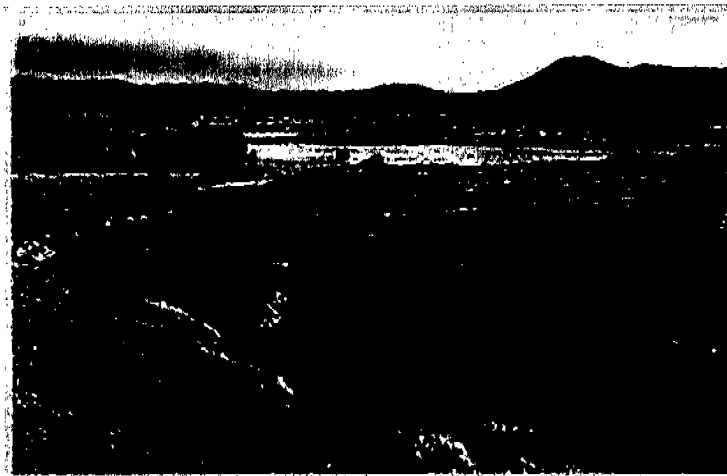


**Genetic Analysis of Rainbow Trout
(*Oncorhynchus mykiss*) from Silver Creek Preserve
of The Nature Conservancy, Idaho.**



Silver Creek by Harold E. Malde

**Richard N. Williams and Madison S. Powell,
Center for Salmonid and Freshwater
Species at Risk, HFCES/University of Idaho,
3059 F National Fish Hatchery Road, Hagerman, ID**

**Matthew R. Campbell, Aquaculture Research Institute
Fish Genetics Lab, University of Idaho, Moscow, ID**

Lab Report HAG – 00-2

August 11, 2000

The Silver Creek Preserve, located 30 miles south of Sun Valley, in south central Idaho is an 875-acre preserve owned and protected by the Idaho Chapter of The Nature Conservancy. Silver Creek is formed entirely from underground springs that flow southeast from the Big Wood River near Bellevue, Idaho. It is not directly connected to the Big Wood River. Silver Creek is one of the most famous spring creeks in the world and is revered by fly fishermen worldwide.

One of the Nature Conservancy's goals is the preservation and enhancement of trout populations that currently occupy the Silver Creek Preserve. One of the questions of interest to the Nature Conservancy, is the taxonomic identity of the rainbow trout (*Oncorhynchus mykiss*) presently found in Silver Creek. Are the rainbow trout in Silver Creek descended from redband trout (the rainbow trout subspecies native to the interior west) indigenous to the southern Idaho region, or are they descended from hatchery produced rainbow trout native to northern California? Do the rainbow trout in Silver Creek today represent one of the forms or a mixture of the two?

This is a logical concern. Curiously there do not appear to be any historical records of a native trout in Silver Creek, leading many biologists to believe that the creek was barren of trout. Alternatively because the lower end of Silver Creek flows into the Little Wood River where indigenous redband trout populations existed, it is also possible that the rainbow trout in Silver Creek are descended from native redband trout. Records do exist, however that document early (1920s – 1930s) plants of McCloud River rainbow trout (B. Esselmann, Idaho Fish and Game, personal communication to RNW; 1990). Although other stocking events followed, Silver Creek has not been stocked since the 1970s. Natural reproduction occurs within the Silver Creek drainage and recent studies show that Silver Creek holds the one of the highest densities of wild rainbow trout in the United States, as many as 5,141 per mile (Nature Conservancy 1996).

Genetic analysis of rainbow trout specimens from Silver Creek will allow us to place the Silver Creek rainbow trout population into one of three possible genetic- taxonomic categories:

1. a genetically pure population of native interior redband trout,
2. a population derived from introduced coastal rainbow trout, or
3. a population that is a mixture of interior (redband) and coastal (rainbow) trout.

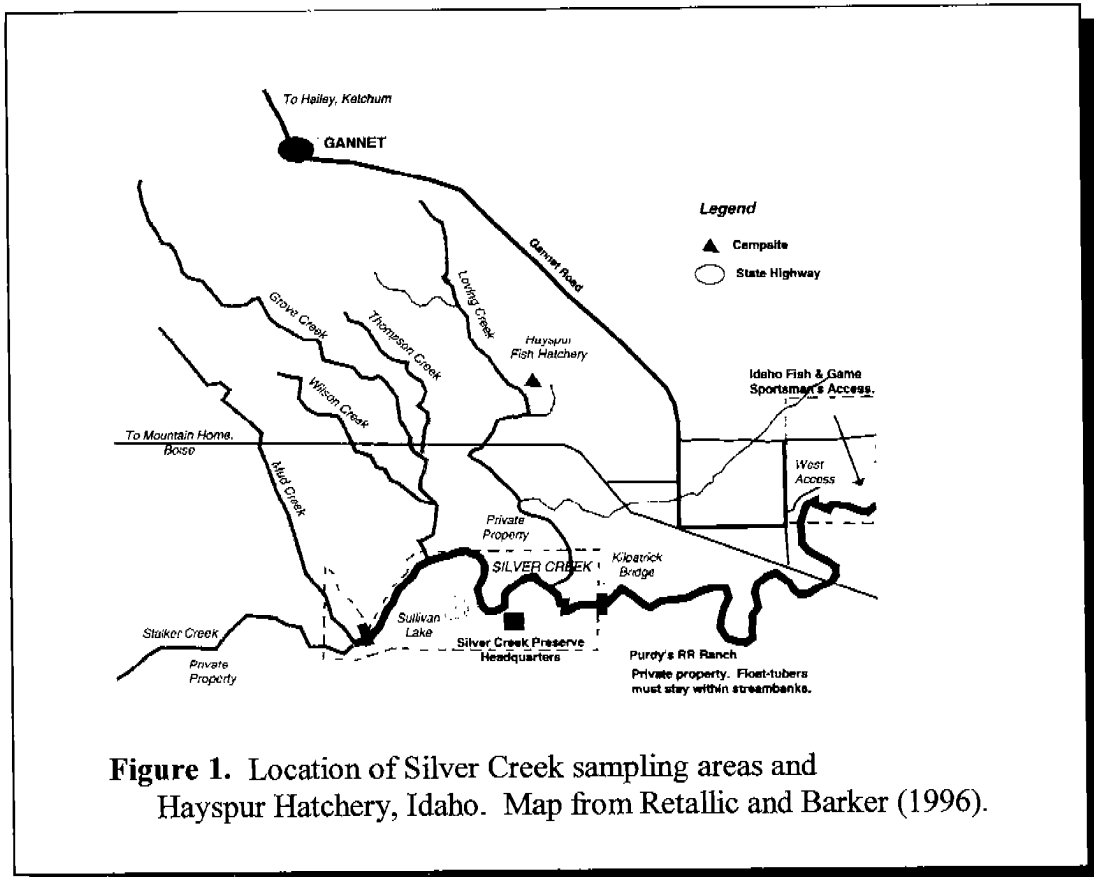
Previous studies of rainbow trout populations have separated interior from coastal forms of rainbow trout using protein electrophoresis, i.e., allozyme analysis (Wishard et al. 1984; Campton and Johnston 1985; Currens et al. 1990; Williams et al. 1996). Unfortunately, allozyme analysis requires sacrifice of the specimen in order to obtain critical tissue samples. More recently, DNA-based genetic techniques that rely on non-lethal sampling of small pieces of fin tissue, have been used to separate interior and coastal forms of rainbow trout (Williams et al. 1996; Williams et al. 1997).

Up until this time, no genetic analyses have been done on rainbow trout within the Silver Creek preserve and it is not known whether gene flow occurs between wild Silver Creek rainbow trout and escapees from the Hayspur Hatchery, two miles north of the preserve on Loving Creek. The goal of this project is to provide baseline information concerning the genetic identity, genetic diversity, and genetic structure of the Silver Creek rainbow trout population and determine to what extent, if any, escaped Hayspur Hatchery fish may contribute to the genetic structure of the Silver Creek population.

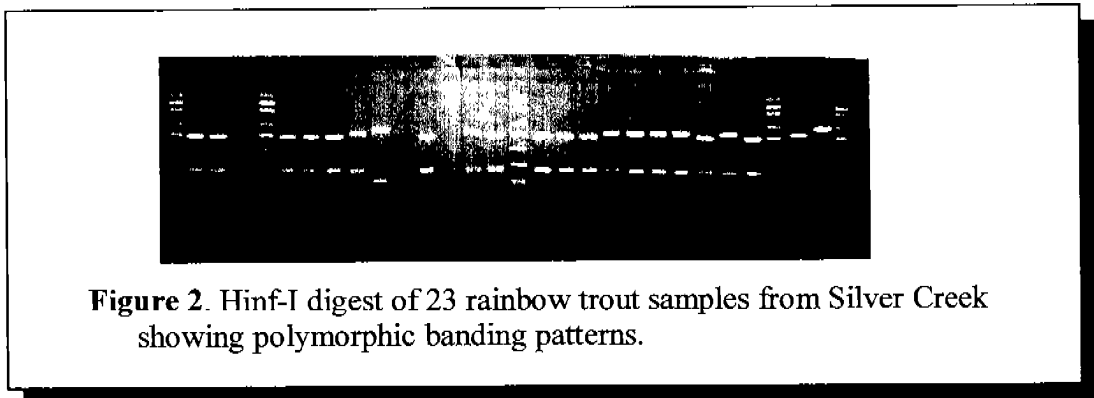
Methods

We analyzed mitochondrial DNA (mtDNA) variation in rainbow trout specimens from Silver Creek and the Hayspur Hatchery (Figure 1). For the purposes of comparative analysis, we also used previously analyzed redband trout samples from the Owyhee River as our redband reference population (Williams et al. 1995). We used restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR) amplified segments of mtDNA to determine the genetic structure of the Silver Creek population and to compare it to the genetic structure of Hayspur Hatchery fish.

In early May 1998, biologists from the University of Idaho and the Idaho Department of Fish and Game sampled rainbow trout from the Silver Creek Preserve owned by The Nature Conservancy. Sampling occurred from Sullivan Springs Crossing to Kilpatrick's Pond within the Silver Creek Nature Conservancy. Small pieces of fin tissue, about the size of a paper punch hole were snipped from each of 46 captured fish. The fish were then released unharmed. Rainbow trout from the Hayspur Hatchery (22 specimens) were sampled in the same manner.



Tissue samples were collected and preserved in lysis buffer before being shipped to the University of Idaho for genetic analysis. Total genomic DNA was isolated from each sample and amplified using the Polymerase Chain Reaction (PCR) with primers specific for the NADH Dehydrogenase 2 (ND2) gene region of the mitochondrial genome. Amplification products were digested with 8 separate restriction enzymes (*Dde-I*, *Dpn-II*, *Hae-III*, *Hha-I*, *Hinf-I*, *Mse-I*, *Msp-I* and *Rsa-I*). Digests were electrophoresed on 3% agarose gels and visualized as band patterns when fluoresced under UV-light (Figure 2).



Each unique band pattern generated by a specific restriction enzyme was assigned a letter. The letter designations for each of the eight restriction enzymes were later combined across enzymes to form a composite haplotype (hereafter haplotype). Haplotypes and haplotype frequencies were compared between samples collected from within the Silver Creek Nature Conservancy, the Hayspur Hatchery, Picabo, ID, and the Owyhee River redband trout reference samples (Haplotype R).

Data were analyzed using the computer programs REAP (McElroy et al. 1991) and PHYLIP (ver. 3.53) (Felsenstein 1993), which generated estimates of genetic divergence among haplotypes and constructed a Least Squares dendrogram, respectively.

Results and Discussion

We obtained full genetic results from 34 specimens from Silver Creek and 22 specimens from the Hayspur Hatchery sample. Variation in band patterns was observed for each of eight restriction enzymes. A unique alphabetic designation was given to the patterns observed for each restriction enzyme (Table 1) and compiled into composite mtDNA haplotypes observed for each of the 56 samples examined.

Table 1. Population, sample size, alphabetic designation of band patterns observed for each of eight restriction enzymes, and mtDNA haplotype(s) observed in each population and for the reference Owyhee River redband trout.

Population	Haplotype	N	Composite Haplotype							
			<i>Dde-I</i>	<i>Dpn-II</i>	<i>Hae-III</i>	<i>Hha-I</i>	<i>Hinf-I</i>	<i>Mse-I</i>	<i>Msp-I</i>	<i>Rsa-I</i>
Silver Creek	I	2	A	A	C	A	C	A	A	A
	J	4	C	A	C	A	A	C	A	A
	K	8	A	A	C	A	C	C	A	A
	L	19	A	A	C	A	A	C	A	A
	M	1	B	A	C	A	C	A	C	A
Hayspur Hatchery	I	10	A	A	C	A	C	A	A	A
	J	6	C	A	C	A	A	C	A	A
	K	6	A	A	C	A	C	C	A	A
Redband	R*	72	A	A	A	A	A	A	A	A

* from Williams et al. 1995.

A difference in haplotype frequency and diversity was observed between rainbow trout samples from Silver Creek and rainbow trout samples from Hayspur Hatchery (Figure 3). Haplotype diversity within Silver Creek was higher with five haplotypes observed in 34 samples. The most common haplotypes observed within Silver Creek were haplotype L (55%) and haplotype K (24%). The remaining three haplotypes occurred in lower frequencies (<12%).

The 22 samples collected from Hayspur Hatchery exhibited lower haplotype diversity with only three haplotypes. The most common haplotype (I) occurred in 46% of the specimens. The other two haplotypes observed in the Hayspur Hatchery samples also occurred in relatively high frequencies, J (27%), and K (27%). Haplotype L, the most common haplotype observed in the Silver Creek sample, was not observed in the Hayspur Hatchery sample.

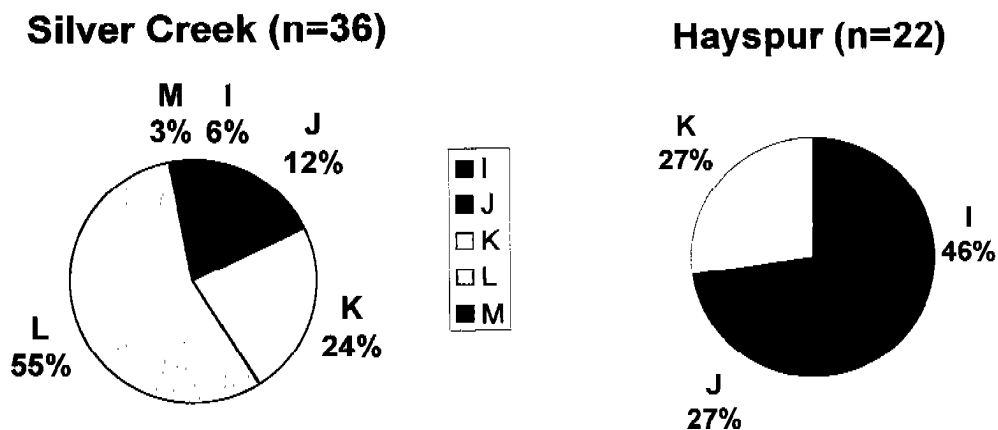


Figure 3. Comparison of mtDNA haplotype frequencies of rainbow trout from Silver Creek and Hayspur Hatchery (3 miles North of the Silver Creek Preserve).

Divergence among the various haplotypes ranged from 0.56 - 2.50 percent sequence divergence. These values are relatively high compared to those that occur in wild populations not influenced by hatchery stocking. Typically, wild populations exhibit only one or a few mtDNA haplotypes that differ but slightly from one another, usually by less than 0.5% sequence divergence. In contrast, rainbow trout populations in the interior west that have interbred with hatchery rainbow

trout, usually exhibit multiple mtDNA haplotypes that differ from one another by up to 1.5 – 2.2% sequence divergence. We observed this pattern in the Silver Creek sample, which had five haplotypes and a maximum sequence divergence estimate of 2.50 among haplotypes, and in the Hayspur sample, which had three haplotypes and a maximum sequence divergence estimate of 1.81 among haplotypes (Table 2).

Table 2. Percent sequence divergence matrix for observed composite haplotypes constructed using REAP (McElroy et al. 1991). Percent sequence divergence is the distance value generated by REAP x 100.

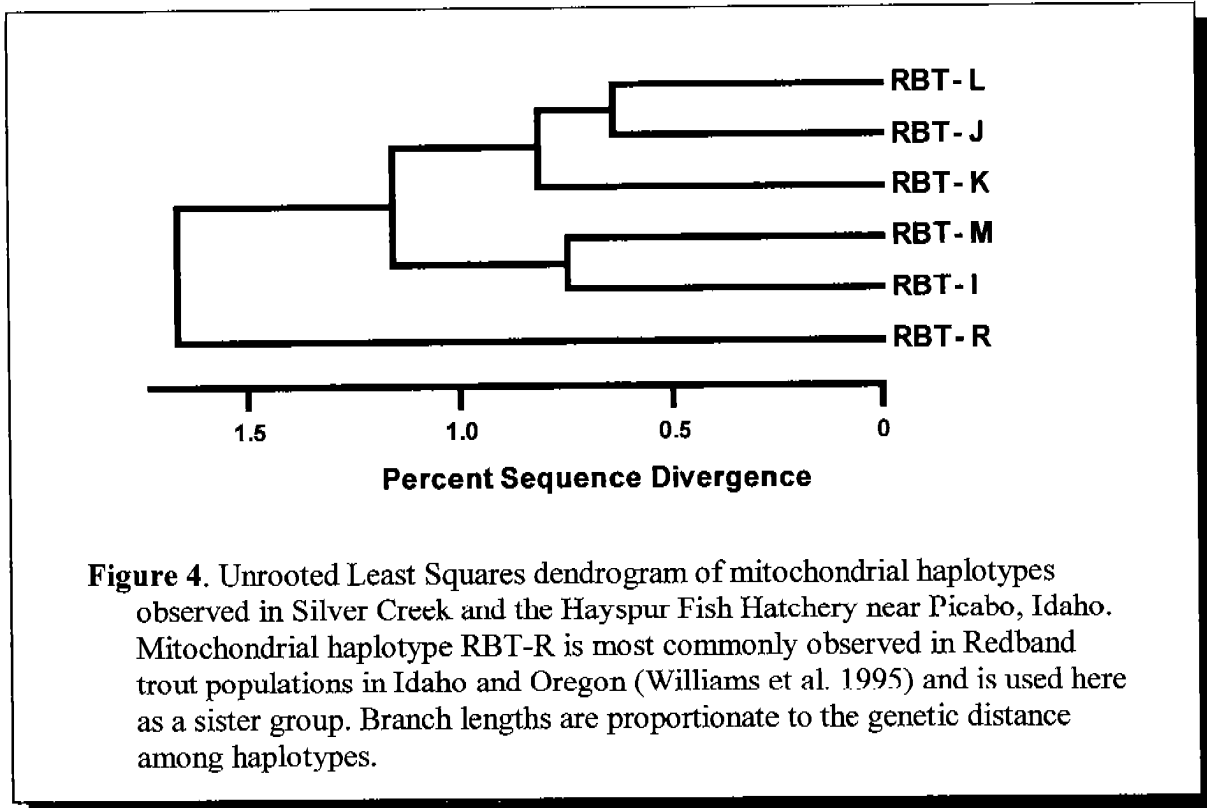
	RBT-I	RBT-J	RBT-K	RBT-L	RBT-M
RBT-J	1.808				
RBT-K	0.617	1.163			
RBT-L	1.220	0.562	0.588		
RBT-M	0.649	2.502	1.282	1.900	
RBT-R	1.220	1.725	1.808	1.163	1.900

We depicted relationships among the five observed haplotypes and the redband reference haplotype in a dendrogram (Figure 4) constructed using the genetic distance estimates (Table 2) as input data into the Kitsch program in PHYLIP (ver. 3.53) with the Least Squares Method. This method joins those haplotypes that have the smallest genetic distance to one another into the first grouping (e.g., Haplotypes L and J in Figure 4), then successively joins the next haplotype that has the smallest genetic distance to the first pair and so on.

Conclusions and Management Implications

Mitochondrial DNA analysis of rainbow trout samples from Silver Creek and the Hayspur Hatchery yielded no evidence of interior redband trout in either sample, but did reveal differences in haplotype frequency and diversity between the two sample populations. Haplotype-L was the predominant haplotype observed in Silver Creek rainbow trout, yet it was absent in the Hayspur Hatchery sample, suggesting that the current rainbow trout strain used at the Hayspur Hatchery is not the same strain that was used to found the Silver Creek population.

Silver Creek's long history of hatchery introductions from multiple sources is evident by the high haplotype diversity observed in the samples.



We observed three mtDNA haplotypes in the Hayspur Hatchery sample (I, J, and K), which were also observed in the Silver Creek sample. These haplotypes may be common to many rainbow trout hatchery strains and their occurrence in the Silver Creek sample may be a relict from earlier introductions of hatchery trout. Alternatively, or concurrently, their occurrence in the Silver Creek sample may be a result of the downstream drift of fish from Hayspur Hatchery (escapees) through Loving Creek into the Silver Creek population.

Based on our previous work on rainbow and redband trout (Williams et al., 1996; 1997), and other fishes (Powell and Faler 1998; Paragamian, Powell and Faler 1999), the rainbow trout in Silver Creek do not represent a single colonization event or assemblage of founders. The sequence divergence between mtDNA haplotypes (and the presumed time for that divergence to accrue in a natural system) is too high.

Initially, the divergence estimates of haplotypes within the Silver Creek and Hayspur samples pointed to either an admixture of redband and coastal forms or simply a mixture of coastal products from several historical inputs. Given the history of Silver Creek and possible introductions to it, including possible introductions from Hayspur, there seems a high likelihood of the latter being the case. The Hayspur Hatchery rainbow trout strain is a complete admixture of several commercial and indigenous rainbow trout strains selected and homogenized for hatchery performance (Williams et al. 1996; Powell and Williams unpublished data). The strain is commonly referred to as "R-9s" in hatchery parlance. The high diversity of mtDNA haplotypes and the high divergence among haplotypes in the Hayspur Hatchery strain, attributes of many established hatchery strains, is a result of the strain's mixed heritage.

Finally, our analysis showed no evidence of interior redband trout in either the Silver Creek or Hayspur Hatchery samples. Redband trout that we've analyzed from other locations in southern Idaho and the Kooteney River have all exhibited predominantly the "R" haplotype (Table 1). What stood out in Figure 4, is that neither of the two clusters of haplotypes (L-J-K, or I-M) directly joined or included the reference redband haplotype. Instead, the two clusters joined together first, then the redband haplotype joined the dendrogram last. This joining order and the large genetic distance between the redband haplotype and all others (1.16 – 1.90% sequence divergence), indicates that the Silver Creek and Hayspur samples do not contain any detectable amount of indigenous redband mitochondrial DNA. Rather, both populations appear to be mixtures of various coastal rainbow trout stocks, a characteristic that is common for many hatchery rainbow trout strains.

Literature Cited

- Campton, D. E., and J. M. Johnston. 1985. Electrophoretic evidence for a genetic admixture of native and nonnative rainbow trout in the Yakima River, Washington. *Transactions of the American Fisheries Society* 114:782-793.
- Currens, K. P., C. B. Schreck, and H. W. Li. 1990. Allozyme and morphological divergence of rainbow trout (*Oncorhynchus mykiss*) above and below waterfalls in the Deschutes River, Oregon. *Copeia* 1990:730-746.
- Felsenstein, J. 1993. PHYLIP: Phylogenetic inference package. University of Washington, Seattle, Washington.
- Nature Conservancy. 1966. Annual Report.
- McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1991. REAP: the restriction enzyme analysis package. Center for Marine Studies, University of Maine, Orono.
- Paragamian, V. L., M. S. Powell, and J. C. Faler. 1999 Mitochondrial DNA analysis of burbot *Lota lota* stocks in the Kootenai River Basin of British Columbia, Montana, and Idaho. *Trans. Am. Fish. Soc.* 128:868-874
- Powell, M. S. and J. C. Faler. 1999. Genetic analysis of threatened chinook salmon (*Oncorhynchus tshawytscha*) populations from the Snake River Basin. HFCES/University of Idaho Technical Report. University of Idaho, Moscow, ID. 16pp.
- Retallic, K., and R. Barker. 1996. Fly Fisher's Guide to Idaho. Wilderness Adventures Press, Gallatin Gateway, Montana.
- Williams, R. N., R. P. Evans, and D. K. Shiozawa. 1995. Genetic analysis of indigenous rainbow trout populations from the Bruneau and Jarbidge Rivers in Nevada. Clear Creek Genetics. Clear Creek Genetics Lab Report, 95-1. Meridian, Idaho.