n/a	Brook	n/a
2	10/15/2002	2-12	IND-02-12	EB	101	9	A	AA	MISS	n/a	Brook	n/a
2	10/15/2002	2-13	IND-02-13	EB	118	15	A	AA	MISS	n/a	Brook	n/a
2	10/15/2002	2-14	IND-02-14	EB	114	13	A	AA	MISS	n/a	Brook	n/a
2	10/15/2002	2-15	IND-02-15	EB	112	12	A	AA	MISS	n/a	Brook	n/a
2	10/15/2002	2-16	IND-02-16	EB	118	14	A	AA	MISS	n/a	Brook	n/a
2	10/15/2002	2-17	IND-02-17	EB	69	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-18	IND-02-18	EB	77	4	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-19	IND-02-19	EB	134	19	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-20	IND-02-20	EB	112	12	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-21	IND-02-21	EB	72	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-22	IND-02-22	EB	71	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-23	IND-02-23	EB	70	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-24	IND-02-24	EB	80	4	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-25	IND-02-25	EB	68	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-26	IND-02-26	EB	100	8	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-27	IND-02-27	EB	145	26	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-28	IND-02-28	EB	106	12	A	AA	MISS	n/a	Brook	n/a
2	10/15/2002	2-29	IND-02-29	EB	141	26	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-30	IND-02-30	EB	100	9	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-31	IND-02-31	EB	122	15	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-32	IND-02-32	EB	92	7	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-33	IND-02-33	EB	148	25	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-34	IND-02-34	EB	63	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-35	IND-02-35	EB	62	2	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-36	IND-02-36	EB	161	34	A	AA	AA	n/a	Brook	n/a

Appendix A. Continued.

Site	Date	Dna	Genetic #	Field ID	Length	Weight	mtDNA Cyt B Rsa/	nDNA p53 Mse/	nDNA GH2	nDNA *Sfo* 18	IDFG Genetic ID	USFWS Genetic ID
2	10/15/2002	2-37	IND-02-37	EB	126	17	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-38	IND-02-38	EB	101	9	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-39	IND-02-39	EB	118	16	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-40	IND-02-40	EB	68	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-41	IND-02-41	EB	72	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-42	IND-02-42	EB	78	4	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-43	IND-02-43	EB	70	3	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-44	IND-02-44	EB	59	1	A	AA		n/a	Brook	n/a
2	10/15/2002	2-45	IND-02-45	EB	117	13	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-46	IND-02-46	EB	103	10	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-47	IND-02-47	EB	64	2	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-48	IND-02-48	EB	69	2	A	AA	AA	n/a	Brook	n/a
2	10/15/2002	2-49	IND-02-49	EB	67	1	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-1	IND-02-124	DBH	177	48	A	AA	AA	AA	Brook	n/a
3	10/9/2002	3-2	IND-02-125	EB	170	37	A	AA	AA	AA	Brook	n/a
3	10/9/2002	3-3	IND-02-126	EB	134	23	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-4	IND-02-127	EB	130	20	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-5	IND-02-128	EB	74	4	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-6	IND-02-129	EB	62	2	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-7	IND-02-130	EB	132	20	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-8	IND-02-131	EB	75	4	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-9	IND-02-132	EB	72	4	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-10	IND-02-133	EB	78	5	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-11	IND-02-134	EB	80	5	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-12	IND-02-135	EB	72	3	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-13	IND-02-136	EB	85	5	A	AA	MISS	n/a	Brook	n/a
3	10/9/2002	3-14	IND-02-137	EB	76	3	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-15	IND-02-138	EB	70	3	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-16	IND-02-139	EB	76	4	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-17	IND-02-140	EB	72	4	A	AA		n/a	Brook	n/a
3	10/9/2002	3-18	IND-02-141	EB	141	24		AA	AA	n/a	Brook	n/a
3	10/9/2002	3-19	IND-02-142	EB	204	72	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-20	IND-02-143	EB	191	72	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-21	IND-02-144	EB	83	7	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-22	IND-02-145	EB	130	22	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-23	IND-02-146	EB	72	5	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-24	IND-02-147	EB	78	6	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-25	IND-02-148	EB	75	5	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-26	IND-02-149	EB	74	6	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-27	IND-02-150	EB	70	4	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-28	IND-02-151	EB	83	5	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-29	IND-02-152	EB	55	2	A	AA	AA	n/a	Brook	n/a
3	10/9/2002	3-30	IND-02-153	EB	70	3	A	AA	AA	n/a	Brook	n/a
4	10/9/2002	4-1	IND-02-50	DBH	287	219	B	AB	AB	AB	F_1	n/a
4	10/9/2002	4-2	IND-02-51	DB	201	84	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-3	IND-02-52	DB	216	85	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-4	IND-02-53	DB	168	40	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-5	IND-02-54	DB	183	47	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-6	IND-02-55	DB	161	34	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-7	IND-02-56	DB	163	35	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-8	IND-02-57	DB	199	65	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-9	IND-02-58	DB	161	38	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-10	IND-02-59	DB	170	37	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-11	IND-02-60	DB	105	11	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-12	IND-02-61	DB	157	29	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-13	IND-02-62	DB	133	23	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-14	IND-02-63	DB	143	18	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-15	IND-02-64	DB	193	58	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-16	IND-02-65	DB	188	66	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-17	IND-02-66	DB	175	52	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-18	IND-02-67	DB	144	25	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-19	IND-02-68	DB	157	31	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-20	IND-02-69	DB	129	16	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-21	IND-02-70	EB	170	47	B	AB	AB	AB	F_1	F_1
4	10/9/2002	4-22	IND-02-71	DB	185	50	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-23	IND-02-72	DB	98	9	B	BB	BB	n/a	BULL	n/a

Appendix A. Continued.

Site	Date	Dna	Genetic #	Field ID	Length	Weight	mtDNA Cyt B Rsa/	nDNA p53 Mse/	nDNA GH2	nDNA *Sfo* 18	IDFG Genetic ID	USFWS Genetic ID
4	10/9/2002	4-24	IND-02-73	DB	133	22	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-25	IND-02-74	DB	141	25	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-26	IND-02-75	DB	140	24	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-27	IND-02-76	DB	202	75	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-28	IND-02-77	EB	188	68	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-29	IND-02-78	DB	165	40	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-30	IND-02-79	DB	144	25	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-31	IND-02-80	DB	153	28	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-32	IND-02-81	DB	99	10	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-33	IND-02-82	DB	144	24	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-34	IND-02-83	DB	141	23	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-35	IND-02-84	DB	164	37	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-36	IND-02-85	DB	138	23	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-37	IND-02-86	DB	182	56	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-38	IND-02-87	DB	195	70	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-39	IND-02-88	DB	128	17	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-40	IND-02-89	DB	163	37	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-41	IND-02-90	DB	151	32	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-42	IND-02-91	DB	144	28	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-43	IND-02-92	DB	138	21	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-44	IND-02-93	DB	156	30	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-45	IND-02-94	DB	136	22	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-46	IND-02-95	DB	109	12	B	BB	BB	n/a	BULL	BULL
4	10/9/2002	4-48	IND-02-167	DB	140	21	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-49	IND-02-168	DB	102	9	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-50	IND-02-169	DB	193	69	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-51	IND-02-170	DB	148	29	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-52	IND-02-171	DB	146	31	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-53	IND-02-172	DB	137	22	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-54	IND-02-173	DB	102	11	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-55	IND-02-174	DB	166	39	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-56	IND-02-175	DB	137	24	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-57	IND-02-176	DB	139	25	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-58	IND-02-177	DB	138	21	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-59	IND-02-178	DB	139	23	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-60	IND-02-179	DB	130	19	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-61	IND-02-180	DB	147	27	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-62	IND-02-181	DB	107	11	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-63	IND-02-182	DB	156	30	B	BB	BB	n/a	BULL	n/a
4	10/9/2002	4-64	IND-02-183	DB	210	75	B	BB	BB	BB	BULL	n/a
4	10/9/2002	4-65	IND-02-184	DB	173	46	B	BB	BB	BB	BULL	n/a
4	10/9/2002	4-66	IND-02-185	DB	169	44	B	BB	BB	BB	BULL	n/a

*Likely that this individual is a >F_1 hybrid

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