

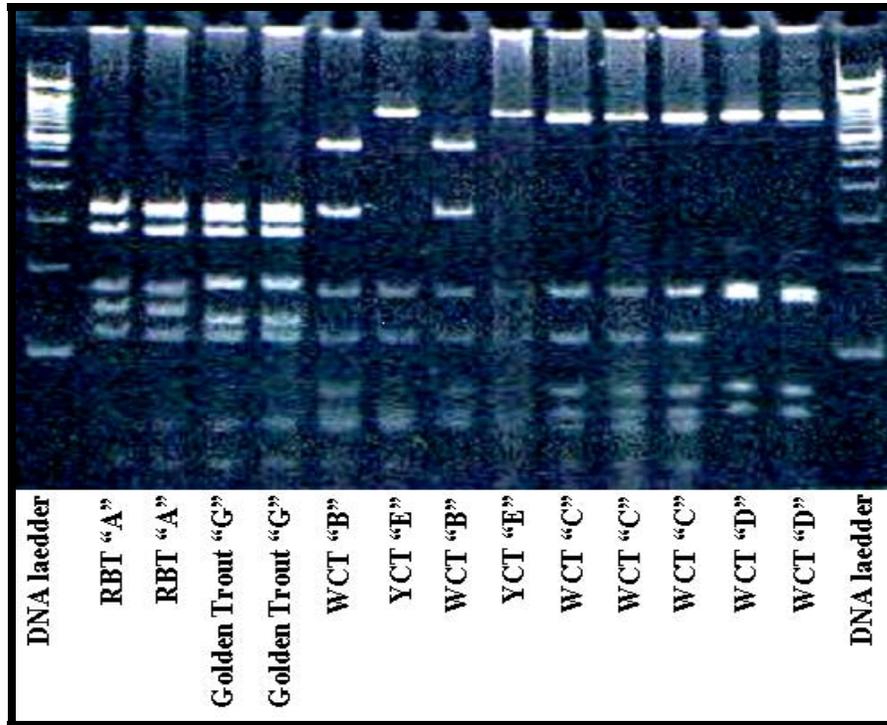
FISHERY RESEARCH



NATIVE SPECIES INVESTIGATIONS

Grant # F-73-R-25

July 1, 2002 to June 30, 2003



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Project 2: Native Species Investigations

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ABSTRACT

The primary goal of the newly constructed Idaho Department of Fish and Game Fish Genetics Laboratory at Eagle, Idaho is to provide detailed genetic information on levels of hybridization and introgression, genetic diversity, and genetic population structure of native fish species throughout Idaho. This genetic information should enable managers to assess current and future genetic risks, preserve existing genetic variability, delineate and prioritize populations for conservation and management purposes, estimate effective population size, identify suitable populations for translocations and reintroductions, identify suitable populations for broodstock development, and address genetic concerns in future Endangered Species Act (ESA) petitions.

This report describes two genetic research projects conducted in fiscal year 2003 involving hybridization and introgression, one on westslope cutthroat trout and one on Yellowstone cutthroat trout, two subspecies of cutthroat trout that provide a significant component to the recreational fishery in Idaho. Importantly, both subspecies have previously been petitioned for listing as threatened under the ESA, and westslope cutthroat trout have just undergone their second status review to determine whether the subspecies is warranted for protection. The genetic information generated from the westslope cutthroat trout research project was used directly by managers in the second status review process. Notably, the U.S. Fish and Wildlife Service recently announced their decision on ESA listing and concluded that westslope cutthroat trout were not warranted for listing. Managers are using the genetic information generated as part of the Yellowstone cutthroat trout project to evaluate different strategies aimed at reducing hybridization and introgression between Yellowstone cutthroat trout and rainbow trout in the upper Snake River drainage.

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INTRODUCTION

In the spring of 2002, the Idaho Department of Fish and Game (IDFG) finished construction of a new fish genetics laboratory at the Eagle Fish Hatchery to provide an efficient, cost-effective means of generating the detailed genetic information necessary for the proper management and conservation of Idaho's native fish species. This report describes two research subprojects completed by the lab during the July 1, 2002 to June 30, 2003 contract period. The first subproject describes a genetic investigation of hybridization and genetic population structure of westslope cutthroat trout *Oncorhynchus clarki lewisi* (WCT) populations within the Middle Fork of the Salmon River. This research was especially timely given the fact that WCT underwent a second status review during the last year by the U.S. Fish and Wildlife Service (USFWS) to consider whether the subspecies was warranted for listing as threatened under the Endangered Species Act (ESA). Findings from this study were formally reported to the USFWS by IDFG for inclusion into the Administrative Record in October 2002 (Information Submittal for Consideration in the Westslope Cutthroat Trout Status Review; Ref: 50 CFR Part 17). In addition to this report, an internal white paper reviewing the genetic considerations and management implications of hybridization and introgression in WCT populations was submitted to the USFWS for inclusion into the Administrative Record (Appendix A).

On August 1, 2003, the USFWS issued its 12 month finding for the amended petition to list the Westslope cutthroat trout as a threatened species under the ESA. The USFWS determined that WCT were not warranted for listing as either threatened or endangered at this time.

The second subproject described in this report is an investigation on the Blackfoot River using genetic data to assess introgression levels within an adfluvial Yellowstone cutthroat *O. clarki bouvieri* (YCT) population and to test phenotypic identifications of YCT, rainbow trout *O. mykiss* (RBT), and hybrids between the two.

Management and conservation of YCT populations are high priorities for IDFG due to population declines throughout their historic native range (Thurrow et al. 1988; Behnke 1992; May 1996). While declines in YCT have been attributed to habitat degradation and overfishing, the stocking of fertile RBT (and subsequent hybridization and introgression of YCT populations) has also been cited as a cause of population declines (Thurrow et al. 1988; Behnke 1992). The research described in this report is important, because current management strategies rely on the ability to make accurate phenotypic identifications to prevent RBT and hybrids from migrating into important spawning tributaries and hybridizing with native YCT.

JOB PERFORMANCE REPORT
SUBPROJECT #1: AN ASSESSMENT OF HYBRIDIZATION AND INTROGRESSION
BETWEEN WESTSLOPE CUTTHROAT TROUT AND BOTH NATIVE AND INTRODUCED
TROUT IN THE MIDDLE FORK SALMON RIVER, IDAHO: CONSERVATION AND
MANAGEMENT IMPLICATIONS

State of: Idaho

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

Project No.: 2

Title: Native Species Investigations

Subproject #1: An Assessment of Hybridization and Introgression Between Westslope Cutthroat Trout and Both Native and Introduced Trout in the Middle Fork Salmon River, Idaho: Conservation and Management Implications

Contract Period: July 1, 2002 to June 30, 2003

ABSTRACT

Westslope cutthroat trout are currently under a second, court-ordered, status review by the U.S. Fish and Wildlife Service to determine whether the subspecies should be listed as threatened under the Endangered Species Act. Hybridization and introgression from stocking of nonnative trout has been cited as the principal biological hazard to the persistence of the subspecies and was referred to by the court as the primary reason for the decision to order a second status review. The Idaho Department of Fish and Game and the University of Idaho have been investigating hybridization and introgression in westslope cutthroat trout populations throughout Idaho. This current research is focused on populations of westslope cutthroat trout in the Middle Fork of the Salmon River drainage. The two primary objectives of this study are 1) to assess whether past stocking of hatchery trout in high mountain lakes has led to hybridization and introgression with westslope cutthroat trout throughout the drainage, and 2) to determine whether natural hybridization and introgression between sympatric westslope cutthroat trout and native rainbow trout occurs within the drainage. During the past two years, nonlethal fin samples were collected from over 1,000 trout in the Middle Fork of the Salmon River drainage. Sample sites were distributed over a large geographic area from the headwaters to the mouths of 12 tributaries, as well as from the mainstem Salmon River, and included areas below mountain lakes that had been stocked and areas that had not been stocked. Samples were genetically tested using species-specific nuclear and mitochondrial DNA markers. Results indicate low levels of hybridization and introgression in many of the tributaries. The identification of probable F₁ hybrids, as well as hybridization and introgression in stocked and nonstocked areas, suggests hybridization likely occurs between westslope cutthroat trout and both native *Oncorhynchus mykiss* as well as introduced nonnative hatchery trout.

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INTRODUCTION

Westslope cutthroat trout *Oncorhynchus clarki lewisi* (WCT) are currently under a second, court-ordered, status review by the U.S. Fish and Wildlife Service (USFWS) to determine whether the subspecies should be listed as threatened under the Endangered Species Act. In June 1997, several conservation groups collectively petitioned the USFWS to list WCT as threatened under the Endangered Species Act (Federal Register 61 FR 64425, 2002). Petitioners cited habitat loss and degradation, fragmentation of existing habitat, stocking of nonnative trout (contributing to predation, competition, and hybridization and introgression), and inadequate management programs as principal causes of population declines.

In the spring of 2000, a formal review by the USFWS concluded that WCT were not warranted for listing given that “viable, self-sustaining WCT stocks remain widely distributed throughout the species’ historic range” and “small headwater populations of WCT are relatively secure.” In October of 2000, petitioners filed suit against the USFWS alleging four claims: 1) USFWS consideration of existing regulatory mechanisms was arbitrary; 2) USFWS consideration of hybridization as a threat to WCT was arbitrary, because they included hybrids when establishing population size and distribution; 3) USFWS consideration of isolation and loss of life histories as threats to WCT was arbitrary, and 4) USFWS failed to account for the threat of whirling disease. In March of 2002, a U.S. District Court judge ruled that the USFWS listing determination for WCT was not supported by the “best available science” and ordered the USFWS to conduct a second status review and reevaluate its listing decision. In response to this order, the Idaho Department of Fish and Game (IDFG) and the University of Idaho initiated a study within the Middle Fork of the Salmon River (MFSR, Figure 1) to assess whether past stocking of nonnative trout has led to hybridization and introgression and whether natural hybridization exists between native WCT and native rainbow trout *O. mykiss* (RBT) in the MFSR.

The introduction of nonnative trout into mountain lakes and their subsequent dispersal into downstream habitats has been cited as a major risk to native fish species throughout the Western United States (Adams et al. 2001). Stocking of hatchery reared WCT, RBT, WCT X RBT hybrids, golden trout *O. mykiss aquabonita* (GT), and Yellowstone cutthroat *O. clarki bouvieri* (YCT) has occurred in some headwater lakes of some MFSR tributaries from the early 1930s through the mid-1980s. There has been concern that populations of native RBT and WCT below those lakes have been negatively impacted by these introductions, primarily through the negative consequences of hybridization and introgression. One of the objectives of this research project is to evaluate hybridization and introgression within populations of WCT and nonnative trout populations below stocked lakes.

A second related objective is to document the occurrence and extent of introgressive hybridization between native WCT and native RBT. Westslope cutthroat trout coevolved with RBT in many drainages throughout their historic range, and sympatric populations of native WCT and native RBT are found throughout Idaho within the Kootenai, Salmon, and Clearwater basins (Behnke 1992). It is likely that both species have developed reproductive isolating mechanisms within their sympatric range, which have allowed the persistence of two distinct species. However, natural hybridization has been documented between WCT and RBT (Huston 1988; Sage et al. 1992) and between steelhead *O. mykiss* and coastal cutthroat trout *O. clarki clarki* (Campton 1981; Campton and Utter 1985; Wenburg 1998; and Jennifer Nielsen, U.S. Geological Survey, personal communication). To address whether natural hybridization occurs,

we compare the incidence of hybridization and introgression in areas in which there is no history of stocking to areas in which there is a history of stocking nonnative rainbow trout.

OBJECTIVES

1. Assess whether past stocking of hatchery rainbow trout, cutthroat trout X rainbow hybrids, golden trout, and Yellowstone cutthroat trout in headwater lakes has led to hybridization and introgression of westslope cutthroat trout populations below these lakes.
2. Assess whether natural hybridization occurs between sympatric *Oncorhynchus mykiss* and Westslope cutthroat trout populations in the Middle Fork Salmon River.

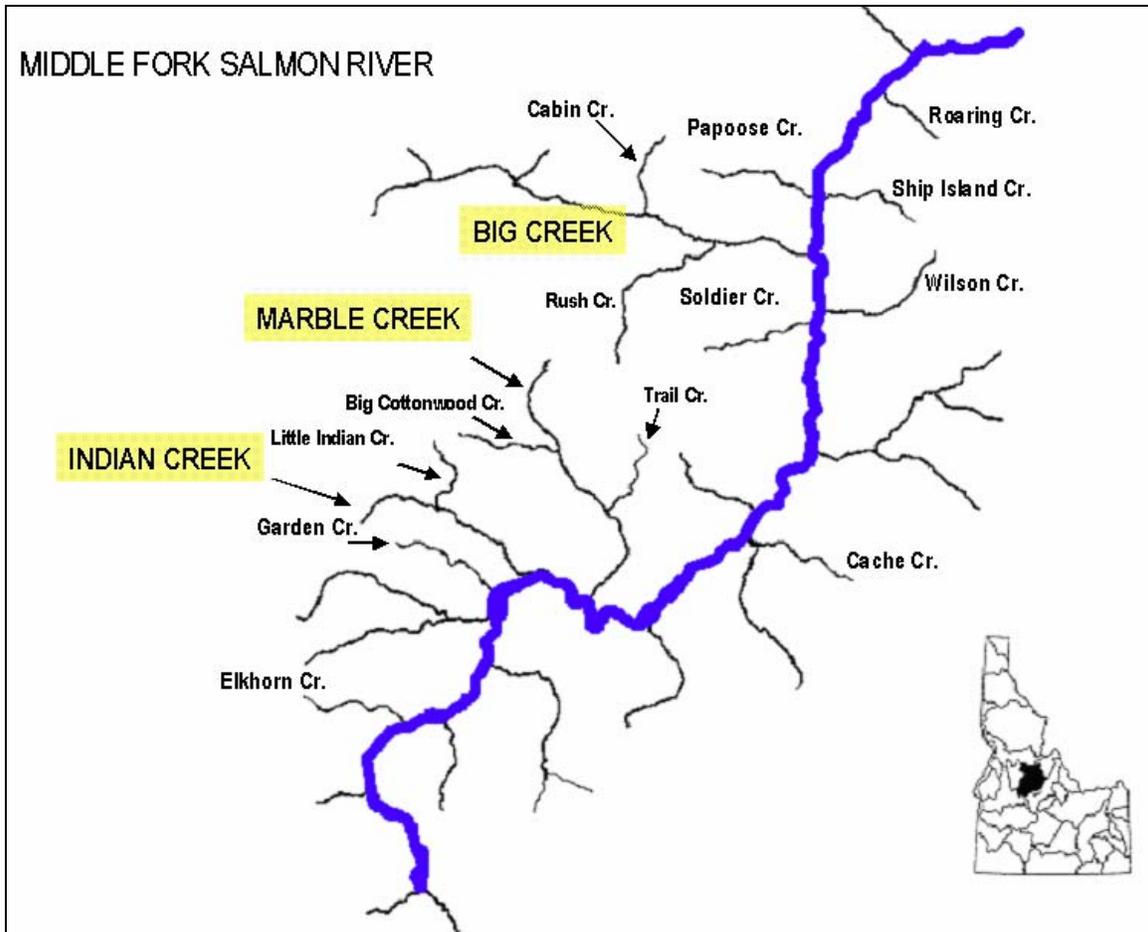


Figure 1. Middle Fork Salmon River, Idaho and tributaries.

METHODS

Sample Collection

To address whether past stocking of nonnative hatchery RBT, RBT X cutthroat trout (both WCT and YCT) hybrids, GT, and YCT in high mountain lakes has led to introgressive hybridization of WCT populations below those lakes (Objective 1), eight creeks within the MFSR (five with a known history of stocking and three with no known history of stocking) were sampled (see Appendix A for stocking history information). Nonlethal fin samples were obtained from 476 *Oncorhynchus sp.* collected by angling (Table 1). Collectors were asked to sample the entire length of the stream where possible and collect approximately 60 samples per stream. All *Oncorhynchus sp.* samples were collected regardless of phenotype.

Eighty-one GT from the Mt. Whitney State Hatchery (Cottonwood Lakes strain) were sampled for use as a reference population since stocking records indicate that this strain was likely used for GT outplants in Idaho.

To address the question of whether natural hybridization occurs between native RBT and WCT in the MFSR (Objective 2), 15 sample locations within three MFSR drainages were sampled. The Indian Creek, Marble Creek, and Big Creek drainages were selected for sampling due to historical information indicating the presence of sympatric populations of native RBT and WCT in these drainages (Thurrow 1985; IDFG parr monitoring database), and the absence or near absence of hatchery RBT stocking (see Appendix A for stocking history information). Sample sites were distributed uniformly from the headwaters to the mouths of Indian, Marble, and Big Creeks (upper, middle, and lower) and included mainstem as well as tributary sites. Nonlethal fin samples were obtained from 771 *Oncorhynchus sp.* collected by angling (Table 2). Samples were collected regardless of phenotype, and sample sizes from each site were approximately 60. All fin clips were stored at room temperature in 100% nondenatured ethanol.

Table 1. Sample locations for Objective 1, stocking history, year sampled, and number of samples collected.

Location	Stream	History of Stocking	Year Sampled	No.
MFSR	Roaring Creek	Yes	2002	43
	Ship Island Creek	Yes	2002	60
	Wilson Creek	Yes	2002	63
	Cache Creek	Yes	2002	58
	Papoose Creek	Yes	2002	65
	Soldier Creek	No	2002	71
	Garden Creek	No	2002	58
	Elkhorn Creek (upper)	No	2002	29
	Elkhorn Creek (lower)	No	2002	20

Table 2. Sample locations for Objective 2, stocking history, year sampled, and number of samples collected.

Location	Stream	History of Stocking	Year Sampled	No.
Big Creek (MFSR)	Mainstem Big Creek (upper)	Yes ^a	2002	66
	Mainstem Big Creek (middle)	No	2002	71
	Mainstem Big Creek (lower)	No	2002	63
	Cabin Creek	No	2001	50
	Cabin Creek	No	2002	66
	Rush Creek	No	2001	34
	Rush Creek	No	2002	60
Marble Creek (MFSR)	Mainstem Marble Creek (upper)	No	2002	60
	Mainstem Marble Creek (middle)	No	2002	60
	Big Cottonwood Cr.	No	2002	60
	Trail Creek	No	2002	60
Indian Creek (MFSR)	Mainstem Indian Creek (upper)	No	2002	60
	Mainstem Indian Creek (middle)	No	2002	62
	Mainstem Indian Creek (lower)	No	2002	61
	Little Indian Creek	No	2002	60

^a Lick Creek Lake (Headwater to upper Big Creek) was stocked once in 1959 with RBT.

Genetic Analysis

Total genomic DNA was extracted from fin clip samples following methods described by Paragamian et al. (1999) and adapted from protocols by Sambrook et al. (1989) and Hillis et al. (1996). Up to five codominant nuclear DNA (nDNA) markers diagnostic between RBT and WCT were screened to assess the genetic status of individual fish and sample locations (Table 3). These included three intron Restriction Fragment Length Polymorphism (RFLP) markers: Recombination Activation Gene (RAG3'), Ikaros Gene (IK), and Protoncogene 53 (p53) (Baker et al. 2002; Campbell et al. 2002), two simple sequence repeat markers (SSR): OM13 and OCC16 (Ostberg and Rodriguez 2002), and one RFLP mitochondrial DNA (mtDNA) marker diagnostic between RBT, YCT, WCT (Mays 2002), and GT (observed in this study).

To test the null hypothesis that samples were drawn from a randomly mating population, Fisher's exact tests for Hardy-Weinberg equilibrium (HWE) were performed using Genepop Version 3.1a (Raymond and Russet 1995). Hardy-Weinberg equilibrium tests were performed for all sample locations independently and combined.

Recent papers have reported the relative number of individuals identified as hybrids rather than allele frequencies as a measure of the extent of introgression within populations (Weigel et al. 2002^a, 2002^b). For comparison purposes, we state the percentage of individuals that were genetically identified as hybrid at each sample location, but we also report the percentage of RBT alleles observed among combined samples of individuals with genotypes indicative of both hybrids and WCT.

Samples from 12 sample locations (upper, middle and lower Big Creek; Rush Creek 2001 and 2002; Cabin Creek 2001 and 2002; Wilson Creek; Ship Island Creek; Papoose Creek; Roaring Creek; and Garden Creek) were additionally screened with four microsatellite loci

(*Ogo4* [Olsen et al. 1998], *Oneu14* [Scribner et al. 1996], *OMM1035*, and *OMM1050* [Rexroad, unpublished]) to examine genetic population structure (Table 3). The forward primer of each pair was fluorescently labeled with 6-FAM, HEX, or NED (Applied Biosystems). Loci were multiplexed to increase throughput efficiency. The following amplifications were performed in a single Polymerase Chain Reaction (PCR): *Ogo4*, *OMM1035* and *OMM1050*, and *Oneu14*. A 15 μ l amplification was performed for each pair of loci using 1 μ l of genomic DNA, 20 mM of each dNTP, 1X reaction Buffer, 2.5 mM MgCl₂, 0.20 μ M of each primer, and of 0.6 Units of Amplitaq polymerase (Applied Biosystems). The following PCR profile was used for all loci: 96° for 2 min, 35 cycles of 95° for 30 s, 60° for 30 s, 72° for 40 s, followed by a final 2 min. extension at 72°. Amplifications were performed using an MJR PTC-100 thermal cycler.

Amplification products were also multiplexed. For electrophoresis, 1.5 μ l of each multiplex was added to 40 μ l of Formamide and 0.33 μ l of internal size standard GeneScan® ROX 500 (Applied Biosystems). Amplified microsatellite fragments were separated using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) and genotyped using GeneScan® 3.0 and Genotyper® 2.1 (Applied Biosystems).

Genepop version 3.1a (Raymond and Rousset 1995) was used to test each microsatellite locus in each population for departures from HWE. Each sample site (including all samples) was a priori tested for random mating. If random mating was rejected (significant departures from HWE), individuals within the sample set were separated into species classifications based upon the RFLP results and run again. A sequential Bonferroni correction (Rice 1989) was used to correct for multiple tests using a significance level of 0.05.

Genetic diversity indices, such as observed heterozygosity and population genetic structure, were evaluated for samples with genotypes indicative of RBT at each site separately. Arlequin 2.0 (Schneider et al. 1997) was used to generate pairwise F_{st} estimates and a hierarchical Analysis of Molecular Variance. The hierarchical AMOVA simultaneously estimates the proportion of genetic variation partitioned within and among populations. To ascertain if the individual samples identified with genotypes indicative of RBT within sites with a known history of stocking were genetically different than RBT samples from sites with no stocking history, a second AMOVA analysis was performed between sets of samples with genotypes indicative of RBT within the stocked and nonstocked sites.

Table 3. Loci, marker type, total size of amplified product in base pairs (bp), enzymes used to yield diagnostic banding patterns, and expected sizes of digest fragments (bands) for rainbow trout, westslope cutthroat trout, Yellowstone cutthroat trout, and golden trout.

Locus	Marker Type	Total size (bp)	Enzyme ^b	RBT expected sizes of fragments ^c	WCT expected sizes of fragments ^c	YCT expected sizes of fragments ^c	GT expected sizes of fragments ^c
Recombination activation gene (RAG3)	Intron/RFLP ^a	1013	<i>Dde-I</i>	544/469	(1) 544/286/183 (2) 324/286/220/183	Same as WSC	Same as RBT
Ikaros Gene (IK) ¹	Intron/RFLP ^a	813	<i>Hinf-I</i>	813	520/293	Same as WSC	Same as RBT
Protoncogene 53 (p53) ²	Intron/RFLP ^a	481	<i>Alu-I</i>	(1) 190/140/100/51 (2) 330/100/51 ^d	330/100/51	Same as WSC	Same as RBT
OM-13	SSR ^b	175/190	N/A	175	190	Same as WSC	Same as RBT
OCC-16	SSR ^b	280/380	N/A	230	380	Same as WSC	Same as RBT
Cytochrome B (mtDNA)	MtDNA/RFLP ^c	1300	<i>Hae-III</i>	310/300/265/165/130/110	(1) 910/165/110/75/50/40 (2) 600/310/165/110/75/50	985/165/110/50/40	300/290/265/195/120/110

¹ The Ikaros gene has been described as a fixed genetic marker between RBT and WCT (one allele is only observed in RBT, never in WCT, and one allele is observed in WCT, never in RBT) (Baker et al. 2002; Rubidge et al. 2002). Work by IDFG in this study indicates that the IK/Hinf-I marker is not fixed within WCT populations in the Middle Fork of the Salmon River. The A allele, previously only observed in RBT, is also observed in low frequency in WCT in the MFSR.

² The p53 marker is an imperfect marker; only one of the alleles exhibit fixed differences between RBT and WCT.

^a Restriction Fragment Length Polymorphism (RFLP). In this technique, introns (noncoding regions of nDNA) are digested with a specific restriction enzyme that yields diagnostic banding patterns (fragments) between species/subspecies-Dominant marker, in which both the paternal and maternal alleles are amplified and visualized (see Baker et al. 2002).

^b Simple sequence repeat primers (type of microsatellite), which amplify alleles of varying length-Dominant marker (see Ostberg and Rodriguez 2002).

^c Same as in ^a, but in this case a mitochondrial DNA (mtDNA) region, Cytochrome B, is amplified and digested with a specific enzyme (see Mays 2002).

RESULTS

Diagnostic nDNA Markers and mtDNA Marker

Preliminary results only include data from RAG3', OM13, and OCC16 since, at the time of this report, not all sample locations have been examined with the markers (p53 and IK). Both p53 and IK are imperfect markers in which only one of the alleles exhibits fixed differences between RBT and WCT (Baker et al. 2002). We, therefore, determined that p53 and IK would be the last two loci examined because of anticipated time constraints.

Hybrids were identified in 18 out of 24 (75.0%) sample locations (Table 4). The highest percentage of hybrids was observed in Ship Island Creek (32/60 = 53.3%). Samples from Wilson Creek, Garden Creek, Rush Creek (2001), Cabin Creek (2001), upper Indian Creek, and Little Indian Creek only contained individuals with genotypes indicative of pure RBT and/or pure WCT. Among sample sites, the majority (21/24 = 87.5%) was observed with less than 10% hybrids, and 17 of the 24 (70.8%) were observed with less than 5% hybrids. This is in contrast to recent work in the Clearwater River Basin, which reported that 22% of the sites examined were identified with >50% hybrids out of the total number of samples examined (Weigel 2002^a). Of the 80 hybrids identified, 17 (21.3%) had genotypes indicative of F₁ hybrids (heterozygous for one WCT allele and one RBT allele at each diagnostic locus examined). The remaining 63 hybrids had genotypes indicative of >F₁ hybrids.

Rainbow trout introgression, the actual percentage of RBT alleles observed among combined samples of individuals with genotypes indicative of both hybrids and WCT at each sample site, is reported in Table 5. Introgression levels within stocked sites ranged from 0% (Roaring Creek and Wilson Creek) to 58.8% (Ship Island Creek). Among nonstocked sites, RBT introgression ranged from 0% to 14.3%. Except for Soldier Creek (14.3% introgression, low sample size, N = 7), all of the remaining nonstocked sites had less than 10% introgression, and 15 of 18 sites had less than 5% introgression.

Fisher's exact test of HWE (all loci combined) rejected the null hypothesis of random mating ($P < .005$) at all sample sites except upper Marble Creek and Big Cottonwood Creek.

The maternal lineage on all samples was determined by amplifying the mitochondrial DNA gene region cytochrome B and digesting it with the restriction enzyme *Hae*-III (Figure 2). This marker has previously been shown to yield banding patterns (fragments) diagnostic between WCT, YCT, and RBT (Mays 2002). An examination of 81 golden trout samples from the Mt. Whitney Hatchery, California with this same marker yielded a polymorphism denoted as "G" in 78 of 81 samples (96.3%) not previously observed within *O. mykiss* (Mays 2002) (Figure 3).

Of 80 hybrids identified, 51 exhibited WCT mtDNA (63.8%), 22 exhibited RBT mtDNA (27.5%, 15 in Ship Island Creek), and three exhibited YCT mtDNA (3.8%). No samples were observed with the golden trout "G" polymorphism. Twelve of the samples identified with genotypes indicative of F₁ hybrids had mtDNA of WCT (66.7%).

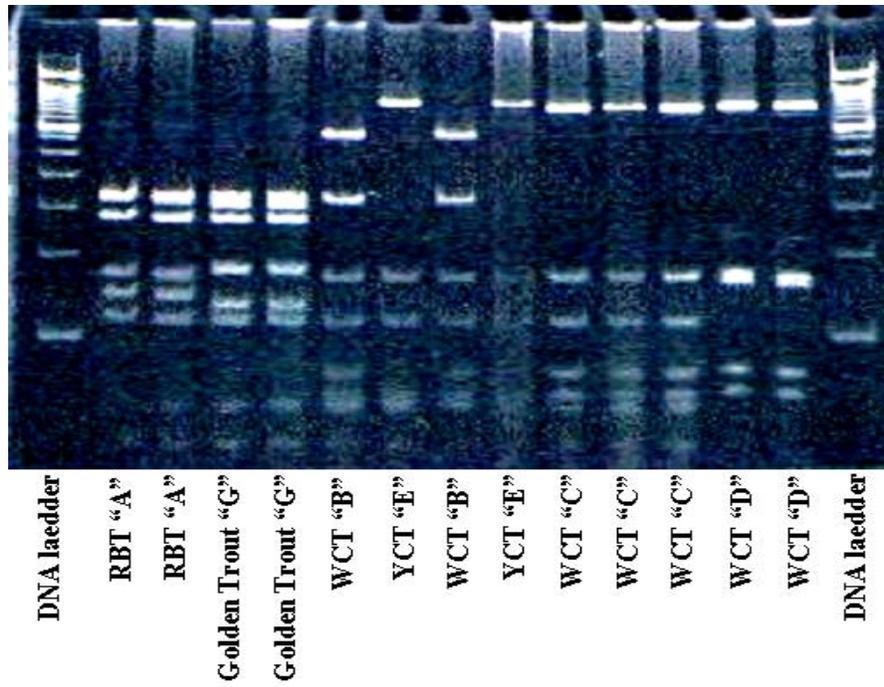


Figure 2. Photograph of a gel illustrating polymorphic banding patterns in a CytB/HaeIII digest.

Table 4. Sample location, sample size, stocking history, percent of sample identified with genotypes indicative of WCT, RBT, >F₁ hybrids and F₁ hybrids, and results of Fisher's Hardy-Weinberg (HWE) test for random mating.

Sample Location	No.	Stocked	Hybrids Detected	Genotypes indicative of WCT	Genotypes indicative of RBT	Genotypes indicative of >F ₁	Genotypes indicative of F ₁	Fisher's HWE (Ho: samples from randomly mating pop.)
Roaring Creek	43	Yes	Yes (6.9%)	35 (81.4%)	5 (11.6%)	3 (6.9%) all YCT mtDNA	0	Reject Ho
Ship Island Creek	60	Yes	Yes (53.3%)	3 (5.0%)	25 (41.7%)	27 (45.0%)	5 (8.3%), 3 WCT mtDNA, 2 RBT mtDNA	Reject Ho
Wilson Creek	63	Yes	No	13 (20.6%)	50 (79.4)	0	0	N/A
Cache Creek	58	Yes	Yes (3.4%)	55 (94.8%)	1 (1.7%)	0	2 (3.4%), both WCT mtDNA	60 (1.000)
Papoose Creek	65	Yes	Yes (4.6%)	7 (10.8%)	55 (84.6%)	2 (3.1%), both WCT mtDNA	1 (1.5%), WCT mtDNA	Reject Ho
Garden Creek	58	No	No	58 (100.0%)	0	0	0	N/A
Soldier Creek	61	No	Yes (4.9%)	4 (6.6%)	54 (88.5%)	2 (3.3%), both WCT mtDNA	1 (1.6%) RBT mtDNA	Reject Ho
Upper Elkhorn Creek	23	No	Yes (4.3%)	21 (91.3%)	1 (4.3%)	0	1 (4.3%) WCT mtDNA	Reject Ho
Lower Elkhorn Creek	21	No	Yes (14.3%)	7 (33.3%)	11 (52.4%)	2 (9.5%) both WCT mtDNA	1 (4.8) RBT WCT	Reject Ho
Big Creek Drainage								
Big Creek (Mainstem-Upper)	64	YES* (See Stocking Record)	YES (4.6%)	28 (43.8%)	33 (51.6%)	3 (4.6%), 1 WCT mtDNA, 2 RBT mtDNA	0	Reject Ho
Big Creek (Mainstem-Middle)	68	No	Yes (2.9%)	41 (60.3%)	25 (36.8%)	2 (2.9%), both WCT mtDNA	0	Reject Ho
Big Creek (Mainstem-Lower)	61	No	Yes (1.6%)	22 (36.1%)	38 (62.3%)	1 (1.6%), RBT mtDNA	0	Reject Ho
Rush Creek-2001	31	No	No	1 (3.2%)	30 (96.8%)	0	0	N/A
Rush Creek-2002	60	No	Yes (13.3%)	37 (61.7%)	15 (25.0%)	8 (13.3%), WCT mtDNA	0	Reject Ho
Cabin Creek-2001	48	No	No	7 (14.6%)	41 (85.4%)	0	0	N/A
Cabin Creek-2002	63	No	Yes (3.2%)	42 (66.7%)	19 (30.2%)	2 (3.2%), both WCT mtDNA	0	Reject Ho
Marble Creek Drainage								
Marble Creek (Mainstem-Upper)	52	No	Yes (13.4%)	45 (86.6%)	0	5 (9.6%), 3 WCT mtDNA, 1 RBT mtDNA, 1N/A	2 (3.8%), 1 WCT mtDNA, 1 N/A	Fail to reject Ho
Marble Creek (Mainstem-Middle)	58	No	Yes (5.1%)	28 (48.3%)	27 (46.6%)	1 (1.7%), WCT mtDNA	2 (3.4%), both WCT mtDNA	Reject Ho
Big Cottonwood Creek	60	No	Yes (5.0%)	57 (95.0%)	0	1 (1.7%), WCT mtDNA	2 (3.4%), both WCT mtDNA	Fail to reject Ho
Trail Creek	44	No	Yes (2.3%)	10 (22.7%)	33 (75.0%)	1 (2.3%), WCT mtDNA	0	Reject Ho
Indian Creek Drainage								
Indian Creek (Mainstem-Upper)	56	No	No	56 (100%)	0	0	0	N/A
Indian Creek (Mainstem-Middle)	59	No	Yes (3.4%)	35 (59.3%)	22 (37.3%)	2 (3.4%), both RBT mtDNA	0	Reject Ho
Indian Creek (Mainstem-Lower)	57	No	Yes (1.8%)	26 (45.6%)	30 (52.6%)	1 (1.8%), WCT mtDNA	0	Reject Ho
Little Indian	54	No	No	54 (100.0%)	0	0	0	N/A

Table 5. Percentage of RBT alleles observed among combined samples of individuals with genotypes indicative of both hybrids and WCT.

Sample Location	No.	Stocked	% RBT alleles/total
Roaring Creek	38	Yes	0
Ship Island Creek	37	Yes	58.8%
Wilson Creek	13	Yes	0
Cache Creek	56	Yes	1.0%
Papoose Creek	10	Yes	11.5%
Garden Creek	58	No	0
Soldier Creek	7	No	14.3%
Upper Elkhorn Creek	23	No	1.0%
Lower Elkhorn Creek	7	No	9.5%
Big Creek (Mainstem-Upper)	31	Yes	7.5%
		(See Stocking Record)	
Big Creek (Mainstem-Middle)	43	No	1.6%
Big Creek (Mainstem-Lower)	23	No	3.6%
Rush Creek-2001	31	No	0
Rush Creek-2002	45	No	8.2%
Cabin Creek-2001	48	No	0
Cabin Creek-2002	44	No	2.7%
Marble Creek (Mainstem-Upper)	52	No	4.2%
Marble Creek (Mainstem-Middle)	31	No	4.3%
Big Cottonwood Creek	60	No	2.2%
Trail Creek	11	No	3.0%
Indian Creek (Mainstem-Upper)	56	No	0
Indian Creek (Mainstem-Middle)	37	No	3.6%
Indian Creek (Mainstem-Lower)	27	No	1.9
Little Indian Creek	54	No	0

Microsatellite loci

Thirty of 32 tests for HWE showed significant deviation from expected allele frequencies after a Bonferroni correction; all were due to a deficiency of heterozygotes. The heterozygote deficiencies were most likely due to a Wahlund effect as a result of sampling WCT and RBT populations that are not randomly mating (as opposed to homozygous dominant selection). These results supported the splitting of samples into two groups (RBT and WCT) based on previously determined genotypic designations. All microsatellite loci were found to be in HWE for all populations when retested with the RBT groupings. Heterozygosity and sample sizes of RBT from each location are listed in Table 6.

Table 6. Sample size and heterozygosity of RBT groups at each location.

Location	Group	Sample Size	Heterozygosity
Wilson Creek	RBT	50	0.735
Papoose Creek	RBT	41	0.616
Cabin Creek-2001	RBT	26	0.653
Ship Island Creek	RBT	18	0.639
Rush Creek-2001	RBT	31	0.54
Upper Big Creek	RBT	26	0.606
Middle Big Creek	RBT	24	0.614
Cabin Creek-2002	RBT	17	0.692
Lower Big Creek	RBT	23	0.592
Rush Creek-2002	RBT	14	0.624

Pairwise F_{st} estimates of RBT between sample locations ranged from <0.001 to 0.063 (Table 7). Lower estimates of genetic differentiation were more frequently observed between sample locations within drainages (upper, middle, and lower mainstem Big Creek pairwise comparisons ranged from .003 to .029) than between drainages (i.e. lower mainstem Big Creek vs. Papoose Creek, $F_{st} = .056$), although there were exceptions (Ship Island Creek vs. Wilson Creek, $F_{st} = .014$). The highest F_{st} value was observed between Papoose Creek and middle mainstem Big Creek (0.063).

Table 7. Pairwise F_{st} estimates between sample sites (RBT group).

	<i>Genotypes indicative of RBT</i>									
	Wilson Creek	Ship Island Creek	Papoose Creek	Cabin Creek 2001	Rush Creek 2001	Upper Big Creek	Middle Big Creek	Cabin Creek 2002	Lower Big Creek	Rush Creek 2002
Wilson Creek	*									
Ship Island Creek	0.014	*								
Papoose Creek	0.041	0.050	*							
Cabin Creek-2001	0.025	0.031	0.047	*						
Rush Creek-2001	0.028	0.028	0.033	0.013	*					
Upper Big Creek	0.018	0.028	0.051	0.013	0.026	*				
Middle Big Creek	0.041	0.045	0.063	0.016	0.025	0.029	*			
Cabin Creek-2002	0.020	0.034	0.046	<0.001	0.023	0.017	0.006	*		
Lower Big Creek	0.037	0.054	0.056	0.014	0.031	0.017	0.003	<0.001	*	
Rush Creek-2002	0.028	0.032	0.029	0.005	0.014	0.011	0.009	<0.001	<0.001	*

The AMOVA analysis revealed little variation among populations. Most genetic variation was found within populations (99.52%) and not between populations (Table 8). A comparison of RBT within stocked versus unstocked sample locations also indicated the majority of genetic variation (99.9%) was partitioned within populations rather than between stocked vs. unstocked sites (Table 9).

Table 8. AMOVA results for RBT groups.

Source of variation	d.f.	Sum of squares	Variance Components	Percentage of variation
Among populations	7	3.684	0.00041 Va	0.08
Within populations	524	261.692	0.49941 Vb	99.92
Total	531	265.376	0.49982	

Table 9. AMOVA results for RBT groups: stocked vs. nonstocked.

Source of variation	d.f.	Sum of squares	Variance Components	Percentage of variation
Among groups	1	0.552	0.00010 Va	0.02
Among populations within groups	6	3.137	0.00042 Vb	0.08
Within populations	462	230.698	0.49935 Vc	99.90
Total	469	234.387	0.49987	

DISCUSSION

Preliminary results from this study demonstrate low levels of hybridization and introgression in many sample locations throughout the MFSR. Observed hybridization and introgression levels are likely due to both natural hybridization between native RBT and WCT, as well as anthropogenically influenced hybridization between stocked, nonnative trout and WCT.

Direct evidence of introgressive hybridization from stocking comes from the identification of hybrids in Roaring Creek with YCT mtDNA. Possible indirect evidence of hybridization and introgression from stocking comes from the detection of hybrids between RBT and WCT in streams with a history of stocking RBT or cutthroat trout X RBT hybrids. Findings for Ship Island Creek, with a known history of stocking, support the hypothesis that the observed hybridization and introgression is likely the result of past hatchery stocking. At this study site, over 50% of the samples were identified as hybrid. However, hybrids were also identified in sample locations with no known history of stocking (13/17 = 76.5%), including large drainages like Marble Creek and Indian Creek. Additionally, and perhaps more importantly, samples with genotypes indicative of F₁ hybrids (21.3%) were identified in both stocked and nonstocked areas, indicating recent hybridization events.

As stated earlier, WCT and RBT (when occurring in natural sympatry) likely have developed isolating mechanisms, which limits hybridization and introgression and maintains species integrity. However, it is well documented that habitat conditions play an important role in maintaining reproductive isolating mechanisms between sympatric species (Coyne and Orr 1999). A number of tributaries within the MFSR have experienced habitat alterations from anthropogenic causes such as mining or agriculture (Thurow 1987, 2000), as well as from natural causes such as fires and floods. The identification of hybrids in sample locations with no history of stocking (e.g., Marble Creek) may indicate areas where natural isolating mechanisms have broken down, increasing the reproductive contact between native RBT and WCT populations (e.g., species are competing for available spawning habitat).

Unrecorded stocking events and the possibility that introduced nonnative trout and hybrids may have strayed into areas that have not been stocked make it difficult to conclusively determine whether the observed hybridization and introgression in nonstocked sites is a product of natural hybridization between native, interior RBT and WCT or hybridization between introduced RBT and WCT in drainages. Conversely, if natural hybridization does occur, the interpretation of hybridization and introgression identified in areas that have been stocked is also complicated.

One approach in attempting to distinguish “natural” versus “unnatural” hybridization is to look for differentiation among different forms of RBT (hatchery coastal vs. native interior). Recent research using microsatellite markers have been able to demonstrate intraspecific hybridization and introgression between native interior RBT and introduced hatchery RBT (Knudsen et al. 2002). While only limited microsatellite analyses have been conducted thus far, results do not indicate significant differences among RBT groupings in the populations examined. Under the hypothesis that stocked vs. nonstocked RBT groups would have different genetic signatures due to the influence of nonnative hatchery RBT (the majority of which are likely of coastal origin), one might expect that a higher partitioning of the total genetic variance would be observed between composite RBT groups from stocked versus unstocked sample locations rather than within sample locations. The opposite was observed, however. In comparisons between samples from stocked and unstocked groupings as well as all sample locations combined, most of the total variation observed was seen within rather than between sample locations. Further microsatellite analyses as part of this project may provide better discrimination between interior and coastal forms of *O. mykiss* and should assist in a more refined understanding of genetic population structure of *O. mykiss* and *O. clarki* in the MFSR.

Additional mtDNA analyses could also assist in discriminating between interior and coastal forms of *O. mykiss*. Mitochondrial DNA RFLP analyses have demonstrated that native, interior populations of RBT typically exhibit only one or a few mtDNA haplotypes that differ slightly from one another (usually by less than 0.5% sequence divergence) and that in contrast, RBT populations that have interbred with hatchery RBT usually exhibit multiple mtDNA haplotypes that differ from one another by up to 1.5–2.2% sequence divergence (Williams et al. 1996). Similar mtDNA RFLP analyses on RBT populations within the MFSR should be able to help determine the origin of observed introgressive hybridization.

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JOB PERFORMANCE REPORT
SUBPROJECT #2: BLACKFOOT RIVER YELLOWSTONE CUTTHROAT PROJECT:
GENETIC DETECTION OF HYBRIDS TO CONFIRM PHENOTYPIC IDENTIFICATIONS

State of: Idaho

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

Project No.: 2

Title: Native Species Investigations

Subproject #2: Blackfoot River Yellowstone Cutthroat
Project: Genetic Detection of Hybrids to
Confirm Phenotypic Identifications.

Contract Period: July 1, 2002 to June 30, 2003

ABSTRACT

The management and conservation of Yellowstone cutthroat trout is an important priority of the Idaho Department of Fish and Game. This is especially true in light of the fact that the species still represents a significant component of the recreational trout fishery in Idaho despite the decline of many populations of pure Yellowstone cutthroat trout throughout their historic native range. The decline in Yellowstone cutthroat trout populations has been attributed to the extensive history of stocking nonnative, hatchery raised rainbow trout, which have hybridized with or replaced cutthroat trout populations in many areas. The objectives of this study were to assess the genetic purity of adfluvial Yellowstone cutthroat trout in the Blackfoot River and test phenotypic identifications used to distinguish rainbow trout and hybrids from Yellowstone cutthroat trout at the adult migration trap (weir) on the Blackfoot River. The purpose of the weir is to limit the movement of rainbow trout and hybrids upstream, thereby reducing risks associated with competition and hybridization. From April through June of 2003, nonlethal fin-clips (N = 129) were randomly collected from putative Yellowstone cutthroat trout, rainbow trout, and hybrids migrating through a weir just upstream of Blackfoot Reservoir. All fish sampled were identified as Yellowstone cutthroat trout, rainbow trout, or hybrid using a rating system based on phenotypic characters. Samples (N = 124) were genetically tested using species-specific nuclear and mitochondrial DNA markers. Five samples yielded insufficient DNA quantity or quality to perform genetic analyses. Results indicated that phenotypic identifications were highly accurate (100% for rainbow trout, 100% for hybrids, and 98.3% for Yellowstone cutthroat trout). Of the total fish examined, three were genetically identified as rainbow trout and two were identified as F₁ hybrids. The remaining 119 samples were genetically identified as Yellowstone cutthroat trout. No rainbow trout introgression (the actual incorporation of genes from one taxon into the population of another) was observed. Results indicate that the operation of a weir may be a valuable tool in limiting the flow of new rainbow trout alleles into adfluvial Yellowstone cutthroat trout populations in the Blackfoot River.

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INTRODUCTION

The management and conservation of Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* (YCT) is an important priority of the Idaho Department of Fish and Game (IDFG). This is especially true in light of the fact that the species still represents a significant harvest component of the recreational trout fishery in Idaho despite the decline of pure populations throughout its historic native range (Thurow et al 1988; May 1996). In August 1998, several conservation groups petitioned the U.S. Fish and Wildlife Service (USFWS) to list YCT as a threatened species under the Endangered Species Act (ESA). Currently, IDFG recognizes YCT as a “species of special concern” (Thurow et al. 1988). The decline in YCT populations has been largely attributed to the extensive history of stocking nonnative, hatchery raised rainbow trout *Oncorhynchus mykiss* (RBT), which have hybridized with or replaced YCT populations in many areas (Leary et al. 1984b; Allendorf and Leary 1988; Behnke 1992).

The IDFG is currently involved in identifying and enumerating remaining pure populations (Meyer 2003) and removing hybrids and RBT from key drainages that support YCT spawning (Host 2002). One such drainage is the Blackfoot River drainage located in southeast Idaho (Figure 3). During the 2002 spawning season, IDFG operated an adult migration trap (weir) just upstream of Blackfoot Reservoir (Figure 3) to enumerate the adult spawning population and to remove RBT and hybrids from the migrant population to reduce the potential threat of hybridization. Yellowstone cutthroat trout, RBT, and hybrids between the two were distinguished at the weir using phenotypic characteristics (Teuscher 2002).

The purpose of this project was to assess hybridization and introgression levels within the adfluvial YCT population and to test phenotypic identifications. To accomplish this, fin clips were randomly collected from adfluvial adult YCT, RBT, and suspected hybrids that returned to the weir. A diagnostic mitochondrial DNA (mtDNA) marker and four nuclear DNA (nDNA) markers were used to assess the level of hybridization and introgression within these samples.

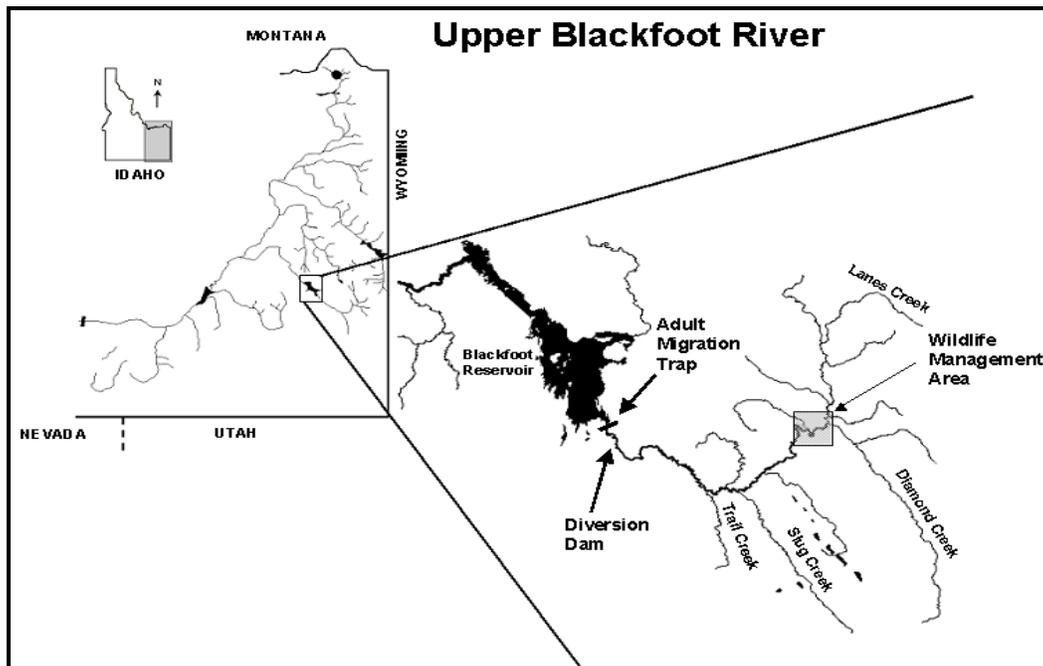


Figure 3. The upper Blackfoot River drainage and adult migration trap.

OBJECTIVES

1. Assess the genetic purity of adult adfluvial Yellowstone cutthroat trout in the Blackfoot River.
2. Test the ability of biologists to distinguish Yellowstone cutthroat trout, rainbow trout, and hybrids at the adult migration trap on the Blackfoot River using phenotype-based measures.

METHODS

Sample Collection

Nonlethal fin clips (N = 129) were haphazardly collected from April through June from putative YCT, RBT, and hybrids migrating through the weir (adult migration trap) just upstream of Blackfoot Reservoir (Figure 3). All fish sampled were identified as YCT, RBT, or hybrid using a subjective rating system based on phenotypic characters (e.g. coloration, spotting number and distribution, etc.). Fish that phenotypically looked like RBT were scored as “1” and considered “pure” RBT. Fish that phenotypically looked like RBT but that had cutthroat slash markings were scored as a “2” and were considered hybrids. Fish that had characteristics of both RBT and YCT were scored as a “3” and were also considered hybrids. Fish that phenotypically looked like YCT but that had spots on the head were scored as a “4” and were considered “pure” YCT. Finally, fish that phenotypically looked like YCT were scored as “1” and considered “pure” YCT (Teuscher 2002).

Genetic Analyses

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). The DNA was resuspended in 100 µl TE. Restriction Fragment Length Polymorphism (RFLP) analyses were conducted using one mitochondrial DNA (mtDNA) marker digested with *Hinf I* (Cytochrome b; Mays 2002) and three nuclear intron markers: Recombination Activation Gene-RAG3' digested with *Dde I* enzyme (New England Biolabs, Inc.), Ikaros Gene-1K digested with *Hinf I* (New England Biolabs, Inc.), and Protoncogene 53-p53 digested with *Alu I* (New England Biolabs, Inc.; Baker et al. 2002; Campbell et al. 2002). A simple sequence repeat (SSR) nDNA marker, Occ16, diagnostic between RBT and YCT was also amplified for each sample (Ostberg & Rodriguez 2002).

Digests were electrophoresed on 3% agarose gels and visualized as band patterns when fluoresced under UV-light (Figures 4 and 5). Each unique band pattern generated by each marker/restriction enzyme pair was assigned a letter. Alphabetic designations were assigned to each unique allele in the case of nDNA or each unique polymorphism in the case of mtDNA. For the markers used in this study, “A” usually refers to a banding pattern unique to RBT, whereas “B” or “C” typically refers to a banding pattern unique to YCT. For the nDNA markers, the genotype “AA” refers to an individual that is homozygous for RBT alleles; “BB,” “BC,” or “CC” refers to an individual that is homozygous for YCT alleles, and “AB” or “AC” refers to an

individual that is heterozygous with both a RBT and YCT allele. The letter designations for each of the five marker/restriction enzyme pairs were later combined to infer if a sample was putatively pure or hybridized.

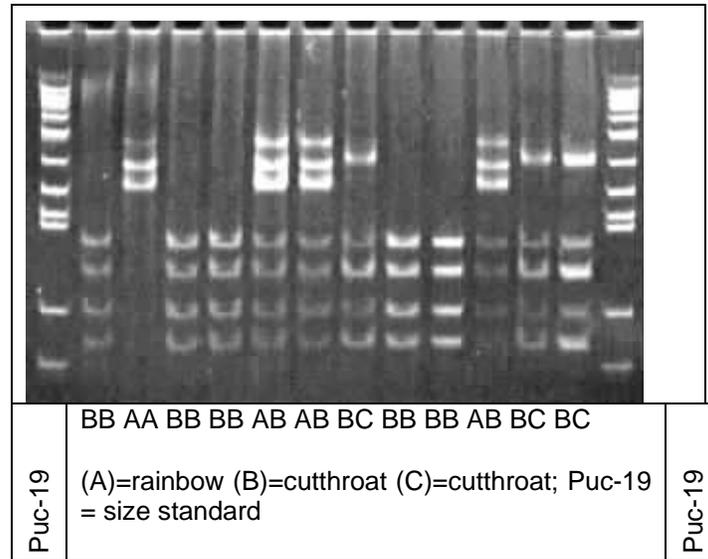


Figure 4. RAG3' digest of YCT and RBT samples showing typical diagnostic banding patterns.

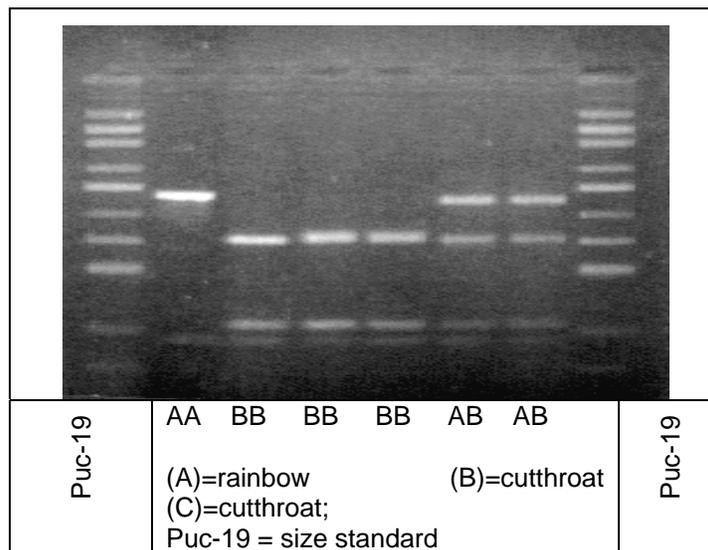


Figure 5. IK digest of YCT and RBT samples showing typical diagnostic banding patterns.

All markers listed above with the exception of p53-AluI pair display fixed differences between YCT and RBT. The nDNA p53 marker is an imperfect marker, in which only one of the alleles exhibits fixed differences between YCT and RBT. It is still a useful marker and provides meaningful information regarding hybridization and introgression. Using the four nuclear markers listed above, a genetic sample size of $(125 * 4 * 7 = 3500)$ was attained. This exceeds the required amount (genetic sample size = 460), needed to attain 99% confidence of detecting greater than 1% introgression in a population.

Hardy-Weinberg equilibrium proportions were tested at each marker/restriction enzyme pair using Genepop on the Web (Raymond & Rousset 1995) to assess if more than one population was sampled. Significant deviations from Hardy-Weinberg equilibrium (e.g., a deficiency of heterozygotes) would indicate that multiple populations were sampled.

RESULTS

In total, 129 samples were extracted for genetic analyses, and 124 samples yielded sufficient DNA for PCR and RFLP analyses. The samples in which no DNA was obtained (or less than three markers were amplified) were: P3, S6, S7, T7, and T10 (Appendix C, refer to genetic ID). These five samples would have to be re-extracted in order to generate complete genotypes, although this is probably unnecessary given the adequate sample size.

Of the 124 samples with complete genotypes, two samples with genotypes indicative of F_1 hybrids (P27, R22) were identified, and three samples with genotypes indicative of RBT (U1, V1, V2) were identified (Appendix C). All five of these samples were correctly identified based on phenotypic characteristics (samples P27 and R22 were identified only as “hybrids”). The remaining 119 samples had genotypes indicative of pure YCT (Appendix C). Only two of these 119 samples were incorrectly identified as “hybrids” based on phenotypic characteristics. Samples R22, V1, and V2 were interesting in that they exhibited a mtDNA polymorphism “D” (when run with the ND2/Hinf-I marker), which we previously had observed in westslope cutthroat trout *O. clarki lewisi*. We ran these three samples with an additional diagnostic restriction enzyme *Hae-III* (diagnostic between RBT, WCT, and YCT) that yielded a RBT polymorphism (“A”). Thus, both samples with genotypes indicative of F_1 hybrids had mtDNA of RBT, indicating hybridization between a female RBT and a male YCT. All samples with genotypes indicative of YCT exhibited mtDNA of YCT.

To provide additional confidence that samples genetically identified as F_1 hybrids (P27 and R22) and samples genetically identified as RBT (U1, V1, and V2) were correct, four additional, newly developed SSR markers (Occ 35, Occ 36, Occ 38, and Occ42) diagnostic between RBT and YCT were also amplified for each of these samples (Ostberg In Press). Genotypic classification results were identical as before with samples P27 and R22 heterozygous at each locus with both a RBT and YCT allele (indicative of F_1 hybrids), and samples U1, V1, and V2 homozygous for RBT alleles at each locus (indicative of pure RBT).

A test for HWE was performed using the software program Genepop on all samples together (including hybrids and RBT), as well as only samples with genotypes indicative of YCT. All four loci were significantly out of HWE ($p < .05$) when tested on all samples (Table 10).

Table 10. Hardy Weinberg: Probability test (Hybrids and RBT included) F_{IS} .

Locus	P-value	S.E.	W&C	R&H	Matr
p53	0.0393	0.0009	+ 0.391	+ 0.393	-
IK	0.0000	0.0000	+ 0.854	+ 0.861	-
RAG3'	0.0000	0.0000	+ 0.245	+ 0.469	-
Occ 16	0.0000	0.0000	+ 0.743	+ 0.749	-
All (Fisher's method):	Chi ² : Infinity Df: 8 Prob: High Sign.				
All loci, all populations	Chi ² : Infinity Df: 6 Prob: High Sign.				

In the second test, hybrids and RBT were removed, and only the RAG3' marker was tested since it is the only marker out of the four examined that is variable within YCT (2 alleles are observed, "B" and "C"). We failed to reject that the samples came from more than one randomly mating population at the RAG3' locus ($p > .05$) (Table 11).

Table 11. Hardy Weinberg: Probability test (Hybrids and RBT removed) F_{IS} .

Locus	P-value	S.E.	W&C	R&H	Matr
RAG3'	0.0503	0.0027	+ 0.191	+ 0.192	-

DISCUSSION

Results from this study indicate that phenotypic identifications were highly accurate in distinguishing RBT (3/3 = 100%), YCT (117/119 = 98.3%), and hybrids (2/2 = 100%). Similar results for phenotypic identifications have also been observed in Henrys Lake hatchery operations (Campbell et al. 2001) and in weir operations on the S.F. Snake River (Host 2003).

Results also demonstrate low numbers of hybrids (2/124 = 1.6%) and no RBT introgression within the adult spawning run ascending the lower river in 2003. Previous research in 2000 from populations above the weir reported introgression levels of 10.6% (% RBT alleles/total examined) and identified five hybrids out of 24 samples analyzed (20.8%), two of which were confirmed as backcross hybrids ($>F_1$) (University of Idaho, unpublished data). However, while the previous study reported higher levels of RBT introgression, it actually had less power than the current study in identifying RBT introgression, since only 24 samples were examined and only two diagnostic nDNA markers were utilized. Importantly, samples from the 2000 and 2002 studies were collected from different locations on the Blackfoot River, and it is likely that the discrepancy in findings observed between the two studies is the result of having sampled separate populations between years and perhaps multiple populations within a given year, at least one of which is introgressed with RBT alleles and at least one of which appears to contain pure YCT. A limitation of most diagnostic genetic markers used in these types of hybridization studies is that they are not variable (only one allele is observed in YCT and only one is observed in RBT). This eliminates the ability to determine whether multiple YCT or RBT

populations have been sampled. In this study, we did use one nDNA marker variable in YCT (RAG3' has two YCT alleles, B and C). However, we would have to run additional, highly variable nDNA loci to have confidence that we were not committing a type II error (failing to reject the null hypothesis that we have sampled one population when we have actually sampled more than one). At this point, we can only conclude that we have no evidence at the RAG3' marker that we sampled more than one YCT population. However, we do have high confidence in concluding that we have not sampled one population of randomly mating fish (YCT, RBT, and hybrids), and that we are likely sampling pure YCT, pure RBT, and hybrids between the two. This has been observed before in the upper Snake River drainage. For example, a laboratory report to IDFG from the University of Montana that identified back-cross hybrids (using variable allozyme loci) within 30 samples of trout collected from the South Fork Snake River reported that the sample "appears to have fish originating from more than one population, because 16 of 30 fish in the sample appear to be genetically pure Yellowstone cutthroat and 10 appear to be pure rainbow trout, which is more than expected from chance based on the estimated allele frequencies at the diagnostic loci. The remaining fish had genotypes at one or more diagnostic locus indicating them to be of hybrid origin. Thus, the fish in the sampled area appear to have originated from a pure Yellowstone cutthroat trout population, a pure rainbow trout population, and at least one hybridized population of these fishes" (letter to Bill Schrader, IDFG, August 25, 1998).

These data indicate that either hybridization is a relatively recent event in these areas, or that there are reproductive isolating mechanisms that are preventing widespread hybridization and introgression between these species. Population surveys in 2000 identified large numbers of juvenile RBT in the river above the weir on the Blackfoot River (IDFG, unpublished results). It may be that the levels of RBT hybridization and introgression will increase in the adfluvial YCT population as these RBT become reproductively mature. This will have to be monitored. Alternatively, prezygotic isolating mechanisms, such as differences in spawn timing, may keep hybridization and introgression levels low within the adfluvial population. Fish phenotypically identified as RBT migrated through the trap generally earlier than fish identified as YCT (Dave Teuscher, IDFG, personal communication). This has also been observed in previous studies. Henderson et al. (2000) reported that on the South Fork Snake River, "The median spawning date for mainstem-spawning YS cutthroat trout (June 9) was significantly later than for RBT (May 19) and hybrids (May 18)."

However, this is not to suggest that managers should not be concerned about hybridization and introgression of YCT in the Blackfoot River. Evidence of F₁ hybrids in the sample of fish migrating above the weir obviously demonstrates that there is some temporal overlap in spawning time between YCT and RBT. If naturally reproducing RBT populations increase, or if environmental conditions that play a role in maintaining isolating mechanisms change in the future, it is likely that introgression levels, as well as the number of introgressed populations, could increase in the future.

MANAGEMENT RECOMMENDATIONS AND IMPLICATIONS

Results from this study support the use of phenotype-based procedures to distinguish returning RBT, YCT, and hybrids. If weirs are dependable in intercepting all migrating fish, then they should be valuable tools in limiting the flow of new RBT alleles into the YCT populations above them.

Managers should consider that while a weir may help limit the flow of RBT alleles into the populations above them, self-reproducing populations of RBT above the weirs remain a threat to the continued genetic integrity of upstream YCT populations. Further studies should be aimed at developing a better understanding of the resident and adfluvial YCT populations in the Blackfoot River and the threat that RBT populations pose to YCT genetic purity in the Blackfoot River.

Finally, certainly in cases where managers believe that multiple populations may have been sampled, hybridization and introgression studies should include analyses with highly variable markers (i.e. microsatellites) that are capable of answering more detailed questions concerning population structure. Without the delineation of populations in complex systems like the Blackfoot River, interpreting results of these studies and making proper management decisions from these results will remain difficult.

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APPENDICES

Appendix A. Genetic Considerations

Factors that influence hybridization and introgression between introduced nonnative trout and indigenous westslope cutthroat trout: Genetic considerations and management implications Matthew Campbell, Geneticist, IDFG

Introductions of nonnative trout for fisheries management purposes have occurred throughout the range of westslope cutthroat trout for more than 100 years. It has been well documented that these introductions have often led to hybridization and introgression, a potentially serious, ongoing genetic hazard throughout much of the species present range (Weigel et al. 2002; Sage et al. 1992; Leary et al. 1995). However, there is also research that has failed to show evidence of hybridization and introgression within populations even though nonnative trout have been previously stocked (Williams et al. 1996, Mays 2001).

There are many factors that determine whether nonnative trout (e.g., rainbow trout, Yellowstone cutthroat, golden trout) introductions will result in hybridization (i.e. the interbreeding of introduced nonnative trout with indigenous westslope cutthroat trout) and introgression (i.e. the incorporation of genes of nonnative trout into the gene pool of a westslope cutthroat population).

One or more of the following factors may influence levels of hybridization and introgression:

- The number of nonnative trout stocked;
- The number of times stocked, time of year stocked, time since last stocking, age/size at stocking, strain or subspecies stocked, survival of stocked fish, size of the indigenous westslope cutthroat population, and fishing pressure on stocked streams;
- Presence/Absence of isolating mechanisms (both pre-mating and post-mating mechanisms). For instance, the presence or absence of isolating mechanisms may depend on whether rainbow trout are stocked on westslope cutthroat populations that are naturally sympatric with native populations of *O. mykiss*, or whether they are stocked on westslope populations that have not previously lived in sympatry with *O. mykiss*;
- Dispersal patterns and reproductive success of introduced trout and hybrids;
- Ecological conditions can influence many aspects of stocked rainbow trout survival, the presence/absence of isolating mechanisms, fitness of hybrids, gene flow between populations, as well as the geographical distribution of introduced nonnative trout, native trout, and hybrids within an area.

There are also numerous complicating factors that determine whether the percentage of nonnative alleles within a population, the number of hybrids in a population, or the number of hybridized populations will increase, decrease, or remain unchanged over time. The fate of nonnative trout alleles introduced into a westslope cutthroat trout population depend on the extent to which introduced trout and westslope cutthroat trout hybridize, the subsequent reproductive fitness of hybrids and the extent to which the hybridizing populations depart from Hardy-Weinberg expectations of an ideal population.

For example, if 20 rainbow trout (breeding adults) are introduced onto a cutthroat population (80 breeding adults, no other individuals) before any mating, the sample of fish is composed of 20% rainbow trout (RBT) alleles and 80% westslope cutthroat trout (WCT) alleles. If the introduced RBT randomly mate with the WCT and the subsequent hybrids are as fit as the parents, then the percentage of RBT alleles and WCT alleles will not change from generation to generation. What will change, early on, is the number of hybrids in the population. Before any mating the number of hybrid individuals is zero. As random mating progresses, the number of hybrids in the population increases each generation until eventually all of the individuals are hybrids and the RBT alleles are randomly distributed throughout the population (a hybrid swarm). The percentage of RBT alleles does not increase, however (the potential effects of drift are ignored for this example). Sample observations would indicate 20% RBT alleles and 80% WCT alleles, which is the true frequencies for the population. If enough diagnostic genetic markers are available to detect introgression in the individual (requiring approximately 15 loci, 30 alleles to detect 20% RBT introgression) then a genetic screen will likely demonstrate that all individuals sampled are hybrids to some degree, and the level of introgression among the individuals will be consistent with a binomial distribution of RBT alleles across the population. The more diagnostic loci available, the greater power to detect introgression at low levels in the population and individual.

The increase or decrease of RBT introgression (the percentage of RBT alleles within a population) depends on whether new RBT alleles are continually introduced into the population, the relative fitness of hybrid genotypes, genetic drift, and the potential for the increased mating among related individuals (phenotypic advantage). As new RBT alleles enter the population (stocking) and if hybridization and introgression occurs, the percentage of RBT alleles in the population will increase. If hybrid genotypes/RBT alleles are more fit than WCT genotypes/alleles (outbreeding enhancement or heterosis), then the percentage of RBT alleles in the population will increase even after stocking has stopped due to this selective advantage. Alternatively, if hybrid genotypes/RBT alleles are less fit than WCT genotypes/alleles (outbreeding depression or negative heterosis), then the percentage of RBT alleles in the population will decrease after stocking has stopped, depending on the level in which they are expressed and selected against within the population. Genetic drift (change in allele frequency from generation to generation due to statistical chance) may also change the percentage of RBT alleles within a population, especially if the population is small. However, genetic drift is nondirectional, providing equal opportunity for RBT or WCT allele frequencies to change significantly. Rainbow trout alleles will also increase in the WCT population if rainbow trout or hybrid phenotypes are preferred partners for mating (both equally or unequally among sexes). The increase in mating success will result in an overall increase in RBT alleles in the population and a departure from random mating evidenced by examining linkage and/or gametic disequilibrium among individuals.

Appendix A. Continued.

Whether the number of populations that are introgressed in an area increases depends on a number of factors including the stocking history (how long ago were nonnative trout stocked, whether nonnative trout are stocked in places now that they were not in the past), whether the stocking of nonnatives has resulted in self-sustaining populations, the dispersal of stocked trout and hybrids, and the amount of natural gene flow that occurs between WCT populations. If stocking took place in areas that had not been stocked prior to the first study, then subsequent resampling and genetic analysis may find an increase in the number of populations that show introgressive hybridization. If RBT are introduced into an area with WCT and there is subsequent introgressive hybridization, gene flow will move RBT alleles into surrounding populations. In some areas, stocking has resulted in self-sustaining RBT populations (Hitt et al. submitted). If these introduced populations increase in size and/or individuals disperse and immigrate, both the percentage of RBT alleles within populations, as well as the number of introgressed populations can increase if those immigrants are reproductively successful.

It is important that managers continue to screen WCT populations for hybridization and introgression and continue to investigate the ecological and genetic factors that influence the consequences of nonnative introductions. In some cases the outcome of stocking nonnative trout on indigenous WCT populations has been severe enough as to have led to the formation of hybrid swarms (Hitt et al. submitted). However, it is likely that a number of factors, including existing reproductive isolating mechanisms (e.g. those found in naturally sympatric populations) or environmental conditions which select against nonnative trout and hybrids, have limited the incidence of hybridization and spread of introgression in a number of drainages, and has thus preserved genetic integrity of the native parental populations. This is not to suggest that the practice of stocking fertile, nonnative trout on indigenous WCT populations should continue. The States of Idaho, Montana, Oregon, and Washington have already adopted policies focused either on the cessation of stocking nonnative trout in WCT waters, or the use of sterile triploid rainbow trout in hatchery supported fisheries which are adjacent or connected to waters supporting westslope cutthroat trout.

It is also important that managers monitor and document possible changes in the level of introgression within a population or changes in the number of populations in which hybridization and introgression is observed. Populations in which introgression has increased over time should not receive the same conservation status and should be managed differently than populations in which introgression levels have remained stable or are decreasing. Documenting areas in which population-level introgression is increasing or where the number of hybridized populations is increasing is essential, because it may highlight areas in which management actions should change (e.g., stopping further introductions of hatchery rainbow trout, Rubidge et al. 2001).

Ideally, research studies that examine temporal changes among vagile animals should attempt to compare samples collected from the exact same location and at the same time of year. Additionally, samples sizes should be similar and the genetic methods used should be similar in their precision and accuracy of detecting hybridization and introgression. Preferably, the exact same diagnostic loci would be used so that frequencies of specific diagnostic alleles could be monitored over time in the population.

Recent research in the Flathead River system in Montana (Hitt et al. submitted) and in the Kootenay River drainage in British Columbia (Rubidge et al. 2001) has reported the rapid spread of RBT introgression into WCT populations previously reported as free from detectable levels of introgressive hybridization. Some researchers who have addressed the question of how to define a 'pure' WCT population have argued that management plans that attempt to set some arbitrary limit of admixture (introgressive hybridization) below which a population will be considered 'pure' (e.g. 1%, 10%) are problematic because, as cited above, the amount of admixture in many WCT populations is rapidly increasing. Research reporting the rapid spread of introgression is significant and will have to be considered carefully by the agencies responsible for managing these particular WCT populations. However, as reviewed previously, it is highly unlikely that every WCT population that has experienced some level of hybridization and introgression would experience an increase in the percentage of RBT introgression over time or that introgression would spread rapidly from one population to many populations throughout a drainage. Importantly, the reportedly continuing spread of RBT introgression within the Flathead River system is likely due to the establishment of self-reproducing populations of introduced rainbow trout and the dispersal of hybrids into areas containing pure cutthroat populations (Hitt et al. submitted). In the case of the observed increase in hybridization and introgression within the tributaries of the upper Kootenai River, those authors mention that "the most likely reason for the apparent increase is the continued and expanded introductions of rainbow trout into the Kooconusa Reservoir and adjacent tributaries" (Rubidge et al. 2002).

It is also important to separate out two different issues with regards to setting limits of introgression. One issue would be the scientific rigor and precision associated with estimating the level of introgression in a population using molecular genetic information. It may be reasonable to set a limit of introgression below which a population will be considered 'pure' if it is appropriate to be conservative due to imprecision associated with the genetic markers. Genetic markers used to detect introgressive hybridization are often assumed to be "fixed" between RBT and WCT (meaning that a certain marker is only observed in RBT and never observed in WCT or vice versa). However, markers continually have to be tested to ensure that they are in fact fixed within populations. The recent work by Rubidge et al. (2001) reports that the nuclear DNA marker Ikaros (IK) digested with *Hinf-I* yields fixed differences between RBT and WCT. Work by IDFG on WCT populations in the Middle Fork of the Salmon River indicates that the *IK/Hinf-I* marker is not fixed within these populations, stressing the importance of using multiple diagnostic genetic markers when assessing introgressive hybridization.

Hitt (2002) (using dominant PINE markers) described procedures for being conservative in describing a population as admixed or not following procedures outline by Forbes and Allendorf (1991). When individuals from a population only show a "RBT" band (based on its electrophoretic mobility through a gel) at one marker/locus, then the population is considered pure and the observed "RBT" band is considered a WCT allele with the same electrophoretic mobility as the true diagnostic RBT allele. Hitt (2002) described six populations as being unhybridized WCT populations despite the fact that they exhibited "RBT" bands. These "RBT" bands were used as evidence for RBT introgression in other populations when other diagnostic markers also demonstrated RBT introgression.

A second issue regarding setting limits of admixture involves the setting of introgression levels at some level from which populations should be prioritized and conservation and management decisions made (e.g., *Cutthroat Trout Management: A Position Paper, Genetic Considerations Associated with cutthroat trout management* UDWR 2000; <http://www.nr.utah.gov/dwr/PDF/cuttpos.PDF>). This document was developed by the states of Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming to help guide managers working with cutthroat trout. Cutthroat trout with a measured introgression level of less than 1% are designated as “core conservation populations” and are considered pure. The less than 1% limit allows for possible imprecision associated with genetic markers. A second category, “conservation population,” is used for populations with less than 10% introgression (but may extend to a greater amount depending upon circumstances and the values and attributes to be preserved). The less than 10% criterion is not suggesting that populations with introgression levels between 1% and 10% be considered ‘pure’ or managed as a ‘pure’ population, rather it is an agreed upon decision to manage populations a certain way given that a particular level of introgression is observed (in this case, <10%). Importantly, the primary management goal of the “conservation population” designation is to protect and conserve populations that, while existing in a introgressed condition, still contain a unique or essential portion of ecological, behavioral, physiological, or genetic diversity found within the subspecies.

A concern with setting such threshold criteria based on percentages is that those criteria may not accurately describe the true hybridization status of a sample location. The percentage corresponds to the number of nonnative alleles observed among the total alleles examined, and is only useful in situations where the researcher is using dominant markers and can determine there is no evidence the sample consists of more than one population. Certainly in the cases of sympatric populations of native RBT and native WCT, even those in which a certain level of hybridization and introgression has occurred, the documentation of the percentage RBT alleles out of the total examined does not accurately describe the status of the population. The same is true in situations where F_1 hybrids are observed, but no backcross hybrids are observed. For instance, if 30 individuals are sampled, and 10 of them have genotypes indicative of F_1 hybrids, 10 have genotypes indicative of WCT, and 10 have genotypes indicative of RBT, the results could be interpreted to say the population is introgressed at a level of 50%, when in fact, these results demonstrate no RBT introgression. This particular situation would be important to document and manage since it represents a loss in reproductive effort for both species, but it has very different management and conservation implications than a hybrid swarm consisting of a mixture of 50% WCT alleles and 50% RBT alleles. A more informative way of describing hybridization and introgression within sympatric populations is to first delineate populations and then to describe the observed genotypes and their frequencies within those populations. For naturally sympatric, non-randomly mating populations of RBT and WCT (according to HWE test), one way of reporting RBT introgression would be the # of RBT alleles observed out of total examined from samples indicative of only WCT and hybrids (individuals with genotypes indicative of RBT would be excluded).

Another concern with the current threshold criteria establishing “core conservation populations”, <1% introgression and “conservation populations”, 1-10% introgression, is that they do not distinguish natural versus un-natural hybridization and introgression or suggest that naturally hybridized populations should be prioritized and managed differently than populations that have been genetically impacted by introductions of non-native trout. Recent research in Idaho (IDFG report to USFWS, preliminary report from USGS (Jennifer Nielsen) to IDFG), Oregon and Washington (Howell and Spruell report to USFWS) has suggested that hybridization between sympatric native *O. mykiss* and native *O. clarki* probably occurs in a number of areas across the species range and often results in low, but in some cases high, levels of introgression in some drainages. Presumably, ecological conditions occurring at both spatial and temporal scales, likely play a significant role in the maintenance of isolating mechanisms between the two species and the occurrence of hybridization and introgression. Clearly, more research is needed to understand both the genetic as well as the ecological conditions that influence hybridization and introgression of sympatric *O. mykiss* and *O. clarki* populations.

Recommendations

We recommend:

- That biologists/managers prioritize WCT populations that have not been genetically altered by non-native trout introductions.
- That biologists/managers reduce the threat of hybridization and introgression to WCT populations by continuing with current management guidelines that prohibit the stocking of fertile non-native trout in waters supporting WCT, and where feasible and necessary reducing or eliminating hybrids and self-reproducing populations of introduced non-native trout. Management strategies to reduce the number of hybrids and rainbow trout in WCT populations could include such things as changes to fishing regulations to allow for the harvest of hybrids and rainbow trout, and the use of weirs on spawning tributaries to remove migrating hybrids and rainbow trout from spawning populations.
- That biologists/managers continue to genetically test WCT populations that have not been previously examined.
- That biologists/managers assess whether non-native trout introgression is likely to increase within a WCT population over time, and whether the number of introgressed populations in an area is likely to increase over time. Likewise, biologists/managers need to monitor populations over time.
- That hybridization and introgression studies attempt to delineate populations within a drainage/sampling location and report hybridization and introgression both in terms of % RBT alleles observed out of the total samples examined, as well as describing genotypes and genotype frequencies (# of F_1 s, $>F_1$ s, etc.).

Appendix A. Continued.

- That biologists/managers assess, using microsatellite and mtDNA analyses, the extent to which observed introgression is coming from introduced RBT and native *O. mykiss*.
- That biologists/managers conserve and protect an introgressed westslope cutthroat trout population when:
 - It is unlikely that introgression will increase in the population over time or lead to the introgression of surrounding populations;
 - When individuals in the population exhibit the traits (phenotypic, behavioral, genetic) that characterize the species westslope cutthroat trout and;
 - When it represents an important component to the overall biological diversity (phenotypic, life-history, genetic) present within the species.

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Appendix B. Stocking Histories

Stocking histories were assessed using IDFG's historical stocking database on the web <http://www2.state.id.us/fishgame/fish/fishstocking/stocking/index.cfm> (1967-Present), IDFG unpublished stocking records (1913-1966), and the catalogue of lakes and streams of Idaho. All streams and headwater lakes in the Big Creek, Marble Creek, and Indian Creek drainages, as well as in the additional eight sampled streams, were identified using the topography software program TOPO! Version 2.7.5 and subsequently checked for stocking histories. Stocking information that referred to streams or lakes in Idaho but did not contain catalogue #'s, county information, or other descriptions that allowed verification of stream location was not used.

Stream or Lake	Species Stocked	Date	Size (inches)	# of Fish	Comments
Big Creek					
Big Creek	Cutthroat Trout	09/30/1962	1-1.25	22000	Flows into Middle Fork of Salmon R.
Big Creek	Cutthroat Trout	10/11/1958	1.25	18000	Flows into Middle Fork of Salmon R.
*Lick Creek Lake (Headwaters to Big Creek)	Rainbow Trout	07/23/1959	1-1.25	3000	Flows into Lick Creek
Roosevelt Lake	Cutthroat Trout	10/16/1967	1	2320	Flows into Monumental Creek
Roosevelt Lake	Cutthroat Trout	09/22/1958	1.25	7200	Flows into Monumental Creek
Roosevelt Lake	Cutthroat Trout	09/04/1948	1-1.25	5000	*Flows into Monumental Creek
Roosevelt Lake	Cutthroat Trout	09/09/1936	?	20090	*Flows into Monumental Creek
Marble Creek	None	NA	NA	NA	No record of stocking
Indian Creek	None	NA	NA	NA	No record of stocking
Garden Creek	None	NA	NA	NA	No record of stocking
Elkhorn Creek	None	NA	NA	NA	No record of stocking
Soldier Creek	None	NA	NA	NA	No record of stocking
Roaring Creek					
Roaring Creek Lake No. 1	Cutthroat Trout	08/30/1965	1	1900	Flows into Roaring Creek
Roaring Creek Lake No. 1	Cutthroat Trout	07/14/1955	1	2000	Flows into Roaring Creek
Roaring Creek Lake No. 2	Cutthroat Trout	08/30/1965	1	3800	Flows into Roaring Creek
Roaring Creek Lake No. 2	Cutthroat Trout	07/14/1955	1	500	Flows into Roaring Creek
McGuire Lake	Cutthroat Trout	08/19/1977	0-3	1152	Flows into Roaring Creek
McGuire Lake	Cutthroat Trout	08/08/1974	0-3	1008	Flows into Roaring Creek
McGuire Lake	Cutthroat Trout	08/17/1971	0-3	1080	Flows into Roaring Creek
McGuire Lake	Cutthroat Trout	08/03/1968	0-3	1000	Flows into Roaring Creek
Ship Island Lake					
Ship Island Lake	Cutthroat Trout (C7)	08/10/1937	?	6000	*Flows into Ship Island Creek
Ship Island Lake	Rainbow Trout	08/10/1937	?	6000	*Flows into Ship Island Creek
Ship Island Lake No. 2	Westslope Cutthroat	08/26/1998	0-3	1000	Flows into Ship Island Creek
Ship Island Lake No. 2	Westslope Cutthroat	09/22/1992	0-3	750	Flows into Ship Island Creek
Ship Island Lake No. 2	Westslope Cutthroat	08/30/1989	0-3	1000	Flows into Ship Island Creek
Ship Island Lake No. 2	Golden Trout	09/10/1970	0-3	2500	Flows into Ship Island Creek
Ship Island Lake No. 2	Golden Trout	09/06/1969	0-3	2970	Flows into Ship Island Creek
Ship Island Lake No. 4	Grayling	08/26/1998	0-3	500	Flows into Ship Island Creek
Ship Island Lake No. 4	Grayling	09/28/1992	0-3	250	Flows into Ship Island Creek
Ship Island Lake No. 4	Grayling	08/30/1989	0-3	500	Flows into Ship Island Creek
Ship Island Lake No. 4	Henry's Cutthroat	09/02/1986	0-3	1000	Flows into Ship Island Creek
Airplane Lake (S.I.L. #5)	Westslope Cutthroat	08/31/2001	0-3	1000	Flows into Ship Island Creek
Airplane Lake (S.I.L. #5)	Westslope Cutthroat	08/26/1998	0-3	1000	Flows into Ship Island Creek
Airplane Lake (S.I.L. #5)	Westslope Cutthroat	08/08/1996	0-3	1000	Flows into Ship Island Creek
Airplane Lake (S.I.L. #5)	Westslope Cutthroat	09/22/1992	0-3	750	Flows into Ship Island Creek
Airplane Lake (S.I.L. #5)	Westslope Cutthroat	08/30/1989	0-3	1000	Flows into Ship Island Creek
Airplane Lake (S.I.L. #5)	Rainbow Trout	07/24/1963	1	1500	Flows into Ship Island Creek
Airplane Lake (S.I.L. #5)	Cutthroat Trout	08/10/1937	1	1800	Flows into Ship Island Creek
Shoban Lake (S.I.L. #6)	Westslope Cutthroat	08/26/1998	0-3	1000	Flows into Ship Island Creek
Shoban Lake (S.I.L. #6)	Westslope Cutthroat	08/08/1996	0-3	1000	Flows into Ship Island Creek
Shoban Lake (S.I.L. #6)	Westslope Cutthroat	09/22/1992	0-3	750	Flows into Ship Island Creek
Shoban Lake (S.I.L. #6)	Westslope Cutthroat	08/30/1989	0-3	1000	Flows into Ship Island Creek

Appendix B. Continued.

Stream or Lake	Species Stocked	Date	Size (inches)	# of Fish	Comments
Shoban Lake (S.I.L. #6)	Westslope Cutthroat	09/03/1986	0-3	1000	Flows into Ship Island Creek
Shoban Lake (S.I.L. #6)	Westslope Cutthroat	09/06/1983	0-3	1000	Flows into Ship Island Creek
Shoban Lake (S.I.L. #6)	Westslope Cutthroat	08/19/1977	0-3	1152	Flows into Ship Island Creek
Shoban Lake (S.I.L. #6)	Cutthroat Trout	07/24/1963	1	900	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Westslope Cutthroat	08/31/2001	0-3	325	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Westslope Cutthroat	08/26/1998	0-3	1000	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Westslope Cutthroat	08/08/1996	0-3	1000	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Westslope Cutthroat	09/22/1992	0-3	750	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Westslope Cutthroat	08/30/1989	0-3	1000	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Henry's Cutthroat	09/03/1986	0-3	1000	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Cutthroat Trout	08/19/1977	0-3	1152	Flows into Ship Island Creek
Sheepeater Lake (S.I.L. #7)	Cutthroat Trout	07/24/1963	1	900	Flows into Ship Island Creek
Wilson Creek					
Buck Lake	Westslope Cutthroat	08/25/1998	1	250	Flows into Wilson Creek
Buck Lake	Grayling	09/03/1992	0-3	250	Flows into Wilson Creek
Buck Lake	Westslope Cutthroat	09/03/1992	0-3	250	Flows into Wilson Creek
Buck Lake	Westslope Cutthroat	08/31/1989	0-3	500	Flows into Wilson Creek
Buck Lake	Grayling	09/02/1986	0-3	250	Flows into Wilson Creek
Buck Lake	Westslope Cutthroat	08/09/1986	1	250	Flows into Wilson Creek
Buck Lake	Henry's Cutthroat	09/04/1983	0-3	500	Flows into Wilson Creek
Buck Lake	Rxc	09/16/1980	0-3	270	Flows into Wilson Creek
Buck Lake	Grayling	07/15/1974	0-3	2144	Flows into Wilson Creek
Harbor Lake	Westslope Cutthroat	08/31/2001	0-3	3000	Flows into Wilson Creek
Harbor Lake	Westslope Cutthroat	08/26/1998	0-3	3000	Flows into Wilson Creek
Harbor Lake	Westslope Cutthroat	08/08/1996	0-3	3000	Flows into Wilson Creek
Harbor Lake	Westslope Cutthroat	09/28/1992	0-3	2550	Flows into Wilson Creek
Harbor Lake	Westslope Cutthroat	08/30/1989	0-3	3000	Flows into Wilson Creek
Harbor Lake	Mt. Lassen Rainbow	09/03/1986	0-3	3000	Flows into Wilson Creek
Harbor Lake	Rainbow Trout	09/06/1983	0-3	3000	Flows into Wilson Creek
Harbor Lake	Rxc	09/17/1980	0-3	3078	Flows into Wilson Creek
Harbor Lake	Rainbow Trout	08/19/1977	0-3	2880	Flows into Wilson Creek
Harbor Lake	Rainbow Trout	08/17/1974	0-3	2625	Flows into Wilson Creek
Harbor Lake	Cutthroat Trout	08/17/1971	0-3	3060	Flows into Wilson Creek
Harbor Lake	Cutthroat Trout	08/03/1968	0-3	3000	Flows into Wilson Creek
Harbor Lake	Rainbow Trout	08/10/1964	1	2625	Flows into Wilson Creek
Harbor Lake	Rainbow Trout	08/05/1959	1	2800	Flows into Wilson Creek
Harbor Lake	Rainbow Trout	08/31/1951	1-2	3200	*Flows into Wilson Creek
Heart Lake	Westslope Cutthroat	08/31/2001	0-3	1675	Flows into Wilson Creek
Heart Lake	Westslope Cutthroat	08/26/1998	0-3	2000	Flows into Wilson Creek
Heart Lake	Westslope Cutthroat	08/08/1996	0-3	2000	Flows into Wilson Creek
Heart Lake	Westslope Cutthroat	09/28/1992	0-3	1700	Flows into Wilson Creek
Heart Lake	Westslope Cutthroat	08/31/1989	0-3	2000	Flows into Wilson Creek
Heart Lake	Mt. Lassen Rainbow	09/03/1986	0-3	2000	Flows into Wilson Creek
Heart Lake	Rainbow Trout	09/06/1983	0-3	2000	Flows into Wilson Creek
Heart Lake	Rxc	09/17/1980	0-3	2052	Flows into Wilson Creek
Heart Lake	Rainbow Trout	08/19/1977	0-3	1960	Flows into Wilson Creek
Heart Lake	Rainbow Trout	08/17/1974	0-3	1750	Flows into Wilson Creek
Heart Lake	Cutthroat	08/17/1971	0-3	2025	Flows into Wilson Creek
Heart Lake	Cutthroat	08/03/1968	0-3	2000	Flows into Wilson Creek
Heart Lake	Rainbow Trout	08/05/1959	1	2800	Flows into Wilson Creek
Paragon Lake	Westslope Cutthroat	09/01/1998	1	1000	Flows into Wilson Creek
Paragon Lake	Westslope Cutthroat	08/08/1996	1	1000	Flows into Wilson Creek
Paragon Lake	Westslope Cutthroat	09/28/1992	1	850	Flows into Wilson Creek
Paragon Lake	Westslope Cutthroat	08/31/1989	1	1000	Flows into Wilson Creek
Paragon Lake	Henry's Cutthroat	09/03/1986	1	1000	Flows into Wilson Creek
Paragon Lake	Henry's Cutthroat	09/06/1983	1	500	Flows into Wilson Creek
Paragon Lake	Rxc	09/17/1980	1	540	Flows into Wilson Creek
Ramshorn Lake	Westslope Cutthroat	08/31/2001	0-3	350	Flows into Wilson Creek
Ramshorn Lake	Westslope Cutthroat	09/01/1998	0-3	1000	Flows into Wilson Creek

Appendix B. Continued.

Stream or Lake	Species Stocked	Date	Size (inches)	# of Fish	Comments
Ramshorn Lake	Westslope Cutthroat	08/08/1996	0-3	1000	Flows into Wilson Creek
Ramshorn Lake	Westslope Cutthroat	09/22/1992	0-3	750	Flows into Wilson Creek
Ramshorn Lake	Westslope Cutthroat	08/31/1989	0-3	1000	Flows into Wilson Creek
Ramshorn Lake	Henry's Cutthroat	09/03/1986	0-3	500	Flows into Wilson Creek
Ramshorn Lake	Henry's Cutthroat	09/06/1983	0-3	500	Flows into Wilson Creek
Ramshorn Lake	Rxc	09/17/1980	0-3	540	Flows into Wilson Creek
Welcome Lake	Westslope Cutthroat	08/31/2001	0-3	1225	Flows into Wilson Creek
Welcome Lake	Westslope Cutthroat	08/26/1998	0-3	3000	Flows into Wilson Creek
Welcome Lake	Westslope Cutthroat	08/08/1996	0-3	3000	Flows into Wilson Creek
Welcome Lake	Westslope Cutthroat	09/28/1992	0-3	1700	Flows into Wilson Creek
Welcome Lake	Westslope Cutthroat	08/31/1989	0-3	3000	Flows into Wilson Creek
Welcome Lake	Mt. Lassen Rainbow	09/03/1986	0-3	2000	Flows into Wilson Creek
Welcome Lake	Rainbow Trout	09/06/1983	0-3	2000	Flows into Wilson Creek
Welcome Lake	Rxc	09/17/1980	0-3	2052	Flows into Wilson Creek
Welcome Lake	Rainbow Trout	08/19/1977	0-3	1960	Flows into Wilson Creek
Welcome Lake	Rainbow Trout	08/17/1974	0-3	1750	Flows into Wilson Creek
Welcome Lake	Cutthroat Trout	08/17/1971	0-3	2025	Flows into Wilson Creek
Welcome Lake	Cutthroat Trout	08/01/1968	0-3	2000	Flows into Wilson Creek
Welcome Lake	Rainbow Trout	08/10/1964	1	2625	Flows into Wilson Creek
Wilson Lake	Rainbow Trout	08/10/1964	1	2625	Flows into Wilson Creek
Wilson Lake	Westslope Cutthroat	08/31/2001	0-3	1000	Flows into Wilson Creek
Wilson Lake	Westslope Cutthroat	08/26/1998	0-3	1000	Flows into Wilson Creek
Wilson Lake	Westslope Cutthroat	08/08/1996	0-3	1000	Flows into Wilson Creek
Wilson Lake	Westslope Cutthroat	09/22/1992	0-3	750	Flows into Wilson Creek
Wilson Lake	Westslope Cutthroat	08/30/1989	0-3	1000	Flows into Wilson Creek
Wilson Lake	Henry's Cutthroat	09/03/1986	0-3	1000	Flows into Wilson Creek
Wilson Lake	Henry's Cutthroat	09/06/1983	0-3	1000	Flows into Wilson Creek
Wilson Lake	Rxc	09/17/1980	0-3	1026	Flows into Wilson Creek
Wilson Lake	Cutthroat	08/19/1977	0	576	Flows into Wilson Creek
Wilson Lake	Cutthroat	08/19/1977	0-3	1152	Flows into Wilson Creek
Wilson Lake	Cutthroat	08/08/1974	0-3	1008	Flows into Wilson Creek
Wilson Lake	Cutthroat	08/17/1971	0-3	1080	Flows into Wilson Creek
Wilson Lake	Grayling	08/17/1971	0	1440	Flows into Wilson Creek
Wilson Lake	Cutthroat	08/03/1968	0-3	1000	Flows into Wilson Creek
Papoose Creek					
Papoose Creek Lake	Westslope Cutthroat	09/13/2000	0-3	986	Flows into Papoose Creek
Papoose Creek Lake	Westslope Cutthroat	08/15/1994	0-3	500	Flows into Papoose Creek
Papoose Creek Lake	Westslope Cutthroat	08/18/1991	0-3	1000	Flows into Papoose Creek
Papoose Creek Lake	Westslope Cutthroat	08/13/1988	0-3	1000	Flows into Papoose Creek
Papoose Creek Lake	Westslope Cutthroat	08/06/1985	0-3	1000	Flows into Papoose Creek
Papoose Creek Lake	Henry's Cutthroat	08/21/1982	0-3	1000	Flows into Papoose Creek
Papoose Creek Lake	Cutthroat Trout	08/04/1978	0-3	1050	Flows into Papoose Creek
Papoose Creek Lake	Golden Trout	10/02/1977	0-3	976	Flows into Papoose Creek
Papoose Creek Lake	Cutthroat Trout	09/05/1975	0-3	1244	Flows into Papoose Creek
Papoose Creek Lake	Golden Trout	09/06/1969	0-3	1080	Flows into Papoose Creek
Papoose Lake No. 2	Cutthroat Trout	08/02/1978	0-3	175	Flows into Papoose Creek
Papoose Lake No. 2	Cutthroat Trout	09/05/1975	0-3	1244	Flows into Papoose Creek
Cache Creek					
Cache Creek Lake No. 1	Westslope Cutthroat	09/01/1999	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 1	Westslope Cutthroat	08/14/1996	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 1	Westslope Cutthroat	09/05/1990	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 1	Grayling	09/09/1987	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 1	Henry's Cutthroat	09/02/1984	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 1	Rxc	07/29/1981	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 1	Cutthroat Trout	08/18/1977	0-3	288	Flows into Cache Creek
Cache Creek Lake No. 1	Cutthroat Trout	08/09/1974	0-3	1008	Flows into Cache Creek
Cache Creek Lake No. 1	Cutthroat Trout	08/16/1971	0-3	1080	Flows into Cache Creek
Cache Creek Lake No. 1	Cutthroat Trout	08/03/1968	0-3	1000	Flows into Cache Creek
Cache Creek Lake No. 2	Rxc	07/29/1981	0-3	250	Flows into Cache Creek

Appendix B. Continued.

Stream or Lake	Species Stocked	Date	Size (inches)	# of Fish	Comments
Cache Creek Lake No. 2	Cutthroat Trout	08/18/1977	0-3	288	Flows into Cache Creek
Cache Creek Lake No. 2	Cutthroat Trout	08/09/1974	0-3	1008	Flows into Cache Creek
Cache Creek Lake No. 2	Cutthroat Trout	08/16/1971	0-3	1080	Flows into Cache Creek
Cache Creek Lake No. 2	Cutthroat Trout	08/03/1968	0-3	1000	Flows into Cache Creek
Cache Creek Lake No. 3	Westslope Cutthroat	09/01/1999	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 3	Westslope Cutthroat	08/14/1996	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 3	Westslope Cutthroat	09/05/1990	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 3	Westslope Cutthroat	09/09/1987	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 3	Henry's Cutthroat	09/02/1984	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 3	Rxc	07/29/1981	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 3	Cutthroat Trout	08/18/1977	0-3	288	Flows into Cache Creek
Cache Creek Lake No. 3	Cutthroat Trout	08/09/1974	0-3	1008	Flows into Cache Creek
Cache Creek Lake No. 3	Cutthroat Trout	08/16/1971	0-3	1080	Flows into Cache Creek
Cache Creek Lake No. 3	Cutthroat Trout	08/03/1968	0-3	1000	Flows into Cache Creek
Cache Creek Lake No. 3	Cutthroat Trout	08/29/1954	1.5	2000	Flows into Cache Creek
Cache Creek Lake No. 4	Cutthroat Trout	08/09/1974	0-3	1008	Flows into Cache Creek
Cache Creek Lake No. 4	Cutthroat Trout	08/16/1971	0-3	2025	Flows into Cache Creek
Cache Creek Lake No. 4	Cutthroat Trout	08/03/1968	0-3	2000	Flows into Cache Creek
Cache Creek Lake No. 4	Cutthroat Trout	09/24/1959	2.0	2100	Flows into Cache Creek
Cache Creek Lake No. 4	Cutthroat Trout	08/29/1954	1.5	2000	Flows into Cache Creek
Cache Creek Lake No. 5	Grayling	09/11/2001	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 5	Grayling	08/19/1999	0-3	500	Flows into Cache Creek
Cache Creek Lake No. 5	Grayling	08/14/1996	0-3	500	Flows into Cache Creek
Cache Creek Lake No. 5	Grayling	09/05/1990	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 5	Westslope Cutthroat	09/09/1987	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 5	Henry's Cutthroat	09/02/1984	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 5	Rxc	07/29/1981	0-3	250	Flows into Cache Creek
Cache Creek Lake No. 5	Cutthroat Trout	08/18/1977	0-3	288	Flows into Cache Creek
Cache Creek Lake No. 5	Cutthroat Trout	08/09/1974	0-3	1008	Flows into Cache Creek
Cache Creek Lake No. 5	Cutthroat Trout	08/16/1971	0-3	1080	Flows into Cache Creek
Cache Creek Lake No. 5	Rainbow Trout	09/03/1968	0-3	1520	Flows into Cache Creek
Cache Creek Lake No. 5	Cutthroat Trout	08/03/1968	0-3	1000	Flows into Cache Creek
Cache Creek Lake No. 6	Cutthroat Trout	08/09/1974	0-3	1008	Flows into Cache Creek
Cache Creek Lake No. 6	Cutthroat Trout	08/16/1971	0-3	1080	Flows into Cache Creek
Cache Creek Lake No. 6	Cutthroat Trout	08/03/1968	0-3	1000	Flows into Cache Creek
Cache Creek Lake No. 7	Cutthroat Trout	08/09/1974	0-3	1008	Flows into Cache Creek
Cache Creek Lake No. 7	Cutthroat Trout	08/16/1971	0-3	1080	Flows into Cache Creek
Cache Creek Lake No. 7	Cutthroat Trout	08/03/1968	0-3	1000	Flows into Cache Creek

* Indicates either that the county # was given w/out catalogue number, but it was the only name for that body of water, or name was given without county number but according to the topography map of Idaho, was the only one in all of Idaho with that name.

Appendix C. Raw Genetic Scores Blackfoot Study.

Field ID	Genetic ID	CYT B	P53	IK	RAG3'	OCC16	Genetic ID
5-29-R4-P2	DT-02-2	C	BB	BB	BB	BB	YCT
5-29-R4-P3	DT-02-3	C	BB	BB	CC	BB	YCT
5-29-R5-P4	DT-02-4	C	BB	BB	CC	BB	YCT
5-29-R4-P5	DT-02-5	C	BB	BB	BC	BB	YCT
5-29-R4-P6	DT-02-6	C	BB	BB	BB	BB	YCT
5-29-R5-P7	DT-02-7	C	BB	BB	BB	BB	YCT
5-29-R5-P8	DT-02-8	C	BB	BB	BB	BB	YCT
5-29-R5-P9	DT-02-9	C	BB	BB	BC	BB	YCT
5-29-R4-P10	DT-02-10	C	BB	BB	BC	BB	YCT
5-29-R4-P11	DT-02-11	C	BB	BB	CC	BB	YCT
5-29-R5-P12	DT-02-12	C	BB	BB	BC	BB	YCT
5-29-R4-P13	DT-02-13	C	BB	BB	BC	BB	YCT
5-29-R4-P14	DT-02-14	C	BB	BB	BC	BB	YCT
5-29-R4-P15	DT-02-15	C	BB	BB	CC	BB	YCT
5-29-R5-P16	DT-02-16	C	BB	BB	BC	BB	YCT
5-29-R4-P17	DT-02-17	C	BB	BB	CC	BB	YCT
5-29-R4-P18	DT-02-18	C	BB	BB	BB	BB	YCT
5-29-R5-P19	DT-02-19	C	BB	BB	BB	BB	YCT
5-29-R4-P20	DT-02-20	C	BB	BB	CC	BB	YCT
5-29-R4-P21	DT-02-21	C	BB	BB	BB	BB	YCT
5-29-R4-P22	DT-02-22	C	BB	BB	BC	BB	YCT
5-29-R4-P23	DT-02-23	C	BB	BB	BC	BB	YCT
5-29-R4-P24	DT-02-24	C	BB	BB	BC	BB	YCT
5-29-R4-P25	DT-02-25	C	BB	BB	CC	BB	YCT
5-29-R5-P26	DT-02-26	C	BB	BB	CC	BB	YCT
5-16-R4-P1	DT-02-P1	C	BB	BB	CC	BB	YCT
5-16-R4-P2	DT-02-P2	C	BB	BB	CC	BB	YCT
5-16-R5-P3	DT-02-P3	MISS	MISS	MISS	MISS	MISS	MISS
5-16-R5-P4	DT-02-P4	C	MISS	BB	BC	BB	YCT
5-16-R4-P5	DT-02-P5	C	BB	BB	CC	BB	YCT
5-16-R4-P6	DT-02-P6	C	BB	BB	BC	BB	YCT
5-16-R4-P7	DT-02-P7	C	BB	BB	BC	BB	YCT
5-16-R4-P8	DT-02-P8	C	BB	BB	BC	BB	YCT
5-16-R4-P9	DT-02-P9	C	BB	BB	BC	BB	YCT
5-16-R4-P10	DT-02-P10	C	BB	BB	BC	BB	YCT
5-16-R4-P11	DT-02-P11	C	BB	BB	CC	BB	YCT
5-16-R4-P12	DT-02-P12	C	BB	BB	CC	BB	YCT
5-16-R4-P13	DT-02-P13	C	BB	BB	CC	BB	YCT
5-16-R4-P14	DT-02-P14	C	BB	BB	CC	BB	YCT
5-16-R4-P15	DT-02-P15	C	BB	BB	BC	BB	YCT
5-16-R4-P16	DT-02-P16	C	BB	BB	BB	BB	YCT
5-16-R4-P17	DT-02-P17	C	BB	BB	CC	BB	YCT
5-16-R4-P18	DT-02-P18	C	BB	BB	BC	BB	YCT
5-16-R4-P19	DT-02-P19	C	BB	BB	BC	BB	YCT
5-16-R5-P20	DT-02-P20	C	BB	BB	CC	BB	YCT
5-16-R4-P21	DT-02-P21	C	BB	BB	CC	BB	YCT
5-16-R4-P22	DT-02-P22	C	BB	BB	BC	BB	YCT
5-16-R4-P23	DT-02-P23	C	BB	BB	BC	BB	YCT
5-16-R4-P24	DT-02-P24	C	BB	BB	CC	BB	YCT
5-16-R5-P25	DT-02-P25	C	BB	BB	BC	BB	YCT
5-16-R4-P26	DT-02-P26	C	BB	BB	BC	BB	YCT
5-16-R2-P27	DT-02-P27	A	AB	AB	AB	AB	F ₁ HYBRID

Appendix C. Continued

Field ID	Genetic ID	CYT B	P53	IK	RAG3'	OCC16	Genetic ID
5-16-R5-P28	DT-02-P28	C	BB	BB	CC	BB	YCT
5-16-R4-P29	DT-02-P29	C	BB	BB	CC	BB	YCT
5-16-R4-P30	DT-02-P30	C	BB	BB	BC	BB	YCT
5-30-R5-P1	DT-02-Q1	C	BB	BB	BC	BB	YCT
5-30-R4-P2	DT-02-Q2	C	BB	BB	CC	BB	YCT
5-30-R4-P3	DT-02-Q3	C	BB	BB	CC	BB	YCT
5-30-R4-P4	DT-02-Q4	C	BB	BB	CC	BB	YCT
5-30-R4-P5	DT-02-Q5	C	MISS	BB	BC	BB	YCT
5-30-R4-P6	DT-02-Q6	C	BB	BB	BB	BB	YCT
5-30-R4-P7	DT-02-Q7	C	BB	BB	CC	BB	YCT
5-30-R4-P8	DT-02-Q8	C	BB	BB	BC	BB	YCT
5-30-R5-P9	DT-02-Q9	C	BB	BB	CC	BB	YCT
5-30-R5-P10	DT-02-Q10	C	BB	BB	BC	BB	YCT
5-30-R4-P11	DT-02-Q11	C	BB	BB	BC	BB	YCT
5-30-R4-P12	DT-02-Q12	C	BB	BB	BC	BB	YCT
5-30-R4-P13	DT-02-Q13	C	BB	BB	CC	BB	YCT
5-30-R4-P14	DT-02-Q14	C	BB	BB	CC	BB	YCT
5-30-R4-P15	DT-02-Q15	C	BB	BB	BB	BB	YCT
5-30-R4-P16	DT-02-Q16	C	BB	BB	BC	BB	YCT
5-30-R4-P17	DT-02-Q17	C	BB	BB	CC	BB	YCT
5-30-R4-P18	DT-02-Q18	C	BB	BB	CC	BB	YCT
5-30-R4-P19	DT-02-Q19	C	BB	BB	BC	BB	YCT
5-30-R4-P20	DT-02-Q20	C	BB	BB	BC	BB	YCT
5-2-R5-P2	DT-02-R2	C	BB	BB	BC	BB	YCT
5-2-R5-P3	DT-02-R3	C	BB	BB	CC	BB	YCT
5-2-R4-P4	DT-02-R4	C	BB	BB	BC	BB	YCT
5-2-R4-P5	DT-02-R5	C	BB	BB	BC	BB	YCT
5-2-R4-P7	DT-02-R7	C	BB	BB	CC	BB	YCT
5-2-R5-P8	DT-02-R8	C	BB	BB	BC	BB	YCT
5-2-R4-P9	DT-02-R9	C	BB	BB	CC	BB	YCT
5-2-R5-P11	DT-02-R11	C	BB	BB	MISS	BB	YCT
5-2-R4-P12	DT-02-R12	C	BB	BB	BB	BB	YCT
5-2-R4-P13	DT-02-R13	C	BB	BB	CC	BB	YCT
5-2-R4-P14	DT-02-R14	C	BB	BB	CC	BB	YCT
5-2-R4-P15	DT-02-R15	C	BB	BB	CC	BB	YCT
5-2-R5-P16	DT-02-R16	C	BB	BB	CC	BB	YCT
5-2-R5-P17	DT-02-R17	C	BB	BB	CC	BB	YCT
5-2-R4-P18	DT-02-R18	C	BB	BB	BC	BB	YCT
5-2-R4-P20	DT-02-R20	C	BB	BB	BB	BB	YCT
5-2-R5-P21	DT-02-R21	C	BB	BB	MISS	BB	YCT
5-2-R1-P22	DT-02-R22	A	AB	AB	AC	AB	F ₁ HYBRID
5-2-R4-P23	DT-02-R23	C	BB	BB	BB	BB	YCT
5-2-R4-P24	DT-02-R24	C	BB	BB	CC	BB	YCT
5-2-R5-P25	DT-02-R25	C	BB	BB	BB	BB	YCT
5-2-R5-P26	DT-02-R26	C	BB	BB	CC	BB	YCT
5-2-R4-P27	DT-02-R27	C	BB	BB	CC	BB	YCT
5-2-R3-P28	DT-02-R28	C	BB	BB	MISS	BB	YCT
5-29-R4-P1	DT-02-1	C	BB	BB	CC	BB	YCT
5-15-R4-P1	DT-02-S1	C	BB	BB	CC	MISS	YCT
5-15-R4-P2	DT-02-S2	C	BB	BB	BB	BB	YCT
5-15-R4-P3	DT-02-S3	C	BB	BB	BB	BB	YCT
5-15-R4-P4	DT-02-S4	C	BB	BB	BB	BB	YCT
5-15-R4-P5	DT-02-S5	C	BB	BB	CC	BB	YCT
5-15-R4-P6	DT-02-S6	C	BB	MISS	MISS	MISS	YCT

Appendix C. Continued

Field ID	Genetic ID	CYT B	P53	IK	RAG3'	OCC16	Genetic ID
5-15-R4-P7	DT-02-S7	MISS	MISS	MISS	MISS	MISS	MISS
5-15-R3-P8	DT-02-S8	C	BB	BB	BC	BB	YCT
5-15-R4-P9	DT-02-S9	C	BB	BB	BC	MISS	YCT
5-15-R4-P10	DT-02-S10	C	BB	BB	BC	MISS	YCT
5-15-R4-P11	DT-02-S11	C	BB	BB	CC	BB	YCT
5-15-R4-P12	DT-02-S12	C	BB	BB	CC	BB	YCT
5-15-R4-P13	DT-02-S13	C	BB	BB	CC	BB	YCT
5-4-R4-P1	DT-02-T1	C	BB	BB	BC	BB	YCT
5-4-R4-P2	DT-02-T2	C	BB	BB	CC	BB	YCT
5-4-R5-P3	DT-02-T3	C	BB	BB	BB	BB	YCT
5-4-R4-P4	DT-02-T4	C	BB	BB	BB	BB	YCT
5-4-R4-P5	DT-02-T5	C	BB	BB	CC	BB	YCT
5-4-R4-P6	DT-02-T6	C	BB	BB	BC	BB	YCT
5-4-R4-P7	DT-02-T7	C	MISS	BB	MISS	BB	YCT
5-4-R4-P8	DT-02-T8	C	BB	BB	CC	BB	YCT
5-4-R4-P9	DT-02-T9	C	BB	BB	BC	BB	YCT
5-4-R4-P10	DT-02-T10	MISS	MISS	MISS	MISS	MISS	MISS
4-20-R1-P1	DT-02-U1	A*	BB	AA	AA	AA	RBT
4-20-R4-P2	DT-02-U2	C	BB	BB	BB	BB	YCT
4-20-R4-P3	DT-02-U3	C	BB	BB	BB	BB	YCT
4-20-R4-P4	DT-02-U4	C	BB	BB	CC	BB	YCT
4-22-R1-P1	DT-02-V1	A*	AB	AA	AA	AA	RBT
4-29-R1-P1	DT-02-V2	A*	AA	AA	AA	AA	RBT

*Samples DT-02-U1, V1, and V2 were run twice with the OCC16 marker. The first time (4/2/03) samples were scored as "AB," heterozygous for both an RBT allele and a CUT allele. The second time (4/14/03) samples were scored as "AA," homozygous for RBT alleles. Both a known F₁ hybrid as well as a known RBT were run with the second batch. The scores from the second run are considered final scores.

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