

## Hybridization and Introgression in a Managed, Native Population of Yellowstone Cutthroat Trout: Genetic Detection and Management Implications

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**Abstract.**—Since the mid-1920s, the Idaho Department of Fish and Game has cultured Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* at Henrys Lake to offset declines in natural production and for use in stocking programs throughout Idaho. Since the mid-1970s, they have also produced F<sub>1</sub> hybrids: female Yellowstone cutthroat trout × male rainbow trout *O. mykiss*. The ability of fishery managers, when selecting broodstock, to visually distinguish returning cutthroat trout from F<sub>1</sub> hybrids is, therefore, crucial to avoid accidental introduction of rainbow trout genes into the hatchery-supplemented cutthroat trout population. To evaluate this ability, fish identified by staff as putative cutthroat trout or hybrids (an array of phenotypic characters are used), were sampled during two spawning seasons. Phenotypically identified fish were genetically tested using species-specific restriction fragment length polymorphisms (RFLPs) of nuclear and mitochondrial DNA gene loci and diagnostic allozyme loci. Current levels of rainbow trout introgression in the cutthroat trout population at Henrys Lake were also investigated by analyzing samples collected from the lake and several of its tributaries. Results indicated that staff's phenotypic identifications were highly accurate in distinguishing cutthroat trout from F<sub>1</sub> hybrids when selecting broodstock (no F<sub>1</sub> hybrids were detected among 80 samples identified as pure). However, backcrosses of F<sub>1</sub> hybrids were identified in random collections of adults from the lake as well as fry from Henrys Lake tributaries, indicating introgression. Present levels of rainbow trout introgression are most likely the product of past rainbow trout introductions and limited, intermittent spawning of hatchery-produced F<sub>1</sub> hybrids with wild Yellowstone cutthroat, rather than the accidental crossing of F<sub>1</sub> hybrids with cutthroat trout at the hatchery. Current levels of introgression are inadvertently maintained by (1) the inability of managers to phenotypically identify and exclude as broodstock individuals with low levels of rainbow trout introgression and (2) the limited, intermittent reproductive success of straying, hatchery-produced F<sub>1</sub> hybrids.

Management and conservation of Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* populations have become high priorities for the Idaho Department Fish and Game (IDFG) because of population declines throughout their historical native range (Thurow et al. 1988; Behnke 1992; May 1996). In August 1998 several conservation groups collectively petitioned the U.S. Fish and Wildlife Service to list the Yellowstone cutthroat trout as a threatened species under the Endangered Species Act (ESA; 66FR11244). Currently, Yellowstone

cutthroat trout are recognized as a “species of special concern” by the IDFG (Thurow et al. 1988, IDFG 2000). In addition to habitat degradation and overfishing, population declines have been attributed to extensive historical stocking of nonnative, hatchery raised, rainbow trout *O. mykiss*, which have hybridized with or replaced Yellowstone cutthroat trout populations in many areas (Varley and Gresswell 1988; Behnke 1992; Kruse et al. 2000).

As part of a larger project to describe the current status of Yellowstone cutthroat trout populations in Idaho, the IDFG is investigating the extent of introgressive hybridization with nonnative rainbow trout within the Snake River sub-basin above

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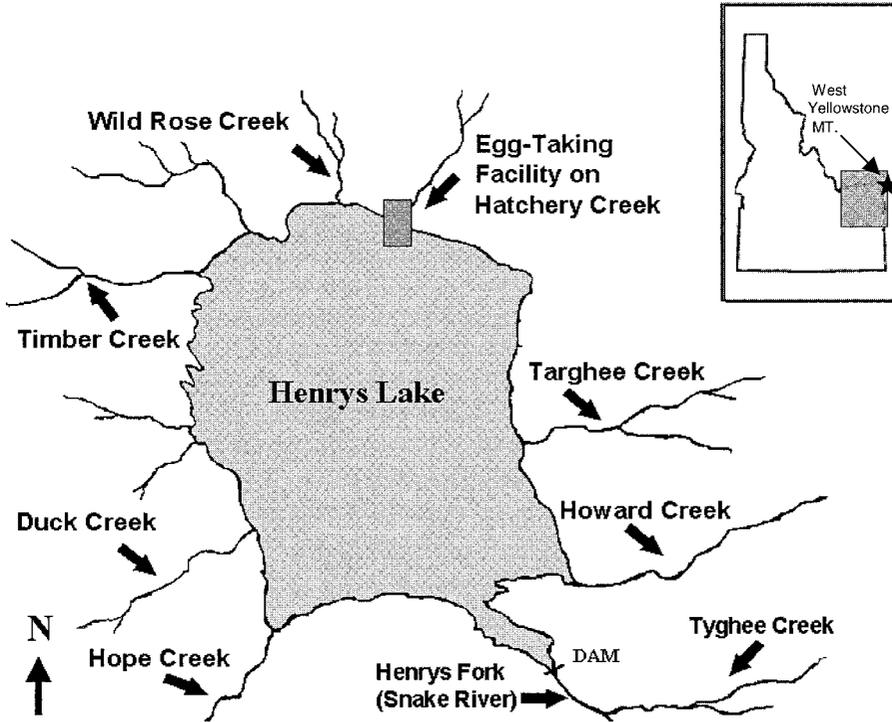


FIGURE 1.—Henrys Lake, Idaho, and its tributaries that were sampled for genetic analysis of Yellowstone cutthroat trout; the egg-taking facility on Hatchery Creek is also depicted. Tyghee Creek (not a tributary to Henrys Lake) was sampled for use as a reference population of Yellowstone cutthroat trout.

Shoshone Falls. Historically, these areas contained abundant (usually predominant) populations of Yellowstone cutthroat trout (Behnke 1992). One of the Yellowstone cutthroat trout watersheds of special concern is the Henrys Lake sub-basin. A genetic assessment of Yellowstone cutthroat trout populations within the Henrys Lake sub-basin and the impact of hatchery operations on these populations were the focus of this investigation.

Henrys Lake is located in the southeast corner of Idaho, approximately 30 km from West Yellowstone, Montana (Figure 1). At an elevation of 1,857 m, the lake covers approximately 2,632 ha (Irving 1954). Yellowstone cutthroat trout are the only native species of *Oncorhynchus* in Henrys Lake (Irving 1954). Rainbow trout have been stocked in the lake in limited numbers for over 50 years. Most recently, 7,200 adult rainbow trout were released into the lake in 1982 (IDFG 1999). However, despite extensive gill netting and creel surveys, no rainbow trout have been detected in the lake during the past 15 years (IDFG, unpublished data).

Since the mid-1920s, IDFG has operated the Hatchery Creek egg-taking facility on Henrys

Lake for the production of Yellowstone cutthroat trout. Hatchery production of cutthroat trout was originally undertaken to offset anticipated declines in natural production due to spawning habitat losses in the lake following construction of a dam at the outlet in 1924. Although a decline in natural recruitment to the lake was expected, it was not documented over the next several decades. Through the 1950s continued natural recruitment was suggested by the fact that the majority of spawning cutthroat trout continued to ascend tributaries other than Hatchery Creek, 77.6% in 1955 and 80.2% in 1956 (Adriano 1956).

However, by the late 1970s and early 1980s, surveys indicated that spawning and rearing habitat in the tributaries had been negatively affected by cattle grazing and irrigation diversions and that very little natural recruitment was taking place (Rohrer 1980). Irrigation diversions on Targhee, Duck, and Howard creeks, three of the largest tributaries to Henrys Lake, resulted in substantial flow reductions by early to midsummer and entrainment losses of fry migrating into canals. Targhee Creek, one of the largest producers of fry in the 1950s (39%), had been dewatered by early summer in

1966 and 1973 (Rohrer 1980). In 1978, an estimated 75–90% of the flow from the main stem of Howard Creek was diverted for irrigation and an estimated 71–95% of fry migrating from upstream areas were consequently lost (Coon 1978).

In response to the substantial decline in natural recruitment to the lake, management plans focused on increasing hatchery production of Yellowstone cutthroat trout. Between 1975 and 1998, IDFG annually stocked an average of 1,000,000 cutthroat trout fingerlings in Henrys Lake and its tributaries (IDFG 1999). The stocking program has been very successful and Henrys Lake has supported high fishing pressure and high catch rates for over 20 years. Between 1978 and 1995, average annual angler effort and catch rates were 144,245 h and 0.33 fish/h (Schrader et al., in press [a]).

For almost 50 years Henrys Lake has also been managed for a trophy hybrid cutthroat trout fishery (female cutthroat trout  $\times$  male rainbow trout), locally referred to as “cuttbows” or “hybrids.” First generation ( $F_1$ ) hybrids grow faster and attain a larger size than pure cutthroat trout (Schrader et al., in press [b]) and are a very popular component of the sport fishery. Limited numbers of hybrids were stocked in the lake beginning in the early 1950s (Irving 1954). A large-scale hybrid stocking program (about 325,000 fingerlings stocked per year) operated on the lake from 1960 to 1971 (Rohrer 1981). The hybrid program was terminated in 1972, when changes in the spot size and spotting pattern of spawning cutthroat trout prompted concerns over rainbow trout introgression in the Yellowstone cutthroat trout population (Rohrer 1981). However, by 1975 angling groups had organized and successfully lobbied the Idaho Fish and Game Commission to reinstate the program (Rohrer 1981). Hybrid stocking has continued since that time, and during the 1990s, IDFG annually stocked an average of 200,000 hybrid fingerlings in Henrys Lake (IDFG 1999).

Each spring, spawning cutthroat trout and hybrids return up the fish ladder on Hatchery Creek to the egg-taking station. The hatchery ladder is opened March 1 and remains in operation through April. All fish ascending the ladder are manually sorted and identified. Hatchery personnel are trained to visually distinguish putatively pure cutthroat trout from hybrids using an array of phenotypic characteristics (Table 1). Cutthroat trout are sorted by sex and held in separate raceways until ripe. Returning hybrids are not used in spawning production. Current management guidelines require that hybrids be stripped of gametes

TABLE 1.—Sixteen phenotypic characters employed by the Henrys Lake Hatchery staff to distinguish Yellowstone cutthroat trout from hybrids.

Character
1. Spots on head (absent/present)
2. Spot shape
3. Spot halo (absent/present)
4. Red lateral (absent/present)
5. White tip fins (absent/present)
6. Throat slash (absent/present)
7. Jaw length
8. Number of spots above lateral line
9. Number of spots near caudal peduncle
10. Size of spots
11. Number of spots
12. Body coloration
13. Scale size
14. Body shape
15. Head length
16. Fork length

before being returned to the lake to eliminate the possibility that individuals that stray and re-ascend other Henrys Lake tributaries successfully reproduce with naturally spawning Yellowstone cutthroat.

Early-run cutthroat trout females (through mid-March) are used for hybrid production. Eggs are stripped and fertilized with milt obtained from domestic Kamloops rainbow trout broodstock at the Hayspur State Fish Hatchery, Bellevue, Idaho. When hybrid egg quotas are met, cutthroat trout males and females are selected and spawned for the remainder of the run.

Both hybrid and cutthroat trout eggs are incubated to the eyed stage at the egg-taking facility on Henrys Lake and then shipped to Mackay State Fish Hatchery, Idaho, for rearing. For example, during the 1999 spawning season, 4,894 putative cutthroat trout returned to the egg-taking facility on Hatchery Creek (Dillon et al., in press). During the same spawning season, 1,734 putative  $F_1$  hybrids also returned and were excluded from spawning. In 1999, 1,162 cutthroat trout females were spawned with cutthroat trout males, and 280 cutthroat trout females were spawned with rainbow trout milt (Dillon et al., in press).

Hatchery-reared hybrids and cutthroat trout are returned to Henrys Lake in September as fingerlings. Hybrids are returned to Hatchery Creek and held at the spawning station for 2–3 d before being released into the lake. Fingerling cutthroat trout are released into Hatchery Creek and several other Henrys Lake tributaries, including Howard Creek, Duck Creek, and Targhee Creek (Figure 1). Yellowstone cutthroat trout produced from Henrys

Lake have also been used for stocking purposes throughout Idaho.

### Study Rationale and Research Objectives

The IDFG management program for Henrys Lake includes two potentially conflicting objectives: (1) production of Yellowstone cutthroat trout to supplement natural reproduction and (2) the production of  $F_1$  hybrids for a recreational trophy fishery. Maintaining genetic integrity of the cutthroat trout population is important, not only from strictly a conservation standpoint, but also because pure cutthroat trout genes are believed to be essential components in producing the benefits observed in true  $F_1$  hybrids (i.e., improved growth and trophy potential resulting from heterosis or hybrid vigor). The long-term success of both of these objectives depends on two important conditions. Hatchery and management personnel must be able to use phenotypic traits to accurately differentiate cutthroat trout from hybrids in the spawning operation. In addition, hybrids produced at Hatchery Creek must not stray into other tributaries and contribute to natural recruitment.

The inability to distinguish hybrids from cutthroat trout would result in the accidental introduction of rainbow trout genes into the hatchery produced cutthroat trout population. This would undermine the management goal of preserving pure Yellowstone cutthroat trout populations in Idaho. Hybridization and subsequent introgression could jeopardize ESA protection of the Henrys Lake population if Yellowstone cutthroat trout become listed. The ESA currently does not recognize hybrids as a component meriting protection (Avisé 1994), and although pending revisions to the hybrid policy will extend protection to some hybrid populations, the revisions could limit protection of many populations with extensive intra- or interspecific introgression (61FR4710).

The straying of hybrids produced at the hatchery into Henrys Lake tributaries could also lead to rainbow trout introgression into the naturally spawning cutthroat trout tributary populations and further threaten current conservation objectives. Although it is now known that hybrids stray into tributaries other than Hatchery Creek, previous managers may have expected limited wild spawning of hybrids with other hybrids or with cutthroat trout. Research in the 1950s demonstrated high levels of homing by cutthroat trout back to specific tributaries in Henrys Lake and low levels of straying (Adriano 1956). Thus, it was reasoned that hybrids, all of which are released as fingerlings in

Hatchery Creek, would exhibit similar low rates of straying. Additionally, although there is some overlap, the spawning season of cutthroat trout in other Henrys Lake tributaries generally occurs later (mid-April through mid-June) than the spawning season of cutthroat trout and hybrids that return to Hatchery Creek. The earlier spawning run in Hatchery Creek is a result of broodstock selection over time for adults that return earlier (Thurow et al. 1988).

In this study, three different genetic techniques were combined to test phenotypic identifications by IDFG hatchery and management personnel and to detect rainbow trout introgression among Yellowstone cutthroat trout in Henrys Lake and its tributaries. Allozyme analysis of 8 to 10 loci diagnostic between rainbow and cutthroat trout were used because the utility of allozyme markers for investigating introgressive hybridization in Yellowstone cutthroat trout populations is well established (Leary et al. 1987, 1989; Allendorf and Leary 1988), and it was convenient to obtain tissue samples from adult fish that had returned to the egg collection facility on Hatchery Creek.

Samples were also collected from fry and sub-adult cutthroat trout, where it was difficult to collect tissues large enough for allozyme analysis. Thus, the utility of a nonlethal genetic technique that involves analysis of nuclear DNA (nDNA) was also investigated. Similar to mitochondrial DNA (mtDNA), large amounts of nDNA can be extracted from small fin clips (Wenburg et al. 1996). We used restriction fragment length polymorphisms (RFLP) analysis of three intron (non-coding) regions of nDNA (Baker et al. 2002, this issue). All three are diagnostic in separating rainbow trout from cutthroat trout when digested with specific enzymes (Baker et al. 2002).

To address the question of whether or not past rainbow trout introductions led to hybridization and introgression within Yellowstone cutthroat trout in Henrys Lake, a species-specific mtDNA RFLP was used. Because the hybrid program involves the unidirectional spawning of rainbow trout males with cutthroat trout females and because mtDNA is maternally inherited, the identification of rainbow trout mtDNA would indicate the past release and continued reproduction of rainbow trout female lineages not used in historical hybrid production.

### Methods

#### *Sample Collection*

*Reference populations.*—Sixty rainbow trout broodstock from the Hayspur State Fish Hatchery,

Idaho, were sampled (fin tissue) for use as a reference rainbow trout population. Two areas were sampled (fin tissue) to obtain pure (no known rainbow trout introgression) Yellowstone cutthroat trout: 60 from Tyghee Creek, Idaho (Figure 1), and 60 from Yellowstone Lake, Wyoming. The sample location on Tyghee Creek was above a natural barrier, this area has never been stocked with rainbow trout (IDFG 1999). Yellowstone Lake, which was sampled with gill nets at six locations (National Park Service), was stocked with rainbow trout in 1902 (3,000) and 1907 (3,800); however, both stockings were unsuccessful at establishing persistent populations (Gresswell and Varley 1988).

*Hatchery Creek spawning facility.*—Tissue samples (liver, muscle, and eye) were collected in 1998 from 20 fish phenotypically identified as Yellowstone cutthroat trout (putatively pure) and 40 fish phenotypically identified as hybrids (putative hybrids;  $F_1$  or greater). Putatively pure fish were chosen by staff according to the same phenotypic criteria used to select broodstock in 1998. The putative hybrids would have been excluded as broodstock during 1998 spawning operations. Tissue samples were held on dry ice before being transported to a  $-80^{\circ}\text{C}$  freezer where they were stored until allozyme analysis was performed. Additional muscle tissue samples were collected from the same 60 fish for subsequent nDNA and mtDNA analysis. Clipped fins were stored at room temperature in lysis buffer (EDTA 0.5M, 2M tris, 5M NaCl, sodium dodecyl sulfate 20%,  $\text{dH}_2\text{O}$ ).

Sixty additional putative Yellowstone cutthroat at the Hatchery Creek spawning station were sampled in 1999 for liver, muscle, and eye tissue. All fish were used as broodstock in spawning operations and were sampled during each of four sampling dates throughout the spawning run. Tissue samples were also collected from the same 60 fish for nDNA and mtDNA analysis.

*Lake sampling.*—A total of 71 trout *Oncorhynchus* spp., caught directly from the lake via gill nets, were sampled for liver, muscle, and eye tissue in 2000. Six gill nets were used, being set around the lake in a range of habitats, varying in depth and vegetative cover. Each gill net contained six different mesh-size panels ranging from 2.0 to 7.5 cm. Every third trout captured in the gill net (regardless of phenotype) was sampled for genetic analysis. Sampled fish ranged from 157 to 554 mm.

*Henrys Lake tributaries.*—In 1998, fry traps were installed in the three largest tributaries (Tyghee Creek, Duck Creek, and Howard Creek; Fig-

ure 1) to capture emerged fry during their migration from spawning gravels to the lake. In each of these tributaries, 10 fry were sampled weekly for a 6-week period. Fry sampling was important because it ensured the collection of individuals that were produced in the tributary rather than from the hatchery. The identification of any rainbow trout alleles within these samples would indicate wild introgressive hybridization.

In 1999 three additional tributaries were sampled (Timber Creek, Hope Creek, and Wild Rose Creek; Figure 1) using a backpack electroshocker. Sampling was conducted over the entire length of the tributary (Wild Rose Creek) or until private property prevented further access. Both fry (age 0) and sub-adults ( $>$ age 0) were collected.

#### Genetic Analysis

*Protein electrophoresis.*—Ten protein loci were examined using horizontal starch gel electrophoresis to determine the frequency of rainbow trout and cutthroat trout alleles in the 120 adult cutthroat trout and hybrid samples collected from Hatchery Creek in 1998 and 1999 and from 71 adult cutthroat trout and hybrids sampled from the lake in 2000 (Table 2). The protocol provided by Aebersold et al. (1987) was used to make the starch gels, gel buffers, and gel stains needed for this analysis; nomenclature for loci and alleles followed Shaklee et al. (1990).

Alleles were scored on each gel by each of two individuals and were identified by their electrophoretic mobility, as designated by Leary et al. (1987). Two loci, aconitate hydratase (*mAH-3\**) and phosphoglucomutase (*PGM-1\**), were scored only for the presence or absence of a rainbow trout allele. The heterozygous condition  $100^*/73^*$  is not distinguishable from the homozygous condition  $100^*/100^*$  at *mAH-3\**. At *PGM-1\**, the cutthroat trout allele is a null allele (not detectable) and heterozygotes cannot be identified.

*Extraction of DNA.*—Mitochondrial and nuclear DNA were extracted from all samples using methods described by Paragamian et al. (1999), as adapted from protocols by Sambrook et al. (1989) and Dowling et al. (1990).

*Amplification of nuclear DNA gene regions.*—Total genomic DNA was isolated (Baker et al. 2002) from each sample and amplified using the polymerase chain reaction (PCR) with primers specific for three nuclear gene regions: recombination activation gene (*RAG 3'*), *ikaros* gene (*IK*), and *protoncogene 53* (*p53*). The primer sets used to amplify the nuclear loci examined in this study

TABLE 2.—Enzymes, enzyme numbers (IUBMBNC 1992), and loci examined in samples collected in 1998, 1999, and 2000. Aconitate hydratase (*mAH-3\**) was scored phenotypically as rainbow trout (RBT) or cutthroat trout (YSC). For the phosphoglucosmutase locus (*PGM-1\**), the cutthroat allele is a null allele (not detectable). Individuals were scored as either rainbow or cutthroat trout because heterozygotes cannot be identified.

Enzyme	Enzyme number	Locus	Mobility of RBT locus	Mobility of YSC locus	Tissue	Buffer
Aconitate hydratase	4.2.1.3	<i>mAH-3*</i>	100*	73*	Muscle	CAME6.8
Aconitate hydratase	4.2.1.3	<i>mAH-4*</i>	100*	110*	Muscle	CAME6.8
Adenosine deaminase	3.5.4.2	<i>ADA-2*</i>	100*	110*	Eye, muscle	CAME6.8, Tris-gly
Creatine kinase	2.7.3.2	<i>CK-C1*</i>	100*	110/90*	Eye	CAME6.8
Dipeptidase	3.4.-.-	<i>PEPA*</i>	100*	101*	Muscle	Tris-gly
Isocitrate dehydrogenase	1.1.1.42	<i>sIDHp-1, 2*</i>	100/114/71/40*	100/71*	Liver, eye	CAME6.8
Malate dehydrogenase	1.1.1.37	<i>mIDHp-1*</i>	100*	-75*	Muscle	CAME6.8
Malic enzyme	1.1.1.44	<i>sMEP-2*</i>	100*	110/90*	Liver	CAME6.8
Phosphoglucosmutase	5.4.2.2	<i>PGM-1*</i>	100/Null*	Null*	Muscle, liver	CAME6.8, Tris-gly
Tripeptide aminopeptidase	3.4.11.4	<i>PEPB-1*</i>	100*	135*	Muscle	Tris-gly

were originally developed to identify diagnostic RFLP patterns to distinguish coastal cutthroat trout *O. c. clarki* from steelhead *O. mykiss* for investigations involving hybridization and introgression between the two species (Baker et al. 2002). In addition to screening both coastal cutthroat trout and steelhead, 12 Yellowstone cutthroat trout samples (unidentified source) were also amplified with these nuclear loci and digested with a suite of restriction enzymes to identify subspecies specific RFLP patterns (polymorphisms; Baker et al. 2002). From this screening, one restriction enzyme for each of these three loci was identified as producing distinctive polymorphisms between Yellowstone cutthroat trout and steelhead (Table 3). To ensure that these polymorphisms represented fixed allelic differences between rainbow trout and Yellowstone cutthroat trout, Yellowstone cutthroat trout from Yellowstone Lake ( $N = 60$ ) and Tyghee Creek ( $N = 60$ ), and rainbow trout from the Hayspur Hatchery ( $N = 60$ ), were screened with the same three nuclear loci/restriction enzyme combinations. Alleles were visualized as band patterns

(restriction fragments) when stained with ethidium bromide and fluoresced under ultraviolet light.

*Amplification of the mitochondrial DNA gene region.*—The NADH dehydrogenase 2 (ND2) gene region of the mitochondrial genome was amplified using PCR. Primers specific for the ND2 region—(461) 5'-ACC CCG CCT GTT TAC CAA AAA CAT-3' and (562) 5'-TAA GCT ATC GGG CCC ATA CC-3'—were purchased from LGL Ecological Genetics (Bryan, Texas). The ND2 mtDNA gene region was amplified in a 40  $\mu$ L reaction consisting of 0.5–3.0  $\mu$ L DNA extract (approximately 2.5 ng/ $\mu$ L), 4.0  $\mu$ L 10 $\times$  buffer (Perkin Elmer), 4.0  $\mu$ L MgCl<sub>2</sub>, 3.2  $\mu$ L bovine serum albumin, 1.0  $\mu$ L DMSO, 4.0  $\mu$ L of each primer, 3.2  $\mu$ L 10.0 mM deoxynucleotide triphosphates (10mM each of dATP, dCTP, dGTP, and dTTP), 0.15  $\mu$ L Perkin-Elmer Taq polymerase, and 13.45–15.95  $\mu$ L dH<sub>2</sub>O.

*Restriction enzyme digestion of amplification products.*—Amplification products were digested with up to eight restriction enzymes (*Dde-I*, *Dpn-II*, *Hae-III*, *Hha-I*, *Hinf-I*, *Mse-I*, *Msp-I* and *Rsa-I*).

TABLE 3.—Loci, accession number, 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 (RBT) and Yellowstone cutthroat trout (YSC).

Locus	Accession number <sup>a</sup>	Total size (bp)	Enzyme <sup>b</sup>	RBT expected sizes of digest fragments <sup>c</sup>	YSC expected sizes of digest fragments <sup>c</sup>
Recombination activation gene (RAG 3')	U73750	1,013	<i>Dde-I</i>	544/469	(1) 544/286/183 (2) 324/286/220/183
Ikaros gene (IK)	U92199	813	<i>Hinf-I</i>	813	520/293
Protoncogene 53 (p53)	M75145	481	<i>Alu-I</i>	(1) 190/140/100/51 (2) 330/100/51 <sup>d</sup>	330/100/51

<sup>a</sup> GenBank genetic sequence database.

<sup>b</sup> All enzymes ordered through New England Biolabs. *Dde-I* (5' A G<sup>^</sup>C T 3'), *Hinf-I* (5' G<sup>^</sup>A N T C 3'), *Alu-I* (5' A G<sup>^</sup>C T 3').

<sup>c</sup> Estimated from 1 kb ladder and published sequences.

<sup>d</sup> Digest fragments (indicative of a particular allele) not observed without putative steelhead (Baker et al. 2002).

Digests were electrophoresed on 3% agarose gels with tris-acetate-EDTA buffer or 6% acrylamide gels with tris-borate-EDTA and visualized as band patterns (fragments) when stained with ethidium bromide and fluoresced under ultraviolet light.

## Results

### Genetic Analysis of Reference Populations

**Nuclear DNA results.**—Results supported the use of both the IK/*Hinf-I* and RAG 3'/*Dde-I* markers as diagnostic markers between the two taxa. Both demonstrated fixed allelic differences between rainbow trout from the Hayspur Hatchery and Yellowstone cutthroat trout from Yellowstone Lake and Tyghee Creek (Figure 2). Rainbow trout samples collected from the Hayspur Hatchery were fixed for the \**a* allele at the IK locus. Yellowstone cutthroat trout from the two reference populations were fixed for the \**b* allele at the IK locus. The \**a* allele at the RAG 3' locus was also fixed in rainbow trout from the Hayspur Hatchery and was absent in Yellowstone cutthroat trout from the two reference populations. The \**b* and \**c* alleles (previously observed at the RAG 3' locus within Yellowstone cutthroat trout) were observed within the samples from Yellowstone Lake and Tyghee Creek, although at significantly different frequencies ( $\chi^2 = 85.84$ ,  $P < 0.05$ ) between the two locations. Alleles \**b* and \**c* were present in Tyghee Creek samples at 92.5% and 7.5%, respectively. Alleles \**b* and \**c* were present in Yellowstone Lake samples at 35.0% and 65.0%, respectively.

The p53/*Alu-I* marker demonstrates fixed allelic differences between steelhead (100% allele \**a*) and Yellowstone cutthroat trout (100% allele \**b*; Baker et al. 2002). However, it proved to be an imperfect diagnostic marker between rainbow trout from the Hayspur Hatchery and Yellowstone cutthroat trout (Figure 2). Alleles \**a* and \**b* were observed within the rainbow trout samples, each at a frequency of 50.0%. The \**b* allele was fixed in all Yellowstone cutthroat trout samples from Yellowstone Lake and Tyghee Creek. Based on these results, we decided that the p53/*Alu-I* marker would be screened together with the RAG 3'/*Dde-I* and IK/*Hinf-I* markers on all Yellowstone cutthroat trout samples from within the Henry's Lake drainage basin.

**Mitochondrial DNA results.**—Amplification products were digested with eight restriction enzymes (*Dde-I*, *Dpn-II*, *Hae-III*, *Hha-I*, *Hinf-I*, *Mse-I*, *Msp-I*, and *Rsa-I*) on all samples collected from Hayspur Hatchery in 1999, Tyghee Creek in 1999,

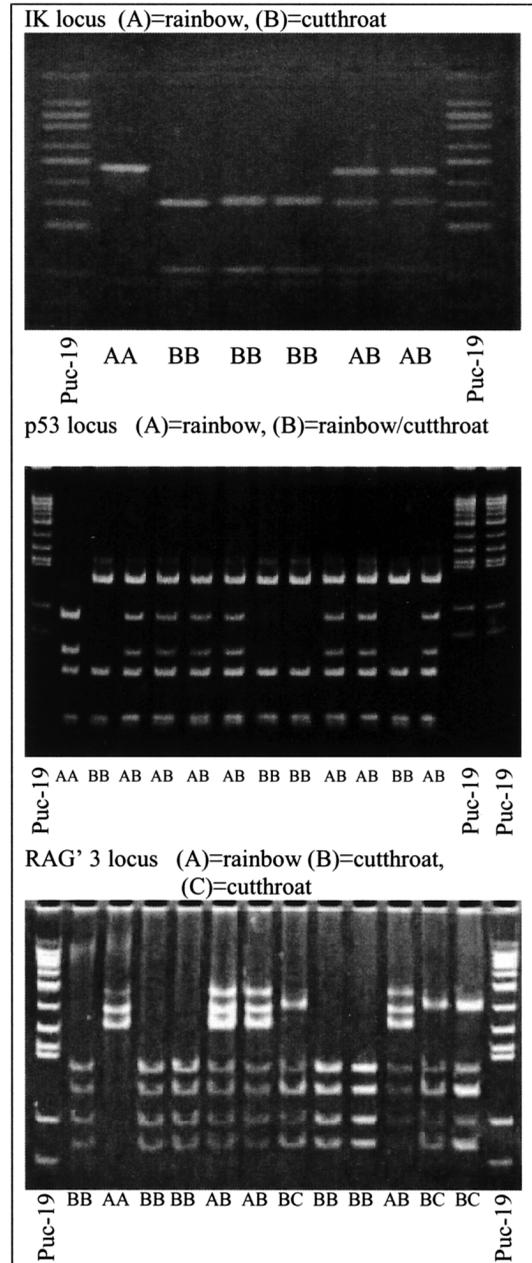


FIGURE 2.—Photographs of three gels illustrating diagnostic alleles (bands) between Yellowstone cutthroat trout and rainbow trout at three nuclear loci: IK locus, digested with *Hinf-I* (3% agarose gel; top panel); p53 locus, digested with *Alu-I* (6% acrylamide gel; middle panel); and RAG 3' locus, digested with *Dde-I* (6% acrylamide gel; bottom panel).

TABLE 4.—Composite haplotypes (simple haplotypes in parentheses) and their frequencies (number [no.] and percent) observed among sample location cutthroat and hybrid trout. Simple haplotypes are combined fragment length patterns from the eight-enzyme digest of NADH dehydrogenase region of the mitochondrial DNA.

Sample location <sup>a</sup> and year	N*	Composite (and simple) haplotypes											
		RBT-01 (AACACAAA)		RBT-02 (CACAACAA)		RBT-03 (AACACCAA)		YSC-04 (BACABBAB)		YSC-05 (BACABBAC)		YSC-06 (BACABBAD)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
HH, 1999	60	26	0.433	30	0.500	4	0.067	0		0		0	
TYC, 1998	60	0		0		0		0		0		60	1.000
YL, 2000	60	0		0		0		26	0.433	34	0.567	0	
HAC, 1998	60	1	0.016	0		0		6	0.100	10	0.167	43	0.717
HAC, 1999	60	0		0		0		9	0.150	4	0.067	47	0.783
HLG, 2000	70	1	0.014	0		0		10	0.143	7	0.100	52	0.743
HWC, 1988	60	0		0		0		3	0.050	15	0.250	42	0.700
DC, 1998	60	0		0		0		8	0.133	30	0.500	22	0.367
TAC, 1998	60	0		0		0		6	0.100	28	0.467	26	0.433
TIC, 1999	21	0		0		0		0		1	0.048	20	0.952
WRC, 1999	33	1	0.030	0		0		3	0.091	4	0.121	25	0.758
HOC, 1999	10	0		0		0		0		2	0.200	8	0.800

<sup>a</sup> DC = Duck Creek; HAC = Hatchery Creek; HH = Hayspur Hatchery; HLG = Henry's Lake (gill net); HOC = Hope Creek; HWC = Howard Creek; TAC = Targhee Creek; TIC = Timber Creek; TYC = Tyghee Creek; WRC = Wild Rose Creek; and YL = Yellowstone Lake.

and Yellowstone Lake in 2000. Six mitochondrial DNA haplotypes were observed among the samples (Table 4). Three haplotypes were found in samples of rainbow trout collected from the Hayspur Hatchery (designated RBT-01, RBT-02, and RBT-03). Three different haplotypes were found in samples of Yellowstone cutthroat trout from the reference populations of Tyghee Creek and Yellowstone Lake (designated as YSC-04, YSC-05, and YSC-06). Haplotype differences between rainbow trout from the Hayspur hatchery and Yellowstone cutthroat trout from Tyghee Creek and Yellowstone Lake were the result of polymorphic differences in the *Dde-I*, *Hinf-I*, *Mse-I*, and *Rsa-I* digests. Among haplotypes YSC-04, YSC-05, and YSC-06, variation was due to polymorphisms only observed in the *Rsa-I* restriction enzyme digest. The remaining enzymes produced monomorphic patterns. Therefore, samples collected from the six additional Henry's Lake tributaries were digested only with the *Rsa-I* enzyme. However, samples that exhibited the rainbow trout polymorphism when digested with *Rsa-I* were also digested with the seven additional restriction enzymes to identify the rainbow trout haplotype.

Frequency and diversity of the three cutthroat trout haplotypes (YSC-04, YSC-05, and YSC-06) differed between sample locations (Table 4). Haplotypes YSC-04 and YSC-05 were the only haplotypes observed in samples from Yellowstone Lake, and both were observed in relatively equal frequencies, 43.3% and 56.7%, respectively. Haplotypes YSC-04 and YSC-05 were not observed

in samples collected from Tyghee Creek. Haplotype YSC-06 was fixed among samples from Tyghee Creek.

#### Genetic Analysis of Samples from Henrys Lake Basin

**Mitochondrial DNA results.**—Three samples collected within the Henry's Lake basin were identified as having rainbow trout mtDNA: Hatchery Creek 1998 ( $N = 1$ ), Henry's Lake gill net 2000 ( $N = 1$ ), and Wild Rose Creek 1999 ( $N = 1$ ; Table 4). The remaining samples ( $N = 358$ ) had Yellowstone cutthroat trout mtDNA (Table 4).

All three cutthroat trout haplotypes were observed among samples collected from Henrys Lake and its tributaries. Haplotype YSC-06 was the most commonly (>70%) observed haplotype in all sample locations within the Henrys Lake basin, except Duck Creek (36.7%) and Targhee Creek (43.3%). Haplotype YSC-05 was the most common haplotype observed in Duck (50.0%) and Targhee (46.7%) creeks. Haplotype YSC-04 was the least common haplotype observed at all sample locations within the Henrys Lake basin.

**Phenotypic identification results.**—The phenotypic subspecific and hybrid identifications (Figures 3, 4) although not 100% accurate, did yield a high level of accuracy in the judgments that were most important to spawning decisions. Genetic analysis (10 diagnostic allozyme loci, 3 diagnostic nuclear loci, mtDNA locus) failed to detect rainbow trout alleles in a high percentage of fish phenotypically identified as pure Yellowstone cut-

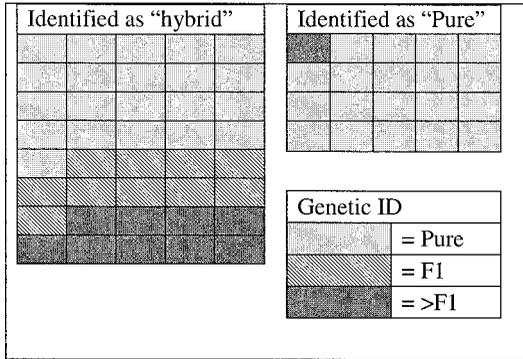


FIGURE 3.—The 1998 Hatchery Creek results: genetic identification of 40 putative (previously identified phenotypically) hybrids (female Yellowstone cutthroat trout  $\times$  male rainbow trout) and 20 putatively pure cutthroat trout.

throat trout (19 of 20 [95.0%] in 1998). The single individual from the 1998 sample that was detected with rainbow trout alleles was homozygous for Yellowstone cutthroat trout alleles at some loci and heterozygous at others, a genotype indicative of a  $>F_1$  hybrid. Of the 60 fish phenotypically identified as pure Yellowstone cutthroat trout in 1999, genetic analysis indicated that only 4 possessed rainbow trout alleles. All four had genotypes indicative of multiple backcrossed hybrids, with each observed with only 1 rainbow trout allele out of the 24 (4.2%) alleles examined.

Of the 40 fish phenotypically identified as hybrids in 1998, our analysis only confirmed 19 (47.5%) fish as  $\geq F_1$  hybrids. Rainbow trout alleles were not detected in the remaining 21 fish. Thus, these 21 fish should have been included in the phenotypic screening as part of the pure Yellowstone cutthroat group.

Of the 19 genetically confirmed hybrids, 10 had genotypes indicative of  $F_1$  hybrids (heterozygous with one cutthroat trout allele and one rainbow trout allele at each locus) and 9 had genotypes indicative of  $F_{>1}$  hybrids. Of these nine fish, eight possessed genotypes that were unlikely to have been produced from matings between  $F_1$  hybrids or matings between  $F_1$  hybrids and cutthroat trout. Rather, they exhibited genotypes indicative of multiple backcross hybrids ( $F_{>2}$ ). For example, one sample had rainbow trout mtDNA but was homozygous for cutthroat trout alleles at all diagnostic nDNA loci. Two fish were homozygous for rainbow trout alleles at one locus, suggesting that they were not offspring of a  $F_1$  and cutthroat trout mating. Three fish were only observed with

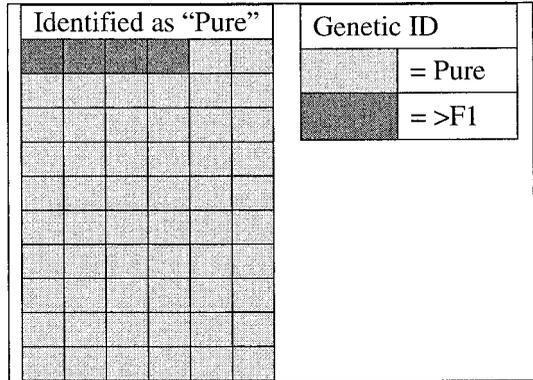


FIGURE 4.—The 1999 Hatchery Creek results: genetic identification of 60 putatively (previously identified phenotypically) pure cutthroat trout.

only 1 rainbow trout allele of 24 examined, a genotype also not indicative of a product of an  $F_1$  and cutthroat trout mating or an  $F_1 \times F_1$  mating. Two fish were homozygous for cutthroat trout alleles at one loci and heterozygous at all remaining loci.

*Henrys Lake gill netting in 2000.*—Samples were genetically analyzed (allozyme loci *mAH-3* and *mAH-4* were excluded from analysis due to poor tissue quality and difficulties in scoring gels) to determine the extent of rainbow trout introgression in an admixture sample of fish from the lake. Of the 71 samples analyzed, 15 (21.1%) had genotypes indicative of  $F_1$  hybrids (heterozygous at each locus examined), 45 (63.4%) had genotypes indicative of pure cutthroat trout (no rainbow trout alleles), and 11 (15.5%) had genotypes indicative of  $F_{>1}$  hybrids (including 9 with genotypes indicative of  $F_{>2}$  hybrids and 1 with mtDNA of rainbow trout).

*Rainbow Trout Introgression within Tributaries*

*Duck Creek, Howard Creek, and Targhee Creek, 1998.*—Rainbow trout alleles were identified in samples from all three tributaries. Individuals with variable genotypes at the diagnostic loci (homozygous for cutthroat trout alleles at some loci, heterozygous at others) were found in each tributary, indicating crosses among hybrids or hybrids and Yellowstone cutthroat. Rainbow trout alleles were found in the highest number of samples from Targhee Creek (15/60 = 25.0%), followed by Howard Creek (8/60 = 13.3%) and Duck Creek (1/60 = 1.7%).

*Timber Creek, Wild Rose Creek, and Hope Creek, 1999.*—Samples collected in Timber, Wild Rose,

and Hope creeks consisted mainly of subadults (>age 0). Subadult sampling is complicated by the fact that the presence of hybrids within these tributaries may be the result of straying of hatchery-produced  $F_1$  hybrids from Hatchery Creek, rather than natural reproduction. Subadult hybrids were identified in all three tributaries (Timber Creek: 10/21 = 47.6%; Wild Rose Creek: 11/34 = 32.4%; and Hope Creek: 4/9 = 44.4%), but only one sample, Wild Rose Creek, was identified as  $F_{>1}$ , having mtDNA of rainbow trout but no rainbow trout nuclear alleles. The remaining 24 samples were heterozygous at both the IK and RAG 3' loci, genotypes indicative of  $F_1$  hybrids.

Fry were sampled in both Wild Rose ( $N = 8$ ) and Timber creeks ( $N = 4$ ). Rainbow trout nuclear alleles were not identified in any of these samples.

### Discussion

The expected level of introgression in Henrys Lake after 30 years of  $F_1$  hybrid (female Yellowstone cutthroat trout  $\times$  male rainbow trout) introductions depends greatly on estimates of variables controlling gene flow within the lake. Three important variables potentially affecting gene flow in Henry's Lake include (1) the accuracy of hatchery managers in distinguishing returning  $F_1$  hybrids from returning pure cutthroat trout when selecting broodstock, (2) the number of  $F_1$  hybrids that stray and successfully spawn among themselves or with wild Yellowstone cutthroat trout, and (3) the relative contributions of wild and hatchery fish to overall recruitment in Henrys Lake.

If managers accurately selected pure cutthroat trout when selecting broodstock, and  $F_1$  hybrids never strayed into other tributaries to spawn with wild cutthroat trout, genetic sampling should indicate only  $F_1$  hybrids and pure cutthroat trout at frequencies similar to those stocked. However, if managers have been ineffective in excluding  $F_1$  hybrids as broodstock and/or many hatchery-produced  $F_1$  hybrids strayed and successfully spawned with wild cutthroat, and recruitment from wild spawning greatly contributed to annual reproduction in the lake, then very few fish should be identified as genetically pure.

Results, however, support neither the interpretation of complete avoidance of genetic introgression, nor that of extensive introgression resulting from introduction of rainbow trout genes into a randomly reproducing population. Observed introgression levels were lower than might be expected following 30 years of hybrid stocking. In a sample of 71 fish taken directly from the lake, 15 (21.1%)

had genotypes indicative of  $F_1$  hybrids and 45 (63.4%) had genotypes indicative of pure cutthroat trout. However, 11 fish (15.5%) were identified as  $F_{>1}$ , and 9 of these had genotypes indicative of  $F_{>2}$  hybrids.

The fact that rainbow trout alleles were not randomly distributed throughout the samples is probably the result of all of the following: (1) high phenotypic selection accuracy in eliminating  $F_1$  hybrids from broodstock at the hatchery, (2) low levels of straying of  $F_1$  hybrids into other tributary streams, and (3) low levels of natural recruitment in wild Yellowstone cutthroat trout and hybrid trout in Henrys Lake. Tributary evaluations conducted in the fall of 2000 still suggest low natural recruitment. Only 138,640 fry were estimated to have emigrated from Duck, Howard and Targhee creeks combined (IDFG, unpublished data). This is only 3–4% of estimated historical annual recruitment to Henrys Lake (Rohrer 1980).

Present levels of rainbow trout introgression are most likely the product of past rainbow trout introductions (evident by the observation of three samples with rainbow trout mtDNA) and limited spawning of hatchery-produced  $F_1$  hybrids among themselves and with wild cutthroat trout within Henrys Lake tributaries. Current introgression levels are maintained, however, by (1) the inability of managers to phenotypically identify and exclude as broodstock individuals with low levels of rainbow trout introgression and (2) the intermittent reproductive success of straying, hatchery-produced  $F_1$  hybrids and naturally reproducing  $F_{>1}$  hybrids (evident by the identification of  $F_{>1}$  hybrids within tributary fry samples).

Clearly, the genetic integrity of Yellowstone cutthroat trout in Henrys Lake has been impacted by management decisions that supported stocking the lake with rainbow trout and  $F_1$  hybrids as well as Yellowstone cutthroat trout from other populations. Irving (1954) argued that a small portion, if any, of the existing cutthroat trout population in the lake was descended from the original stock because Yellowstone cutthroat trout from Yellowstone Lake and Gold Creek, Idaho, had been introduced. Mitochondrial DNA RFLP analysis suggests that the prevalence of two mtDNA haplotypes identified in the population (YSC-04 at 10.4% and YSC-05 at 23.4%) may be the result of introductions of cutthroat trout from Yellowstone Lake.

Past management decisions that focused on the goals of providing two economically important recreational fisheries (hybrid and cutthroat trout)

are understandable and should be judged successful within their own scope. However, these management decisions have a further legacy, which greatly complicates the current management goal of preserving pure, native populations of Yellowstone cutthroat trout in Idaho. The current levels of rainbow trout introgression identified in this study cannot be ignored, nor can they be easily or inexpensively remedied. The significance of these introgression levels will have to be considered carefully by IDFG managers relative to a variety of issues, including possible future ESA listings, possible contamination of other pure cutthroat trout populations through continued stocking programs, and the expense of eradication efforts.

It is important not to underestimate the challenge of reducing current levels of rainbow trout introgression within Henrys Lake. Elimination of all rainbow trout alleles within the Henrys Lake cutthroat trout population is highly unlikely, even with new introductions of cutthroat trout from a genetically pure population. Although replacing the current phenotypic methodology for selecting broodstock with a genetic screen could eliminate individuals with high levels of rainbow trout introgression, it would not be possible to guarantee the elimination of all hybrids as broodstock. The best that a genetic screen could do would be to eliminate hybrids with rainbow trout alleles at the diagnostic loci currently available for examination.

The sterilization program of all hatchery-produced  $F_1$  hybrids begun in 1998 should greatly reduce the introduction of new rainbow trout alleles into the Henrys Lake system. However, it is uncertain what impact this program will have on future levels of introgression, and it offers no possibility for the complete elimination of residual rainbow trout introgression.

The above remedial actions do not seem warranted by this study's findings, which instead support the continuation of current programs in light of low but stable levels of rainbow trout introgression in the Henrys Lake cutthroat trout population. It is important to note that the stability of current levels of introgression depends heavily on continued low levels of natural recruitment into Henrys Lake. This would have to be monitored in lieu of the implementation of any of the more extreme management alternatives mentioned above.

One last implication of the phenotypic methods for selecting broodstock currently used at the Henrys Lake Hatchery needs to be considered. This study demonstrated that rainbow trout alleles were

not detected in 21 of the 40 putative hybrids examined genetically; these 21 fish should have been included with the other putative cutthroat trout. Managers should keep in mind that selecting for specific phenotypes in an attempt to exclude hybrids may have the effect of reducing the genetic variability of the population returning to Hatchery Creek. A physical mark (e.g., an adipose fin clip) on all hatchery-produced cutthroat trout would eliminate the need for additional phenotypic selection.

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