FISHERY RESEARCH

GENETIC INVESTIGATIONS OF BONNEVILLE CUTTHROAT TROUT IN THE BEAR RIVER DRAINAGE, IDAHO:

DISTRIBUTION OF MITOCHONDRIAL DNA DIVERSITY AND RAINBOW TROUT HYBRIDIZATION AND INTROGRESSION

Final Report

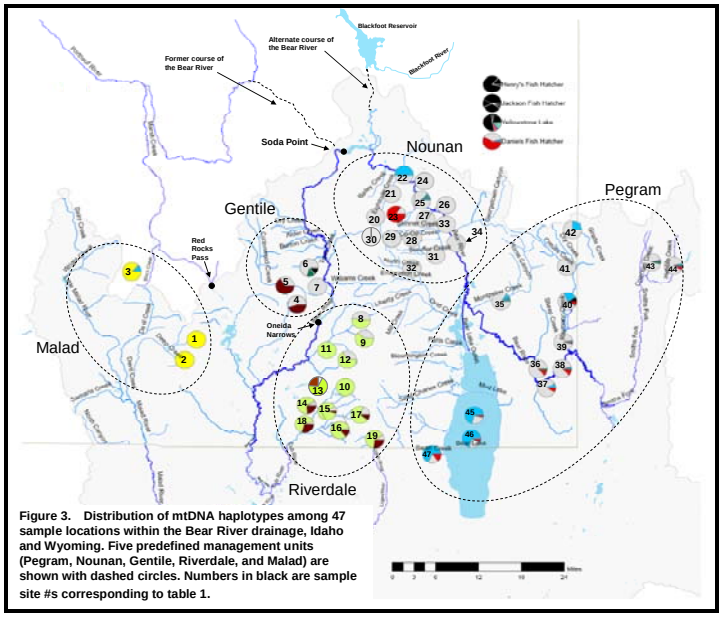

Figure 3. Distribution of mtDNA haplotypes among 47 sample locations within the Bear River drainage, Idaho and Wyoming. Five predefined management units (Pegram, Nounan, Gentile, Riverdale, and Malad) are shown with dashed circles. Numbers in black are sample site #s corresponding to table 1.

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Genetic Investigations of Bonneville Cutthroat Trout in the Bear River Drainage, Idaho: Distribution of Mitochondrial DNA Diversity and Rainbow Trout Hybridization and Introgression

FY2006 Final Report

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ABSTRACT

Due to substantial reductions in the distribution and abundance of Bonneville cutthroat trout *Oncorhynchus clarkii utah* in the Bear River drainage in Idaho, considerable attention has been focused on better understanding the species' demographic, life history, and genetic characteristics to assist with conservation and restoration purposes. This study focused on two areas of population genetics. To assess the impacts from past nonnative hatchery trout stocking on intraspecific and interspecific hybridization/introgression we used a combination of diagnostic nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) markers. To assess genetic population structure we sequenced a highly variable mtDNA gene region that had previously revealed genetic variation within and between populations of cutthroat trout in the Snake River and Bear River drainages. Based on comparisons to hatchery reference populations, we found little evidence of intraspecific hybridization. Only three nonnative mtDNA haplotypes were found among the 750 samples analyzed. Evidence of interspecific hybridization was detected throughout much of the Bear River drainage, but many sites exhibited low levels of rainbow trout *O. mykiss* introgression (<3%). The confirmation of naturally reproducing rainbow trout populations and the identification of F_1 hybrids indicates hybridization is an ongoing problem. Management policies implemented to stock only sterile rainbow trout and to remove existing nonnative rainbow trout populations should continue to prevent the further spread and increase of introgression throughout the drainage. Patterns of genetic structuring generally supported previously defined management units indicating evidence of both current and historical reproductive isolation of groups of populations throughout the drainage. Our results were consistent with previous studies that have demonstrated that cutthroat trout in the Bear River drainage share a more recent common ancestor with Yellowstone cutthroat trout in Idaho than with populations of Bonneville cutthroat trout in the central and southern portions of their range in Utah. Managers will have to carefully consider these findings when considering taxonomic assessments, prioritizing populations for conservation and management purposes, and identifying suitable populations for translocations, reintroductions, and broodstock development programs.

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INTRODUCTION

Bonneville cutthroat trout *Oncorhynchus clarkii utah* are one of 14 described cutthroat trout subspecies native to the western United States (Behnke 1992). Similar to other cutthroat trout subspecies, the distribution and abundance of Bonneville cutthroat trout throughout their native range has been reduced from historical levels by habitat alterations (primarily from irrigation diversions and dams), overfishing, and the introduction of nonnative trout, which have led to hybridization and competition. Bonneville cutthroat trout were petitioned for listing as a threatened species under the Endangered Species Act (ESA) twice since 1979 (45 FR 19857 and 61 FR 7457), and were briefly classified as a category 1 candidate species in 1985 (50 FR 37958). Although the USFWS determined in 2001 that a listing for Bonneville cutthroat trout was not warranted (66 FR 51362), Bonneville cutthroat trout are still recognized as a sensitive species in Idaho, Utah, and Wyoming, and considerable attention has been focused on research to better understand the species' demographic, life history, and genetic characteristics to assist with conservation and restoration purposes (UDWR 2000).

One research area of particular interest has focused on understanding the evolutionary history of Bonneville cutthroat trout. It was originally proposed that all cutthroat trout inhabiting the Bonneville basin originated from an ancestral Yellowstone cutthroat trout *O. clarkii bouvieri* from the upper Snake River basin (Behnke 1992). This theory was based on evidence that the Bear River was historically a tributary to the Snake River, and was diverted as recently as 30,000 years ago to the Bonneville basin, a result of basalt flows during the late Pleistocene (Behnke 1992). Desiccation of ancient Lake Bonneville was believed to have then fragmented Bonneville cutthroat trout into three geographic areas that make up the species' current range, including the Bear River basin, the Snake Valley region on the Utah-Nevada border, and the main Bonneville basin (Loudenslager and Gall 1980; Martin et al. 1985; Behnke 1992). All cutthroat trout found in these areas are presently characterized as "Bonneville cutthroat trout" for conservation and management purposes, including ESA considerations (USFWS 2001).

This theory of a single, recent invasion of cutthroat trout into the Bonneville basin has been refuted with genetic and fossil evidence (Smith et al. 2002). Research indicates that the current distribution of cutthroat trout in the Bonneville basin is a result of at least two independent colonization histories. Populations of Bonneville cutthroat trout found outside the Bear River drainage have likely been in the Bonneville basin since the Pliocene, and rather than originating from Yellowstone cutthroat trout, represent a sister species to Yellowstone cutthroat trout, deriving from a common cutthroat trout ancestor that gained access to the Snake River, Lahontan, and Bonneville basins sometime during the last three million years. Cutthroat trout in the Bear River drainage, however, are much more similar genetically to Yellowstone cutthroat trout than Bonneville cutthroat trout outside the Bear River drainage, reflecting the historical connection between the Bear River and Snake River drainages (Martin et al. 1985; Smith et al 2002).

Very little is actually understood regarding the historical connection and genetic relationships of cutthroat trout between the Bear River and Snake River drainages. A review of the hydrogeological history of these two drainages reveals a complicated account of drainage diversion, isolation, and reconnection, allowing multiple opportunities for population isolation, divergence, and episodes of secondary contact during the last 500,000 years (Scott et al. 1982; Bouchard et al. 1998; Link et al. 1999). Part of the purpose of this research project is to help describe and resolve some of the complicated evolutionary history and contemporary geographical patterns of genetic variation within "Bear River" cutthroat trout.

Assessing the taxonomic status, as well as the conservation status of cutthroat trout in the Bear River, is complicated, however, by the fact that the drainage (including Bear Lake) has been stocked extensively with nonnative Yellowstone cutthroat trout and rainbow trout *O. mykiss*. Stocking began in the drainage more than 100 years ago and led to the speculation by some that most pure Bonneville cutthroat trout populations may have been replaced by hybrids (Popov and Low 1953; Cope 1955; McConnell et al. 1957; Holden et al. 1974; Duff 1988). Some meristic, mitochondrial DNA (mtDNA) and allozyme data in the 1980s suggested that pure populations still existed in the Bear River drainage (Duff 1988). However, no genetic markers have successfully distinguished cutthroat trout in the Bear River from Yellowstone cutthroat trout, inhibiting the ability to assess intraspecific hybridization, and no studies have previously used fixed diagnostic nuclear DNA (nDNA) loci to evaluate hybridization and introgression from rainbow trout.

In this study, we employed mtDNA sequencing to assess both phylogenetic population structure and intraspecific hybridization. A segment of the NADH Dehydrogenase 2 (ND2) gene region was chosen for sequencing because previous RFLP analyses on this gene region had yielded polymorphisms within Bonneville cutthroat trout and Yellowstone cutthroat trout populations (Toline et al. 1999). In addition, the ND2 gene region has been previously sequenced on Yellowstone cutthroat trout populations throughout their range in Idaho (IDFG, unpublished data) and Wyoming (Novak et al. 2005; IDFG unpublished data). Hybridization and introgression from rainbow trout was assessed using six diagnostic nDNA loci (Ostberg and Rodriguez 2002, 2004). These loci have demonstrated their utility in assessing rainbow trout hybridization/introgression in Yellowstone cutthroat trout (Meyer et al. 2006; IDFG unpublished data). Information gained from this study should assist future conservation and management measures for cutthroat trout in both the Snake and Bonneville River basins

OBJECTIVES

1. Assess intraspecific and interspecific hybridization and introgression in cutthroat trout populations in the Bear River drainage.

2. Assess mtDNA diversity and distribution in cutthroat trout populations in Bear River drainage and Snake River drainage.

METHODS

Sampling and DNA Extraction

During 1998-2005, IDFG and U.S. Forest Service personnel collected ~1,200 cutthroat trout fin clips from 44 tributaries in the Bear River basin in Idaho and Wyoming. An attempt was made to sample fish at multiple sites within each tributary (low, medium, and high in the drainage) and a sample size goal of 30 per tributary was attempted, although many sites had less than 30 due to low population densities. Fish were sampled regardless of phenotypic identification and size. All fish sampled for genetic analyses were also photographed. Fin tissue was stored in 100%, nondenatured ethanol until DNA extraction. DNA was extracted using a salt-chloroform method described by Paragamian et al. (1999).

Mitochondrial DNA Amplification and Sequencing

The combined NADH Dehydrogenase 1 and 2 gene regions (3558 b.p.) were amplified following procedures described by Toline et al. (1999). Primers flanking the ND12 region, (ND12L) 5'-GCC TCG CCT GTT TAC CAA AAA CAT-3' at position number 2988 within the 16S rRNA, and (ND12H) 5'-CCG GCT CAG GCA CCA AAT AC-3' at position number 6547 within the CCO I gene, were purchased from Integrated DNA Technology (Coralville, Iowa). The ND12 mtDNA gene region was amplified in a 40 µl reaction consisting of 0.5-3.0 µl DNA extract (approx. 100 ng/µl), 4.0 µl 10X buffer (Perkin Elmer), 4.0 µl MgCl2, 3.2 µl BSA, 1.0 µl DMSO, 4.0 µl of each primer, 3.2 µl 10.0 mM dNTPs (10mM each of dATP, dCTP, dGTP, and dTTP), 0.15 µl Taq polymerase (Perkin-Elmer), and 13.45-15.95 µl dH2O. Polymerase chain reaction conditions consisted of an initial denaturing cycle of 94˚C for 3 minutes, followed by 39 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 1 minute, and extension at 72°C for 4 minutes, with a final extension at 72°C for 5 minutes.

Internal primer sequences were designed using the Primer3 (Rozen and Skaletsky 2000) program on the University of California's San Diego Supercomputer Center Biology Workbench (SDSC workbench; http://workbench.sdsc.edu) and the complete rainbow trout mtDNA sequence (Zardoya et al. 1995) available on Genbank (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov). Internal primer sequences are as follows: ND12 Reverse #1 (5'-CCTGATCCAACATCGAGGT -3'); ND12 Forward #2 (5'- ACCTCGATGTTGGATCAGG -3'); ND12 Reverse #2 (5'- GCGTACTCGGCTAGGAAAAA -3'); ND12 Forward #3 (5'-GGGCAGTGGCACAAACTATT -3'); ND12 Reverse #3 (5'- GGTATGGGCCCGAAAGCTTA -3'); ND12 Forward #4 (5'- TAAGCTTTCGGGCCCATACC -3'); ND12 Reverse #4 (5'-GGGTCGGGGATTTAGTTCAT -3'); and ND12 Forward #5 (5'- ATGAACTAAATCCCCGACCC -3'). Sequencing reactions were performed with a BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems) using the ND12 Forward #4 and ND12 Forward #5 internal primers. These primers were chosen because previous research in our lab indicated that this segment of the ND2 gene region is variable both within and between Bonneville cutthroat trout and Yellowstone cutthroat trout (IDFG, unpublished data). Sequenced products were cleaned using gel filtration plates (Edge Biosystems, Gaithersburg, Maryland) and were run out on a Prism 3730 DNA sequencer (Applied Biosystems). Sequences were edited using Sequencher (version 4.1.2, Gene Codes Corporation, Ann Arbor, Michigan) and the consensus sequences (approximately 600 b.p.) were aligned using the Clustal X program version 1.81 (Thompson et al. 1997) on the SDSC workbench.

Diagnostic Interspecific nDNA Locus Amplification and Electrophoresis

All samples were screened with six codominant nDNA markers (Occ34, Occ35, Occ36, Occ37, Occ38, and OM55) diagnostic between rainbow trout and cutthroat trout (Ostberg and Rodriguez 2002; 2004). All six loci were amplified together in one Polymerase Chain Reaction (PCR) amplification. Amplifications were performed in 10µl reaction volumes consisting of 5 µl of QIAGEN Multiplex PCR Master Mix® (final concentration 1X), 1 µl of primer cocktail (all forward and reverse primers at 100 µM concentration combined together), 3 µl of DNase/RNase free water, and 1 µl of DNA template (varying concentrations). Amplification product was diluted with 200 µl of water, with 1 µl of this dilution added to 0.5 µl of LIZ size standard and 30 µl of formamide, prior to electrophoresis on an ABI 3100 fragment analyzer (Applied Biosystems) and allele differentiation using GeneMapper® 3.5 software (Applied Biosystems). Reverse primer sequences, forward primer sequences with corresponding fluorescent labels, and allele sizes observed in cutthroat trout and rainbow trout are available from the authors upon request.

Interspecific and Intraspecific Hybrid Detection

Individual interspecific hybridization sample classification was based on composite nDNA and mtDNA genotypes following procedures outlined by Ostberg and Rodriquez (In press) and Kozfkay et al. (2007). Samples were classified as "cutthroat trout-like" if they were homozygous for cutthroat alleles at all loci, "rainbow trout-like" if they were homozygous for rainbow trout at all loci, and "hybrid" if they possessed a mixture of alleles from the two parental species. Hybrids were further classified into two categories: first-generation hybrids (F_1) if they were heterozygous at all loci, and later-generation hybrids (F_n) if they possessed a mix of heterozygous and homozygous loci. With six codominant nDNA loci, our probability of mistaking a more advanced backcross hybrid ($>F_1$) as an F_1 hybrid is less than 1% (Boecklen and Howard 1997). Hybridization levels at each site were reported as the number of hybrids detected out of the total number of samples analyzed. Introgression levels at each site was reported as the number of rainbow trout alleles observed in fish classified as cutthroat trout-like and $>F_1$ hybrids out of the total alleles examined. Introgression is the actual incorporation of genes from one taxa into the population of another through hybridization and backcrossing (Kearney 2005). Therefore, alleles from fish classified as rainbow trout-like or F_1 hybrid were not included in introgression estimates.

Assessments of intraspecific hybridization was accomplished by comparing mtDNA haplotypes observed at each site to haplotypes observed in three reference, out-of-basin, Yellowstone cutthroat trout populations that had been previously stocked in the Bear River drainage: Henrys Lake, Idaho; Jackson National Fish Hatchery, Wyoming; and Yellowstone Lake, Wyoming (IDFG historical stocking database, http://fishandgame.idaho.gov/apps/stocking/; USFWS 2001).

Phylogenetic and Population Genetic Analyses

Phylogenetic analyses included haplotypes that were identified as part of this study as well as haplotypes previously identified as part of rangewide evaluation of Yellowstone cutthroat trout in Idaho and Wyoming (IDFG, unpublished data). Genetic relationships among haplotypes were described and displayed two ways. We constructed a bootstrapped (10,000 replicates) neighbor joining tree (Kimura-2 parameter model) from pairwise sequence divergence estimates using the software program MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar et al. 2001). In addition, we also used the software program TCS Version 1.18 (Clement et al. 2000) to prepare a haplotype network using statistical parsimony (Templeton et al. 1992). We assessed the partitioning of genetic variation across two hierarchical levels (among population groups and within populations) using analysis of molecular variance (AMOVA) in Arlequin 2000 (Weir and Cockerham 1984). Population groups were based on five previously described management units (MU): Pegram, Nounan, Dam Complex, Gentile, Riverdale, and Malad (Teuscher and Capurso 2007). A fifth management unit was previously identified (Dam Complex) but we had no samples from that area to test. These management units reflect groups of populations that are believed to be isolated from other groups as a result of major drainage divides and/or dams (Teuscher and Capurso 2007). To assess genetic differentiation between groups, we also performed exact tests of population differentiation (Raymond and Rousset 1995) and calculated pairwise FST estimates using Arlequin 2000.

RESULTS

Intraspecific Hybridization

In comparisons to hatchery reference populations (Henrys Lake and Jackson National Fish Hatchery) and samples from Yellowstone cutthroat trout from Yellowstone Lake, three populations showed possible evidence of hybridization from past out-of-basin stocking. One sample from the Thomas Fork River (1999) exhibited a mtDNA haplotype (HAP8) observed in samples from Henrys Lake, Jackson National Fish Hatchery, and Yellowstone Lake (Table 1). This haplotype had also been observed in samples that we have sequenced previously from the upper Snake River and Yellowstone River drainages in Wyoming (IDFG, unpublished data; Table 2). Two samples from Montpelier Creek (2005) exhibited a mtDNA haplotype (HAP12) that was observed in samples from Henrys Lake. This same haplotype was also observed in one sample from Pearl Creek03. This haplotype appears to be a dominant haplotype in the Henrys Fork drainage, Idaho (Campbell et al. 2002) and we have observed it previously in samples from Tygee Creek, Idaho (Table 2). All of the remaining samples from sample sites within the Bear River drainage exhibited mtDNA haplotypes that were not found in hatchery reference populations or in samples from Yellowstone Lake.

Interspecific Hybridization

Hybrids between rainbow trout and cutthroat trout were identified in 18 of the 54 (33.3%) sample locations analyzed (Figure 1; Table 3). Hybridization (# of hybrids observed/total) ranged as high as 33.3% (3 sites). First-generation hybrids were identified in seven sample locations, indicating recent hybridization. Of these seven sites, five also contained samples with genotypes indicative of both rainbow trout and cutthroat trout. Despite evidence of recent hybridization in some areas, more than half of the sites in which hybridization was detected contained only fish with genotypes indicative of cutthroat trout and $>F_1$ hybrids. Introgression within these sites (# of rainbow trout alleles observed/total) was low (<3.0%). Of the 20 F_1 hybrids detected, four possessed rainbow trout mtDNA and 16 possessed cutthroat trout mtDNA. Of the 47 $>F_1$ hybrids detected, 14 had rainbow trout mtDNA and 33 possessed cutthroat trout mtDNA. No hybrids were detected in sample sites upstream of Sheep Creek (n = 320 samples).

Phylogenetic and Population Genetic Analyses

Eleven mtDNA haplotypes (HAP8, HAP13, HAP15-17, and HAP19-24) were identified in reference to Yellowstone cutthroat trout samples from the Jackson National Fish Hatchery, Wyoming and from Yellowstone Lake, Wyoming (Table 1). All 11 haplotypes clustered together in the neighbor-joining tree (70% bootstrap support; Figure 2). Three mtDNA haplotypes (HAP8, HAP12, and HAP13) were observed in reference samples from Henrys Lake. HAP12 was the most common haplotype observed and was genetically differentiated from HAP8 and HAP13, instead clustering with haplotypes observed in samples from the Bear River drainage and with reference haplotypes previously observed in samples from the Snake River, Idaho. A total of 11 haplotypes were observed within samples from the Bear River drainage (Table 1; Figure 3). As mentioned previously, two of these haplotypes (HAP8 and HAP12) had also been observed in reference populations and were presumed to be nonnative. Two additional haplotypes had also been observed previously outside the Bear River drainage. HAP9 was observed in a single sample from Coantag Creek (Site #44; Figure 3) and is one of the dominant haplotypes previously observed in samples from the Blackfoot River, Idaho (Table 3; IDFG unpublished data). HAP1 (yellow on Figure 3) was observed in the Malad River drainage in samples from

Second and Third Creeks (Sites #1 and #2, Figure 3) and in samples from Mill Canyon Creek (Site #3, Figure 3). This haplotype has been previously observed in Bonneville cutthroat trout samples from the Glenwood Fish Hatchery in southern Utah (Table 3). The remaining seven haplotypes (HAP2, HAP3, HAP5-7, HAP10, and HAP18) have not been observed previously in populations outside the Bear River drainage (IDFG unpublished data). All of the haplotypes observed in the Bear River drainage (except for the two nonnative haplotypes [HAP8 and HAP12] and HAP1 [Malad River drainage]) cluster together in a well supported clade (87% bootstrap support; Figure 2). HAP1 was very genetically distinct from all other haplotypes observed in this dataset and instead clustered with two reference haplotypes previously observed in samples from the Portneuf River (HAP4 and HAP14, 99% bootstrap support).

Of the 11 haplotypes observed within samples from the Bear River drainage, the most common haplotype was HAP2 (grey on Figure 3) at a frequency of 57.7%. It was observed in more sample locations than any other haplotype (N = 48) and was present in all predefined management units except for the Malad (Figure 3). Several haplotypes, however, were not as widely distributed, and were generally limited to specific geographic areas. The next most frequently observed haplotype (HAP7-18.0%; green on Figure 3) was unique to sample locations south of Oneida Narrows (Riverdale MU; Figure 3). HAP10 (brown on Figure 3) was also found in sample locations in the Riverdale MU as well as the Gentile Valley MU (sites 4-7), but was absent from any sample locations upstream of Soda Point, Idaho. HAP3 (blue on Figure 3) was observed in sample locations from both the Nounan and Pegram MUs, but was observed in highest frequency in samples from Bear Lake (sites 46, 46) and Swan Creek (Site 48, Bear Lake tributary). HAP5 (red in Figure 3) was the dominant haplotype observed in samples from the Daniels Fish Hatchery (Table 2), but was a relatively minor haplotype observed in Bear Lake and Bear River sample locations east of Soda Point (Figure 3). HAP5 was the most frequent haplotype observed in six samples from N. Pearl Creek (83.3%; site 24). Finally, as mentioned previously, HAP1 was found in the Malad MU but was not observed in any other sample location within the Bear River drainage.

All predefined management units exhibited admixtures of moderate to highly divergent haplotypes. For example, two haplotypes in the Pegram MU (HAP3 and HAP5) are separated by six mutational steps (Figure 4), with each clustering into a separate, well-supported subclade (Figure 2). Two of the common haplotypes observed in the Riverdale MU are HAP2 and HAP7. These two haplotypes are separated by four mutational steps (Figure 4) and they also cluster into the two separate subclades. The most divergent haplotypes observed in any MU were found in the Malad, with HAP1 and HAP3 separated by 17 mutational steps (Figure 4), each clustering into separate, highly supported clades (Figure 2).

Tests of genetic differentiation between predefined management units indicated significant genetic structure of haplotypes both between and within units (Table 4). When AMOVA analyses were run with all five MUs (Pegram, Nounan, Gentile, Riverdale, and Malad), 55.98% of the genetic variation was observed among the MUs and 44.02% within. All pairwise F_{ST} estimates and exact tests were significant ($P < 0.05$) (Table 5). Pairwise F_{ST} estimates ranged from 0.066 (Pegram versus Nounan) to 0.953 (Nounan versus Malad). In a separate analysis, with Bear Lake samples (Bear Lake98, Bear Lake03 and Swan Creek) removed from the Pegram MU and treated independently, pairwise exact tests and pairwise F_{ST} estimates indicated no differentiation among Pegram and Nounan MUs (Table 6). Pairwise comparisons of grouped Bear Lake samples versus the Pegram and Nounan MUs indicated significant differentiation (Fst = 0.512 and 0.558, respectively).

DISCUSSION

Intraspecific Hybridization

The stocking of nonnative, out-of-basin, Yellowstone cutthroat trout in the Bear River drainage began more than 100 years ago, and although some speculated that these introductions might have resulted in the hybridization of native populations, no previous genetic studies had assessed intraspecific hybridization throughout the range of Bear River cutthroat trout in Idaho. This is primarily due to the fact that few genetic markers had been identified that distinguish cutthroat trout throughout the Bear River drainage from the different populations of Yellowstone cutthroat trout that had been used for stocking. Researchers had previously demonstrated haplotype frequency differences between selected populations of Bonneville cutthroat trout, Colorado River cutthroat trout *O. clarkii pleuriticus*, and Yellowstone cutthroat trout using RFLP analyses of the ND2 mitochondrial gene region (Toline et al. 1999). Since direct DNA sequencing can provide better resolution than RFLP analyses (Bagley et al. 2002) we chose this method to compare the diversity and distribution of a highly variable segment of the ND2 mitochondrial DNA gene region to assess intraspecific hybridization. We believe that subsequent analyses allowed us to distinguish nonnative haplotypes found in Henrys Lake, Yellowstone Lake, and the Jackson National Fish Hatchery from native haplotypes found in cutthroat trout populations throughout the Bear River drainage in Idaho. Interestingly, despite the long history of nonnative cutthroat trout stocking throughout the drainage, we found very little mtDNA evidence of hybridization with only three sites (3 samples) exhibiting nonnative haplotypes. Each of these sites/creeks had been stocked with hatchery cutthroat trout in the late 1960s, 1970s, and early 1980s (IDFG historical stocking database).

Two caveats should be considered when evaluating these results. Mitochondrial DNA is maternally inherited and evidence of hybridization would be missed if native female cutthroat preferentially spawned with stocked, nonnative, male hatchery cutthroat trout. In addition, sample sizes were low in many sites, meaning that nonnative haplotypes present within individual sample locations may have been missed even if they were present in relatively high frequencies. Keeping in mind these potential limitations, if our results are in fact representative of the actual impact of previous Yellowstone cutthroat trout stocking, they would appear to support previously held notions that 1) Yellowstone cutthroat trout (particularly from Yellowstone Lake) are poorly adapted for introductions outside their native range, especially when they have to compete with native trout (Varley and Gresswell 1988; Behnke 1992); and/or 2) the particularly unique (and harsh) conditions of the desert environment of the Bear River drainage may reduce the ability of nonnative trout (at least Yellowstone cutthroat trout) to establish and reproduce (Behnke 1992).

Interspecific Hybridization

Interspecific hybridization from nonnative rainbow trout was observed throughout much of the Bear River drainage in Idaho. The identification of fish with genotypes indicative of F_1 hybrids indicates that hybridization has occurred recently (within the last generation). The F_1 hybrids identified among our samples, collected between 2000 and 2005, could be from both stocked fertile rainbow trout as well as naturally reproducing rainbow trout that have become established in several streams as a result of past stocking (Teuscher and Capurso 2007). Since 2000, the Department has only stocked rainbow trout throughout Idaho treated to be sterile (average triploidy induction level of 96.2%; Kozfkay et al. 2006). The identification of $>F_1$ hybrids, in particular individuals that appear to be the result of several generations of

backcrossing, indicate that hybridization is not, however, solely of recent origin. Most of these fish appear to be backcrosses to cutthroat trout rather than to rainbow trout (their genetic composition was more similar to cutthroat trout than rainbow trout). This is in contrast to a study involving Yellowstone cutthroat trout/rainbow trout hybridization that suggested that hybrids generally backcrossed to other hybrids or rainbow trout (Henderson et al. 2000). This apparent assortative mating may be influenced by the frequency of pure parental types in the population. Relatively high numbers of rainbow trout were identified in St. Charles Creek, Williams Creek, and Mill Canyon Creek, and the $>F_1$ hybrids identified in these areas appeared to be backcrosses to rainbow trout.

The occurrence of many streams with fish exhibiting only genotypes indicative of cutthroat or $>F_1$ hybrids (no rainbow trout or F_1 hybrids) could be due to the straying of hybrids from "source" populations. Previous research examining hybridization between westslope cutthroat trout and rainbow trout indicates that the spread of rainbow trout introgression within drainages may be facilitated by straying hybrids from nearby populations where rainbow trout have become established (Rubidge and Taylor 2005). Why higher levels of introgression were not observed in many areas may be due to a combination of factors. Over 30 different rainbow trout strains have historically been stocked in the Bear River (Teuscher and Capurso 2007). Many strains may have been ill adapted to local environmental/ecological conditions and simply did not survive to reproduce. Additionally, some stocked fish were likely removed via fisheries. Obviously, the occurrence of hybrids (both F_1 and $>F_1$) suggests that some rainbow trout and hybrids have been and continue to be reproductively successful. Removing naturally reproducing rainbow trout populations and continuing with management policies of stocking only sterile rainbow trout are two strategies that may limit the spread and rate of introgression in the future.

Phylogenetic and Population Genetic Analyses

Phylogenetic analysis of mtDNA haplotypes observed in the Bear River drainage revealed several interesting findings. Consistent with other studies, all haplotypes observed in the Bear River drainage (except for one) are clearly more closely related to Yellowstone cutthroat trout haplotypes than to "Southern Bonneville" haplotypes (Smith et al. 2002). The one exception was observed in the Malad River drainage, where a haplotype was observed that was identical to one previously observed in samples from the Glenwood Hatchery in southern Utah (HAP1-Sevier River brood source). It is unlikely that Malad River has been stocked with "Southern Bonneville" cutthroat trout (IDFG historical stocking database; http://fishandgame.idaho.gov/apps/stocking/), and it seems reasonable that the Malad River drainage may contain remnant "Southern Bonneville" cutthroat trout. Since ancient Lake Bonneville is believed to have desiccated approximately 10,000 years ago, we would not expect to observe substantial mtDNA sequence divergence among these now isolated and geographically distant populations.

We observed haplotypes very similar to HAP1 in the Portneuf River drainage, Idaho (HAP 4 and 14). Presumably, the presence of these haplotypes is the result of the recent (although very temporary) opportunity for faunal exchange between the Bonneville basin and the Snake River basin during the catastrophic Bonneville flood approximately 14,000 to 11,000 years ago (Link et al. 1999). Johnson (2002) offered a similar hypothesis to explain the distribution of "Southern Bonneville" Utah Chub haplotypes in the Portneuf River. What is even more difficult to explain is why no "Southern Bonneville" haplotypes are found anywhere else in the Bear River drainage, a pattern also exhibited in Utah Chub (Johnson 2002). Although the

Bear River was connected to the Bonneville basin through the Oneida Narrows by about 20,000 years ago (Bouchard et al. 1998), it may not have been prior to that time, instead flowing intermittently into a series of lakes just north of the Oneida Narrows collectively referred to as Lake Thatcher (Bright 1963; Bouchard et al. 1998). Perhaps following the evacuation of Oneida Narrows, waterfalls were present that prevented upstream movement of "Southern Bonneville" cutthroat trout into the Bear River drainage in Idaho. It has also been suggested that lacustrine "Southern Bonneville" cutthroat trout in Lake Bonneville may not have easily displaced fluvial cutthroat populations already established throughout much of the Bear River (Dennis Shiozawa, BYU, personal communication).

Whatever the reason for the disjunct distribution of "Southern Bonneville" cutthroat trout, Bear River cutthroat trout are clearly a result of a distinctly different founding event. However, the distribution and divergence of haplotypes in the drainage suggest that this was not a single event either. The geological/hydrological history of the Bear River, Portneuf River, and Blackfoot River drainages indicates that the Bear River has changed course several times throughout its history due to volcanic activity, alternately flowing north through the Portneuf River or Blackfoot River drainages and south into the Lake Thatcher area (Link et al. 1992). The earliest diversion is believed to have occurred possibly 500,000 years ago, when basaltic volcanism in both the Gem and Blackfoot River valleys produced large lava fields that blocked the northward drainage of the Bear River (Scott et al. 1982; Kuntz et al. 1992). The most recent (and final) diversion is believed to have occurred sometime around 50,000 years ago, ultimately resulting in the evacuation of Oneida Narrows, dramatically increasing the volume of water entering Lake Bonneville (Link et al. 1999). In between the initial and most recent diversion, the exact drainage history of the Bear River is unclear. Bouchard et al (1998) used sedimentological evidence to suggest that the Bear River likely flowed into the Thatcher Basin ~140,000 years ago, but was not an input into the lake between ~140,000 to ~50,000 years ago. During this period, Lake Thatcher is believed to have been maintained by local streams (Link et al. 1999). This would have provided opportunities to isolate populations for a considerable period if they had been present in the area.

The distribution of divergent Bear River haplotypes into two well-supported subclades seems to reflect the multiple opportunities for population isolation, divergence, and secondary contact. Bear River haplotypes observed in subclade #B1 might represent lineages that were isolated as part of the initial diversion of the Bear River from the Snake River. Bear River haplotypes observed in subclade #B2, which cluster strongly with haplotypes observed in the Blackfoot and Portneuf river drainages, perhaps reflect a more recent colonization history. The two clades are not geographically correlated. Instead, most sites demonstrate an admixture of haplotypes from both clades, suggesting that historically much of the Bear River drainage must have been connected, allowing gene flow over fairly large distances. However, we do observe genetic structuring in terms of haplotype frequency distribution throughout the Bear River drainage, with a significant portion of total genetic variation observed among population groupings.

The designation of management units based on suspected isolating mechanisms (major drainage divides and/or dams) was largely supported by the genetic results, with a couple of exceptions. Samples from Bear Lake, when removed from the Pegram MU, were significantly differentiated from populations within the Pegram and Nounan MUs, primarily due to the high frequency of HAP3. An analysis of surficial deposits in the Bear Lake Valley suggests that over the last 280,000 years, Bear Lake has largely been connected to the Bear River, although there were several periods spanning 10,000-year intervals during which they were not connected (Laabs and Kaufman 2003), perhaps providing opportunities for population isolation and

divergence. It is important to keep in mind that the Bear Lake cutthroat trout population relies heavily on hatchery supplementation from spawners collected in Swan Creek (Teuscher and Capurso 2007). So essentially the “Bear Lake” grouping in this study consists of samples from one population. Whether this population should exist within a management unit of its own is unclear, although gene flow between lacustrine cutthroat trout in Bear Lake and fluvial cutthroat trout in the Bear River is undoubtedly restricted due to irrigation diversions.

When Bear Lake and Swan Creek samples were removed from the Pegram MU, pairwise comparisons of the Pegram and Nounan MUs indicated no genetic differentiation. Despite the fact that our results suggest that gene flow between these areas likely occurred historically, Stewart Dam, installed over 100 years ago, currently presents a barrier to fish movement between these areas.

Managers will have to carefully consider the genetic structuring observed in the Bear River drainage when evaluating taxonomic assessments, prioritizing populations for conservation and management purposes, and identifying suitable populations for translocations, reintroductions, and broodstock development programs. Preservation of genetic diversity of cutthroat trout in the Bear River drainage will require at minimum the protection of multiple populations within each management unit.

RECOMMENDATIONS

1. Continue with Department policy of stocking only rainbow trout that have been treated to be sterile.

2. Test biologist’s/manager’s ability to phenotypically identify cutthroat trout, rainbow trout, and hybrids, and investigate methods to remove rainbow trout and hybrids.

3. Identify “core conservation populations” and “conservation populations” (UDWR 2000a) within each management unit for conservation and preservation.

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Table 1. Site number (from Figure 1), sample site, frequency of haplotypes per site, and total sample size (N).

Site #	Sample Site	HAP01	HAP02	HAP03	HAP04	HAP05	HAP06	HAP07	HAP08	HAP09	HAP10	HAP11	HAP12	HAP13	HAP14	HAP15	HAP16	HAP17	HAP18	HAP19	HAP20	HAP21	HAP22	HAP23	HAP24	Total (N)
1	SecondCreek	4																								4
2	ThirdCreek	3																								3
3	MillCanyonCreek01	7		1																						8
4	CottonwoodCreek03		10								8															18
5	CottonwoodCreek05		7								14															21
6	NHoopsCreek05		5				1												1							7
7	SouthHoopsCreek		9																							9
8	MinkCreek05		13					14																		27
9	DryCreek00		7					11																		18
10	FosterCreek							2																		2
11	BirchCreek01							3																		3
12	BirchCreek03		2					4																		6
13	SugarCreek01		3					18			1															22
14	MapleCreek01		2					17			6															25
15	MapleCreek03							18			3															21
16	LMapleCreek01		1					5			1															7
17	UMapleCreek03		2					7			1															10
18	CubCreek03		2					22			10															34
19	LoganRiver01		2					14			6															22
20	EightmileCreek01		3																							3
21	EightmileCreek03		4																							4
22	EightmileCreek05		1	1																						2
23	NPearlCreek01		1			5																				6
24	PearlCreek01		5																							5
25	PearlCreek03		6										1													7
26	SSkinnerCreek03		5																							5
27	N.SkinnerCreek01		2																							2
28	S.SkinnerCreek03		5																							5
29	SkinnerCreek01		11																							11
30	SkinnerCreek03		5																							5
31	StauferCreek01		1																							1
32	CanyonCreek01		8																							8
33	CoopCreek01		10																							10
34	BearRiverN		11																							11
35	MontpeilerCreek05		23	2									2													27
36	BearRiverP		21			2	1																			24
37	BearRiver06		39	4		4																				47
38	BearRiver05		50	2		6	1																			59
39	ThomasFrk99		8	3		1			1																	13
40	ThomasFrk04		33	1		1																				35
41	PruessCreek03		4			1																				5
42	GiraffeCreek03		6	2																						8
43	CoantagCreek		21				1			1																23

Table 1. Continued.

Site #	Sample Site	HAP01	HAP02	HAP03	HAP04	HAP05	HAP06	HAP07	HAP08	HAP09	HAP10	HAP11	HAP12	HAP13	HAP14	HAP15	HAP16	HAP17	HAP18	HAP19	HAP20	HAP21	HAP22	HAP23	HAP24	Total (N)
44	HobbleCreek	—	~~66~~	~~3~~	—	~~4~~	~~2~~	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	~~75~~
45	BearLake03		6	20		2																				28
46	BearLake98		8	22		1																				31
47	SwanCreek04		4	15		3																				22
N/A*	DanielsFH		9	1		14																				24
H.R.*	HenrysLake								3				18	1												22
H.R.*	JacksonNFH								9					1		1	3	7								21
H.R.*	YellowstoneLake								5					12						1	1	1	1	1	1	23
	Total	14	442	77	0	44	6	135	18	1	50	0	21	14	0	1	3	7	1	1	1	1	1	1	1	840

Table 2. Reference samples from previous sequencing (IDFG, unpublished data). Sample site, frequency of haplotypes per site and total sample size (N).

Site #	Sample Site	HAP01	HAP02	HAP03	HAP04	HAP05	HAP06	HAP07	HAP08	HAP09	HAP10	HAP11	HAP12	HAP13	HAP14	HAP15	HAP16	HAP17	HAP18	HAP19	HAP20	HAP21	HAP22	HAP23	HAP24	Total (N)
N/A	YellowCCCCREF																						1			1
N/A	BasinCreekREF				2																					2
N/A	BLBACAREF			1																						1
N/A	CLCCCAREF								1																	1
N/A	CottonCreekREF		2																							2
N/A	DanielsREF		1			1																				2
N/A	BlackfootREF									2																2
N/A	GooseCreekREF											1														1
N/A	GlenwoodREF	1																								1
N/A	HarknessCreekREF	1													1											2
N/A	LeHardyRapidsREF								2					1												3
N/A	NewCanyonCr.REF			2																						2
N/A	NovakREF								2								1									3
N/A	TygheeREF												1													1
	Total	2	3	3	2	1	0	0	5	2	0	1	1	1	1	0	1	0	0	0	0	0	1	0	0	24

Table 3. Site number (from Figure 1), sample site, sample size (N), number identified as cutthroat trout-like, rainbow trout-like, F_1 hybrid, mtDNA lineage of F_1 hybrids, $>F_1$ hybrids, mtDNA lineage of $>F_1$ hybrids, total # of hybrids detected, and % rainbow trout (RBT) introgression.

Site #	Sample Sites	N	Cutthroat trout-like	Rainbow trout-like	F_1 Hybrid	mtDNA lineage	$>F_1$ Hybrid	mtDNA lineage	# of hybrids detected	% RBT introgression
1	SecondCreek	4	3	0	1	CUT	0		1 (25.0%)	0.0
2	ThirdCreek	3	3	0	0		0		0	0.0
3	MillCanyonCreek01	15	5	5	1	RBT	4	RBT	5 (33.3%)	9.2*
4	CottonwoodCreek03	22	18	0	0		4	CUT	4 (18.2%)	1.9
5	CottonwoodCreek05	21	16	0	0		5	CUT	5 (23.8%)	1.9
6	NHoopsCreek05	7	7	0	0		0		0	0.0
7	SouthHoopsCreek	9	9	0	0		0		0	0.0
8	MinkCreek05	28	24	0	0		4	CUT	4 (14.3%)	1.2
9	DryCreek00	20	19	0	0		1		1 (5.0%)	0.4
10	FosterCreek	2	2	0	0		0		0	0.0
11	BirchCreek01	3	3	0	0		0		0	0.0
12	BirchCreek03	6	5	0	0		1	CUT	1 (16.7%)	1.4
13	SugarCreek01	26	22	0	0		4	CUT	4 (15.4%)	1.1
14	MapleCreek01	26	25	0	0		1	CUT	1 (3.9%)	0.3
15	MapleCreek03	30	30	0	0		0		0	0.6
16	UMapleCreek03	10	10	0	0		0		0	0.0
17	CubCreek03	35	35	0	0		0		0	0.0
18	LoganRiver01	23	23	0	0		0		0	0.0
19	EightmileCreek01	22	3	19	0		0		0	0.0
20	EightmileCreek03	5	5	0	0		0		0	0.0
21	EightmileCreek05	2	2	0	0		0		0	0.0
22	NPearlCreek01	6	6	0	0		0		0	0.0
23	PearlCreek01	5	4	0	0		1	CUT	1 (20.0%)	1.7
24	PearlCreek03	7	7	0	0		0		0	0.0
25	SSkinnerCreek03	5	5	0	0		0		0	0.0
26	N.SkinnerCreek01	2	2	0	0		0		0	0.0
27	SkinnerCreek01	5	5	0	0		0		0	0.0
28	S.SkinnerCreek03	11	11	0	0		0		0	0.0
29	StauferCreek01	1	1	0	0		0		0	0.0
30	CanyonCreek01	9	6	0	0		3	CUT	3 (33.3%)	2.8
31	CoopCreek01	10	10	0	0		0		0	0.0
32	BearRiverN	12	11	1	0		0		0	0.0
33	Geotown03*	11	0	11	0		0		0	0.0
34	MontpeilerCreek05	30	25	1	0		4	CUT	4 (13.3%	2.0
35	BearRiverP	24	24	0	0		0		0	0.0
36	BearRiver06	49	49	0	0		0		0	0.0
37	BearRiver05	63	62	1	0		0		0	0.0
38	ThomasFrk99	16	16	0	0		0		0	0.0
39	ThomasFrk04	40	40	0	0		0		0	0.0
40	PruessCreek03	5	5	0	0		0		0	0.0
41	GiraffeCreek03	9	9	0	0		0		0	0.0
42	CoantagCreek	39	39	0	0		0		0	0.0
43	HobbleCreek	75	75	0	0		0		0	0.0
44	BearLake03	30	28	0	2		0		2 (3.1%)	0.0
45	BearLake98	35	35	0	0	CUT	0		0	0.0
46	SwanCreek04	24	16	3	1	CUT	4	CUT	5 (20.8%)	N/A
47	Swan 04S	127	119	1	6	CUT	1	CUT	7 (5.5%)	0.001
48	Beaver Cr 03*	13	13	0	0		0		0	0.0
49	St. Charles 03*	39	22	4	4	2,2**	9	RBT	13 (33.3%)	63.3
50	Williams 01*	13	0	12	0		1	RBT	1 (7.7%)	N/A
51	Williams 03*	28	0	23	5	1,4**	0		5 (17.9%)	0.0
	Total	1063	915	81	20		47		67	

* Introgression calculated as the number of RBT alleles observed in samples identified as cutthroat trout-like and hybrid divided by total alleles examined.

** Number with RBT mtDNA listed first, number with CUT mtDNA listed second.

Table 4. Total molecular variance partitioned among groups and among populations within groups.

Structure	Source of Variation	d.f.	Sum of Squares	Variance Components	% Total Variance
5 MUs (Pegram and Bear L./Swan Creek combined)	Among groups	4	448.03	0.94 Va	55.98
FST = 0.56	Within groups	745	550.55	0.74 Vb	44.02
	Total	749	998.58	1.68	
5 MUs (excluding Malad)	Among groups	4	286.39	0.54 Va	46.34
FST = 0.46	Within groups	730	458.79	0.63 Vb	53.66
	Total	734	745.18	1.17	

Table 5. 5 MUs (Pegram and Bear L./Swan Creek combined). Matrix of pairwise F_{ST} (below diagonal) with * indicating significantly different from zero (P <0.05) and pairwise exact tests (above diagonal) with significant tests indicated with a +. Sample sizes in parentheses.

	Pegram (with Bear Lake) (397)	Nounan (85)	Gentile (55)	Riverdale (198)	Malad (15)
Pegram (with Bear Lake)		+	+	+	+
Nounan	0.07*		+	+	+
Gentile	0.12*	0.21*		+	+
Riverdale	0.43*	0.56*	0.48*		+
Malad	0.92	0.95*	0.93*	0.89*	

Table 6. 5 MUs (Pegram and Bear L./Swan Creek separated, Malad removed). Matrix of pairwise F_{ST} (below diagonal) with * indicating significantly different from zero (P <0.05) and pairwise exact tests (above diagonal) with significant tests indicated with a +. Sample sizes in parentheses.

	Pegram (with Bear Lake) (316)	Nounan (85)	Bear Lake (81)	Gentile (55)	Riverdale (198)
Pegram (with Bear Lake)		-	+	+	+
Nounan	0.01		+	+	+
Bear Lake	0.51*	0.56*		+	+
Gentile	0.16*	0.21*	0.45*		+
Riverdale	0.56*	0.56*	0.21*	0.48*	

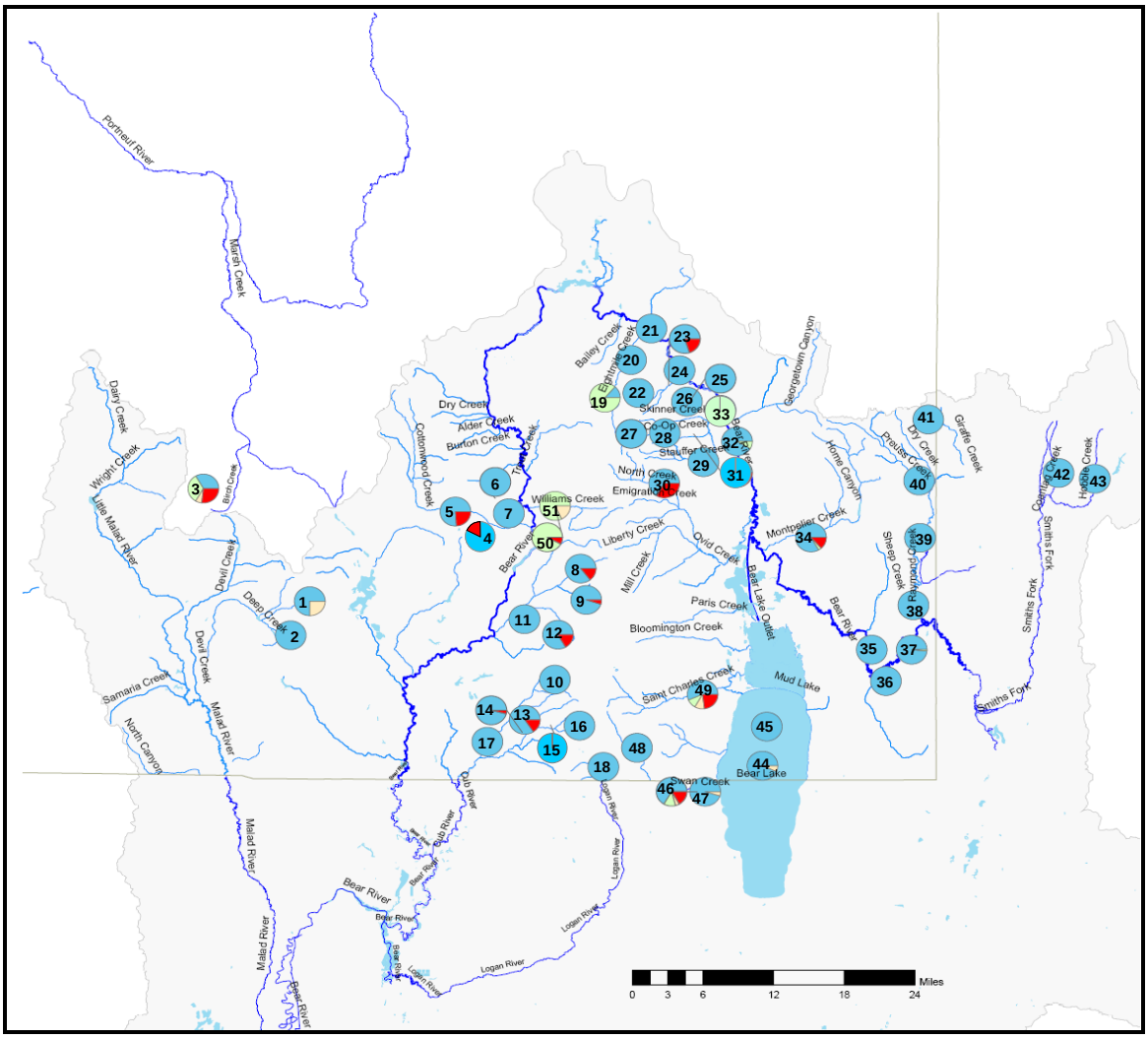

Figure 1. Distribution of cutthroat trout-like genotypes (blue), rainbow trout-like genotypes (light green), F_1 hybrid genotypes (light yellow), and $>F_1$ hybrid genotypes (red) detected among 51 sample locations in the Bear River drainage, Idaho and Wyoming. Numbers in black are sample site #s corresponding to table 3.

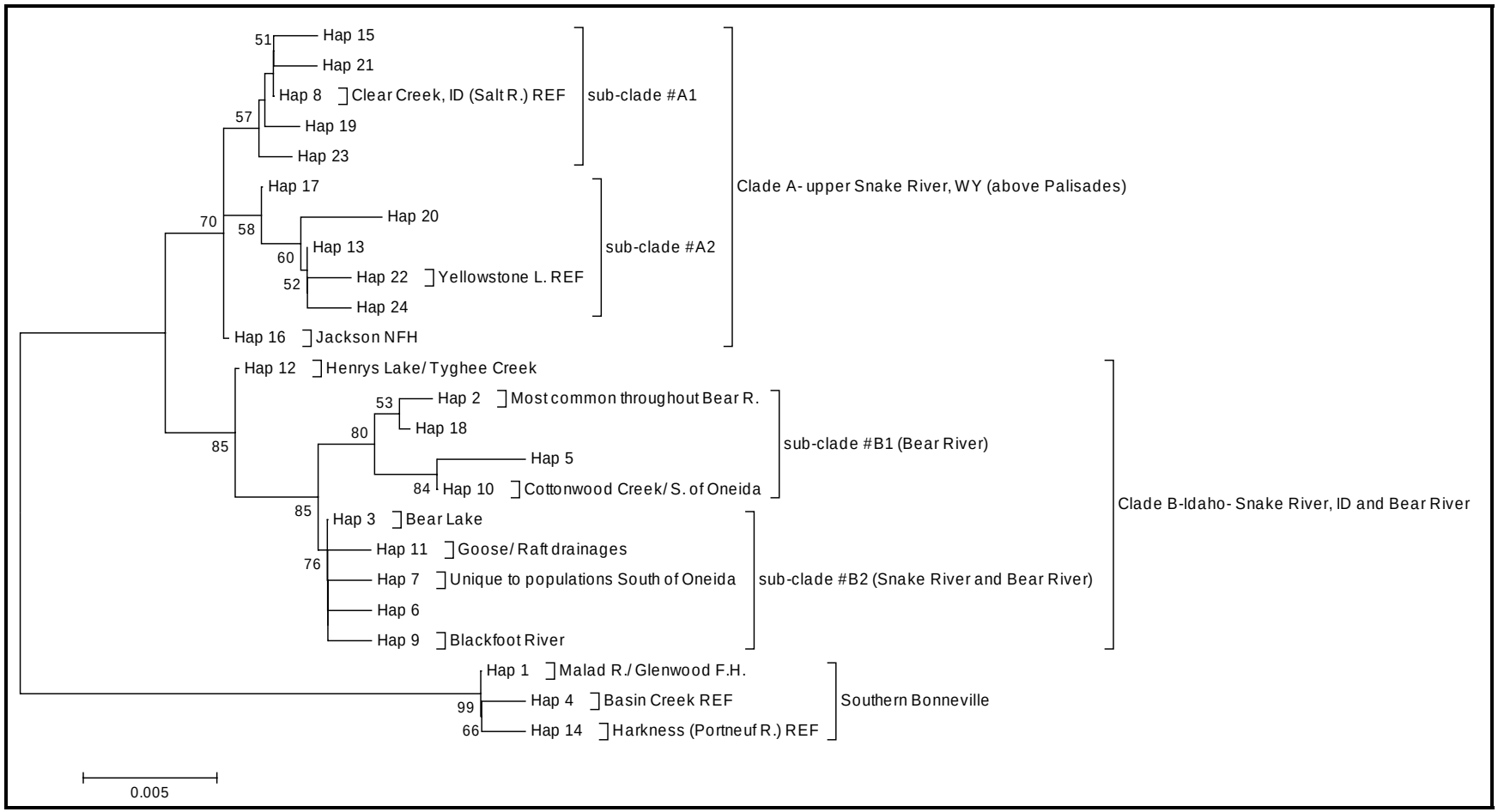

Figure 2. Neighbor-joining tree of 24 haplotypes observed in samples of cutthroat trout from the Bear River, reference hatchery populations, and previously sequenced Yellowstone cutthroat trout.

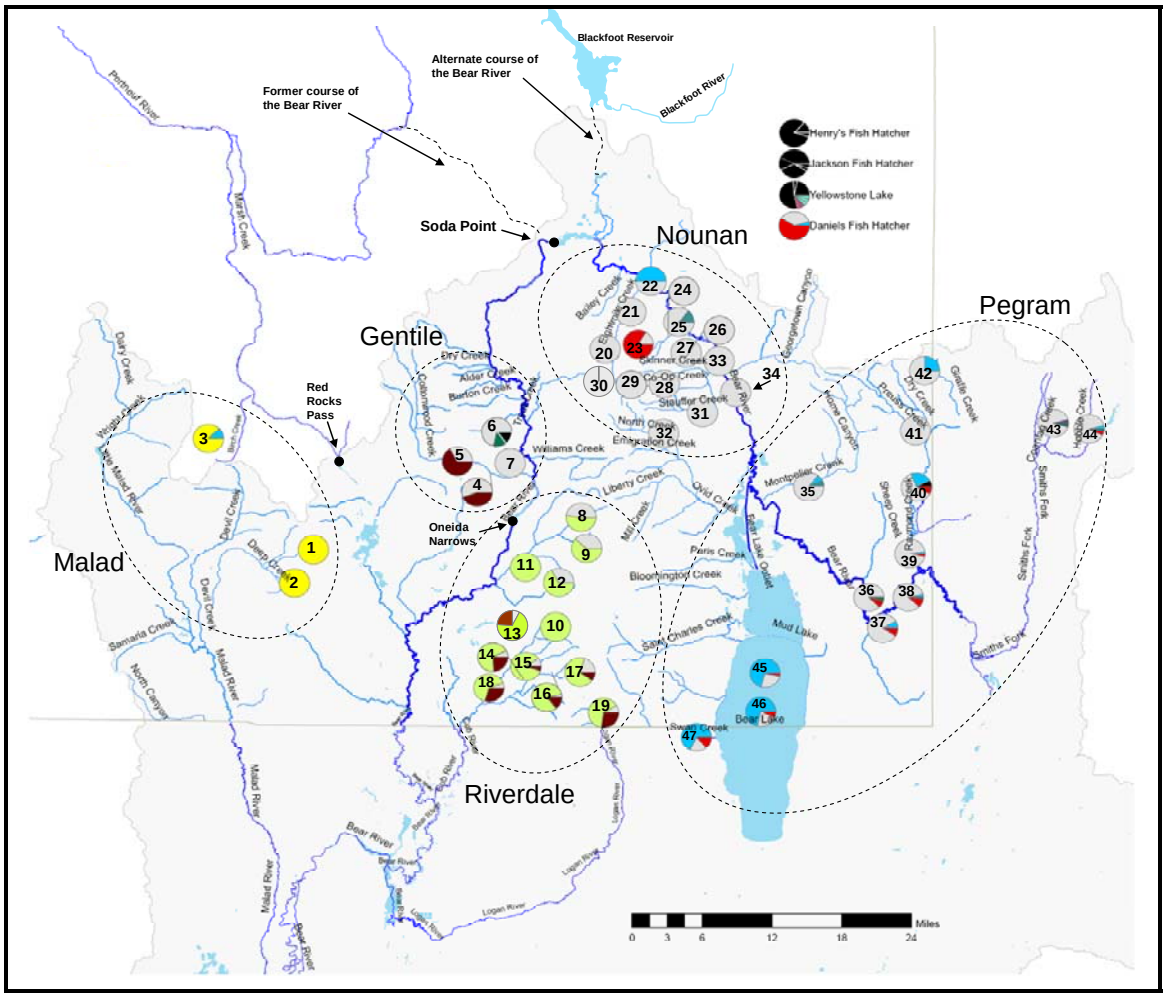

Figure 3. Distribution of mtDNA haplotypes among 47 sample locations within the Bear River drainage, Idaho and Wyoming. Five predefined management units (Pegram, Nounan, Gentile, Riverdale, and Malad) are shown with dashed circles. Numbers in black are sample site #s corresponding to table 1.

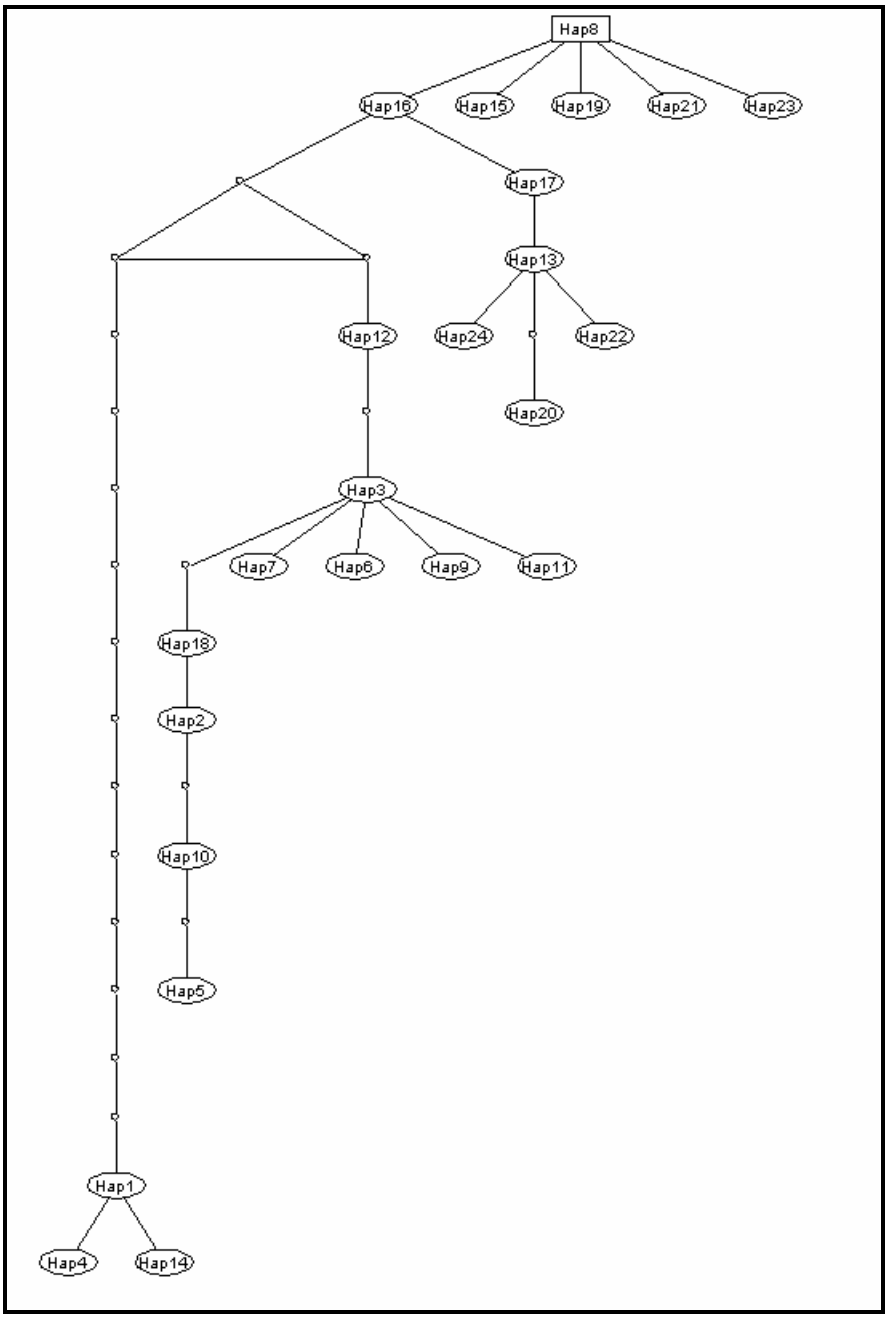

Figure 4. Haplotype network for 24 mtDNA haplotypes observed in samples of cutthroat trout from the Bear River, reference hatchery populations, and previously sequenced Yellowstone cutthroat trout. Network constructed by means of the parsimony criterion using the TCS 1.01 program. Each line in the network represents one mutational change and the circles with numbers inside represent and identify each haplotype. Small empty circles symbolize nodes that indicated inferred steps not found in the sampled populations.

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