

FINAL REPORT:

DESCRIBE EXISTING POPULATIONS AND DETERMINE APPROPRIATE  
SOURCE POPULATIONS FOR RESTORATION OF NATIVE TROUT SUBSPECIES  
IN ROCKY MOUNTAIN NATIONAL PARK UTILIZING MITOCHONDRIAL AND  
NUCLEAR DNA ANALYSIS

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## INTRODUCTION

The Rocky Mountain National Park harbors most of the known populations of the native greenback cutthroat, *Onchorhynchus lewisi stomias* and the native Colorado River cutthroat, *Onchorhynchus lewisi stomias*. Greenback cutthroat trout, whose native range is the east slope of the continental divide in Colorado, are listed as threatened under the federal Endangered Species Act (1978). The recovery plan for greenback cutthroat trout includes Rocky Mountain National Park as an important venue for the creation and maintenance of restoration populations (Young and Harig 2001). Unfortunately, the lakes, rivers and streams of the park have experienced numerous introductions of non-native trout, including brown, brook, rainbow and non-native cutthroat trout. Of these, both the rainbow and non-native cutthroat trout can hybridize with native greenbacks. Restoration efforts have largely eliminated non-native fish from the upper reaches of many streams and rivers, and today there a large number of self-sustaining populations of native greenback trout. It is unclear, however, whether all putatively native trout populations are pure, or if the gene pools are a composite of non-native rainbow, non-native cutthroat and native cutthroat trout.

The objective of the research funded by the National Park Service was to assess of the genetic purity of native greenback trout populations of Rocky Mountain National Park. Using mitochondrial and nuclear DNA markers, we determined genotypes for individuals collected from 39 localities. We discovered “contamination” of native gene pools by rainbow trout in one stream and by non-native (Yellowstone) cutthroat trout in 9 systems. In all but one case of contamination, there is evidence from stocking records for the past introduction of non-native fish.

## METHODS

Chris Kennedy (USFWS) provided a list of 30 priority populations for this contract. Netting or electroshocking was used to collect fish from each population and a portion of the adipose fin removed and preserved in ethanol. DNA was extracted using QIAGEN kits. To screen for non-native Yellowstone/Snake River cutthroat trout and rainbow trout, we used a diagnostic mitochondrial (mtDNA) restriction fragment length polymorphism. The primers used to amplify the cytochrome oxidase I mtDNA region were COI F6 (5'-atctctcagtaccaaacc-3') and COI- aH redo (5'-cacagtgrtaggcgtctgg-3') (Mitton et al. unpublished). To detect Yellowstone mtDNA, we digested the resulting COI polymerase chain reaction (pcr) fragment with the restriction enzyme HincII. In populations where Yellowstone was detected, pcr fragment was double-checked with a second restriction enzyme BsaBI. All samples with Yellowstone mtDNA showed concordant results with both enzymes. To detect rainbow mtDNA, the COI fragment was digested with the enzyme BSU361. Gel pictures are archived in notebooks at CU Boulder.

Since cutthroat and rainbow are approximately 2 million years diverged, enough genetic differences exist that diagnostic nuclear markers discerning the two species are readily available (Ostberg and Rodriguez 2002, 2004). We used one diagnostic nuclear marker, OCC16, to screen all populations for evidence of hybridization with rainbow trout. Using a nuclear marker in addition to the mtDNA marker increases our power to detect the presence of rainbow given a certain sample size. Both mtDNA markers and the nuclear marker were visualized and scored on 1.5% agarose gels. Gel pictures are archived in notebooks at CU Boulder.

Two sets of microsatellite loci were used. The first set of microsatellite data was generated in conjunction with Victoria Pritchard and David Cowley at University of New Mexico. A set of 11 different loci with four populations; two greenback populations from the Arkansas River Drainage (Graneros and Apache), Columbine Creek, and Como Creek. The microsatellite markers used were developed by Genetic Identification Services (<http://www.genetic-id-services.com>; Pritchard et al in prep). The second set used three loci on ten populations; Como Creek, Apache (Arkansas River Drainage), Columbine Creek, Roaring Creek, Hunters Creek, North Inlet, Pettingall Lake, Lake Nanita, Middle Hutcheson Lake, and Upper Hutcheson Lake. The microsatellites used Och5, Och16, and Och17 were developed by Peacock (2004). The data were generated at CU Boulder.

## RESULTS

### *Genetic Contamination?*

Characteristics of 30 priority populations are summarized in Table 1. The overwhelming impression is the Front Range drainages have been subject to a number of introductions of non-native fish. For example, there is evidence for transfer of fish from outside the system into the Big Thompson drainage in early 1920s through the 1940s, followed by the re-introduction of greenback in the late 1950s through the 1990s. None of the sites in the National Park appear to be historic native greenback populations.

Using mitochondrial and nuclear DNA markers, we screened individuals collected from 39 localities to assess genetic purity (Table 2 and Figure 1). We discovered “contamination” of native gene pools by rainbow trout in one stream (Cony Creek) and by non-native (Yellowstone) cutthroat trout in 9 systems. With the exception of Roaring River, there are records of stocking non-native fish for each case of contamination.

***East Slope:*** The waters that drain into the South Platte River and the Arkansas River historically contained greenback cutthroat trout (*O. c. stomias*).

***Big Thompson Drainage and Cache la Poudre Drainage:*** The populations surveyed in Big Thompson, North Fork of the Big Thompson, and Cache la Poudre drainages showed no evidence of rainbow or Yellowstone cutthroat trout genes.

***Fall River Drainage:*** Roaring River contained a low percentage (3%) of Yellowstone mtDNA. Stocking records show that a brook trout removal was done in 1983 and that the system has been re-stocked with BFTC broodstock. This population was thought to be pure greenback. Lawn Lake, Caddis Lake, and Ypsilon Lake showed no evidence for non-native trout genes.

***Glacier Creek Drainage:*** A second drainage with detectable non-native cutthroat trout is Glacier Creek. 31% and 14% of the individuals from Dream Lake and The Loch, respectively, had Yellowstone mtDNA. Dream Lake results are concordant with the record that it was stocked with fish from Upper Hutcheson, which we also found to contain Yellowstone genes. Bear Lake and Lake Husted showed no evidence for non-native genes.

***North St. Vrain Drainage:*** The Cony Creek area, which contains Cony Creek, the three Hutcheson lakes and Pear Lake showed varying levels of contamination. The portion of Cony Creek that is below a major waterfall barrier at approximately 3220m harbors a hybrid swarm between rainbow and cutthroat trout (Metcalf, in preparation) with an extremely low percentage of Yellowstone mtDNA. Presumably the rainbow trout are

present because of past introductions into Hutcheson Lakes and subsequent fish removal efforts failed to eliminate rainbows from the lower reaches of the stream above Calypso Falls. Above the major barrier, no rainbow mtDNA or nuclear DNA was detected; however, the percentage of Yellowstone mtDNA increased from 3% to 10%. Upper Cony Creek, Pear Creek, and Lower, Middle and Upper Hutcheson Lakes do not have any detectable rainbow trout alleles. Yellowstone mtDNA was not detected in Pear Creek above the confluence with Cony Creek. A barrier exists between Cony and Pear creek. Yellowstone genotype frequencies were similar in Lower and Middle Hutcheson Lakes, and Cony Creek; Upper Hutcheson Lake had the highest level of Yellowstone mtDNA in the system at 26%. We do not have nuclear markers that reliably distinguish greenback and Yellowstone cutthroat; however, we can be sure that the gene pool in these lakes, and in Cony Creek, contains alleles of both Greenback and Yellowstone trout. The presence of Yellowstone genes in these populations is not surprising because of documented introductions in 1952 (Table 1). Hunters Creek, Ouzel Lake, and Ouzel Creek showed no evidence of non-native trout genes.

**West Slope:** These waters drain into the Colorado River and historically contained Colorado River cutthroat

*East Inlet Drainage:* The unnamed lake near Paradise Creek contained 38% Yellowstone mtDNA; however, Adams lake contained only native mtDNA haplotypes.

*North Inlet Drainage:* North Inlet and Pettingell contained fairly low levels of Yellowstone mtDNA at 10% and 3% respectively. Ptarmigan Creek and Lake Nanita did not have any detectable non-native genes.

*Colorado River Drainage:* The upper Colorado River (at or above Lulu City) samples contained the highest level of Yellowstone mtDNA found in the 39 populations screened for this contract. Interestingly, this site received both rainbow and Yellowstone cutthroat trout up until about 1960 (Table 2), yet we did not detect any evidence for rainbow trout. Baker Creek, Onahu Creek, and Timber Lake showed no evidence for non-native cutthroat genes.

### ***Levels of variation and isolation***

We did two separate surveys of genetic variation using microsatellites. First, we used eleven loci and characterized three putative historic populations (Apache, Como and Graneros) and one population with a history of past introductions (Columbine). This survey provides a baseline assessment of variation in historical populations based on a relatively large number of loci. Second, we surveyed a larger number of populations with a subset of three genetic markers. This study was designed to assess relatedness of populations and relative amounts of genetic variation to look for the potential effects of past bottlenecks (or founder events associated with re-introductions) and population mixing (especially given the past history of introductions into the drainages).

For the putative historic populations, average heterozygosities were low relative to other published surveys of trout and other animals (Table 4a). Heterozygosity values were similar in putative historic populations (Como, Apache) and the restoration populations surveyed. However, estimates of theta ( $4N_e\mu$ , where  $N_e$  is the effective population size and  $\mu$  is the mutation rate) ranged widely, and were higher in populations with a history of past introductions than in historic populations (Figure 2).

Estimates of genetic differentiation between populations revealed that virtually all populations differ significantly from one another (Table 4). The lone exception was the comparison between Roaring Creek and Pettingal. It is possible that these two populations differ significantly, nonetheless, because so many pair-wise comparisons were performed. Significant  $F_{ST}$  values suggest all of the surveyed populations are isolated and rarely, if ever, swap genes.

## **DISCUSSION**

### ***Genetic Contamination?***

There is evidence for “contamination” of native cutthroat trout gene pools in Rocky Mountain National Park with rainbow and non-native cutthroat trout. Only one stream had rainbow trout, however, and less than a 1/3 had non-native cutthroat trout. In addition, in the populations with non-native cutthroat trout genes, most individuals had native genes.

It is important to stress that non-native cutthroat genes should not be considered as contaminating as rainbow trout genes. The non-native cutthroat genes are most likely derived from Yellowstone fish. Yellowstone and Colorado’s native trout are very closely related. Based on the mtDNA data, the two subspecies probably shared a common ancestry during the late Pleistocene, perhaps as recently as 20,000 years ago, roughly two orders of magnitude more closely related than rainbow trout and cutthroat trout. Such close genetic relatedness implies that the two species probably share identical DNA sequences across large portions of the genome. Furthermore, the hybrid fish and their descendents may enjoy a fitness enhancement because of the masking of part of the genetic load of deleterious recessive alleles in greenbacks due to population bottlenecks and isolation.

Nonetheless, it is clear that non-native cutthroat trout genes have become part of the native greenback cutthroat trout gene pool in Rocky Mountain National Park. For example, restoration efforts have propagated these non-native cutthroat genes when samples from Upper Hutcheson Lake were released into Dream Lake.

### ***Levels of Variability***

Putative historic populations harbored relatively little genetic variation, suggesting the populations surveyed have a relatively small effective population size. None of the populations with a history of introductions appear to have suffered from founder effects because genetic variation in these populations was higher than in the historic populations. This makes sense because most of these populations probably resulted from multiple introductions of different fish. For example, Yellowstone cutthroat trout were stocked into North Inlet in the 1940s (Table 1) and combined with the native trout that were present, and this may explain the extraordinary high value of theta (Figure 4).

### ***Management recommendations***

Significant population genetic structure was evident among all pairs of populations, suggesting most populations are isolated. On the one hand, isolation protects populations from invasion by non-native trout. Extremely low levels of hybridization with rainbow trout (only 1 stream of the 39 surveyed) occurred, and the trout in the system were probably resident (albeit introduced) rather than recent invaders. Such low

incidence of hybridization implies that the restoration populations are safe from non-native fishes, provided Johnny Troutseed is not wandering around the mountains with a bucketful of non-native *Onchorhynchus*. On the other hand, isolation implies that genetic drift and natural selection operate independently in each system, a phenomenon that will promote differentiation among populations. More importantly, isolation implies migrants from another population cannot rescue deleterious events that have occurred in one population because of small effective population size. Moreover, if a population goes extinct, re-colonization can only happen through management assisted restoration.

In the systems with non-native cutthroat trout, if the goal is genetically pure native fish, we recommend continuing the introduction of the genetically pure subspecies over time. Such a strategy will dilute the non-native genes and increase the likelihood of the loss of non-native genes by genetic drift. After a period of time, the non-native genes that survive will be those conferring increased fitness, and this class of genes is likely to represent a very small fraction of the non-native fish genome. **Based on the genetic results of this study and other unpublished results, Como Creek is probably the best source for restoration of populations provided that the abundant genetic variation in the Como Creek population is retained through the process of artificial propagation. In addition, Hunters Creek and the South Fork of the Poudre River also appear to conform closely to expectations of a historic greenback cutthroat population and would therefore be suitable for propagation and restoration purposes.**

### ***Cony Creek***

Only 1 stream harbored rainbow trout (Cony Creek). Importantly, this system is bounded by a barrier prohibiting migration of rainbow trout alleles from Cony Creek into the Hutcheson Lakes. In addition, all of the rainbow trout alleles were detected in hybrid fish, suggesting that pure rainbow trout are no longer present. This hybrid population is the subject of research on the dynamics of genomic interactions resulting from hybridization (Metcalf, in preparation). Examples of relevant conservation questions include: Is selection occurring for native genes or rainbow genes? If we find evidence for selection of rainbow genes, this could be a signal that cutthroat trout restoration populations are suffering from the effects of inbreeding. Do the nuclear and mtDNA from each species appear in the hybrids symmetrically? This is important information when interpreting genetic data that is presented in percentages of a certain marker type (mtDNA vs nuclear DNA). We would like to continue studies on the genetics of trout hybridization in the future; consequently, Cony Creek below the barrier is a valuable population for scientific reasons and we do not recommend eradication of the hybrid fish.

### ***On-going research***

The grant from the National Park Service provided the impetus and support for establishing collaboration among scientists studying the biology of inland cutthroat trout. We are currently working on a large scale analysis of genetic variation for mtDNA sequences and nuclear microsatellites that includes Greenback and Colorado River CT populations in Rocky Mountain National Park and elsewhere, Rio Grande CT populations in New Mexico, and Yellowstone (and related subspecies) CT from Idaho, Wyoming and Montana. Our collaborators include Mark Novak and colleagues from Utah State University and David Cowley and colleagues from the New Mexico State University.

These studies will be published in peer review journals in the future; copies of the manuscripts will be delivered to the National Park Service.

***Future recommendations for research***

Our survey was designed primarily to address the question of whether Rocky Mountain National Park waters harbor native cutthroat trout or non-native fish. The resounding answer is that native cutthroat trout are alive and thriving in the Park. However, there are a number of outstanding questions.

In the course of our survey, we examined variation at a gene under strong selection in populations because of its important role in the immune response. Our preliminary findings suggest two things. First, that the historic population harbor significant genetic variation, implying the populations are genetically “healthy”. Second, the restoration populations derived from hatchery-reared greenback cutthroat trout may have experienced a population bottleneck and suffer the effects of accumulated deleterious alleles. Our incomplete survey of genetic variation at “neutral” microsatellite loci does not provide a good indication of the effects of management strategies. Thus, targeted high-resolution genetic analyses of important functional genes in selected historic and restoration populations are necessary to gauge the effectiveness of restoration strategies.

We strongly urge studies of natural selection during hatchery rearing of greenback cutthroat trout. We suspect, as has been documented for other species of *Onchorhynchus*, that the hatchery setting may impose strong selection and consequently wipe out much of the genetic variation present in the brood stocks derived from historic populations.

Finally, we would like to resample the hybrid population in Cony Creek over time to better understand the dynamics of hybridization between introduced rainbow trout and native cutthroat trout.



**Table 1.** Rocky Mountain National Park populations surveyed using genetic techniques for this contract. Citations for stocking history can be found in

	<b>Population</b>	<b>Continental Divide Slope</b>	<b>Water</b>	<b>Stocking history (per Chris Kennedy, USFWS)</b>
1	Arrowhead Lake*	East	Big Thompson River	1915 – 30,000 native trout 1930's, 1944 – multiple, cutthroat, rainbow and Yellowstone, 1965/66 - unclear if fish present 1992- 5,000 greenback fry
2	Fern Lake*	East	Big Thompson River	1982 – antimycin removal of hybrid fish 1982-1984 – restocked with greenback from BFTC**
3	Big Thompson River - Forest Canyon*	East	Big Thompson River	1922 – 165,000 “native trout” possibly from Trappers Lake (CRC) 1930s – possibly cutthroat and rainbow 1959 – greenback
4	Gorge Lakes Stream/ Little Rock Lake*	East	Big Thompson River	Early 1900's – apparently unsuccessful stocking attempts 1930's – stockings in Arrowhead may have migrated; cutthroat and rainbow
5	Spruce Lake*	East	Big Thompson River	
6	North Fork Big Thompson River	East	North Fork Big Thompson River	
7	Lake Husted	East	North Fork Big Thompson River	1986 and 1990 brook trout removal 1991 – 1992 – gbc stocked from BFTC** and FRH
8	Lake Louise*	East	North Fork Big Thompson River	
9	Upper West Creek (above WC Falls)	East	North Fork Big Thompson River	1978 – antimycin removal of hybrid fish above West Creek Falls 1979 – greenback from Hidden Valley Creek stocked 1984 – 1989 – stocked BFTC**
10	South Fork Cache la Poudre River	East	Cache la Poudre River	Most of this River is in Roosevelt National Forest. 1939 - YCT CDOW stocked 10 adult greenback from roaring creek recently
11	Hague Creek	East	Cache la Poudre River	1936 – black spotted trout stocked 1940 – fry stocked
12	Caddis Lake*	East	Fall River	1958 – rotenone 1959 – 209 fish from Forest Canyon 1960s – 2,000 cutthroat Leadville National Fish Hatchery; 2,000 cutthroat Estes Park Hatchery
13	Lawn Lake*	East	Fall River (above RR)	1983 – Brook trout removal with antimycin and rotenone, restocked with greenback from BFTC** over 3 years
14	Roaring River*	East	Fall River	1983 – Brook trout removal with antimycin and rotenone, restocked with greenback from BFTC** over 3 years
15	Ypsilon Lake*	East	Fall River	1915-1966 extensive stocking: rainbow, brook, native cutthroat, Yellowstone This lake may have become fishless at times and now reflect only later native stockings
16	Bear Lake*	East	Glacier Creek	1975 – antimycin for brook trout removal, restocked with Como Fish 1981 – greenback from BFTC**
17	The Loch	East	Glacier Creek	Owned by Abner Sprague until 1926 1914 – stocked with rainbow and cutthroat 1920's-1950s – extensive stocking, native cutthroat, Yellowstone, unknown
18	Dream Lake*	East	Glacier Creek	1996 – fish removal, restocking from Upper Hutcheson Lake
19		East	North St. Vrain	1938 – fishless

	Cony Creek*		Creek	1940s – Yellowstone cutthroat 1941 – Rainbow reported up to Pear Lake outside the Park until 1981
20	Lower Hutcheson Lake*	East	North St. Vrain Creek	Outside Park management until 1981 1987 – antimycin hybrid fish removal 1989 – greenback restocked BFTC**
21	Middle Hutcheson*	East	North St. Vrain Creek	Outside Park management until 1981 1952 – Yellowstone into Boundary Lakes
22	Upper Hutcheson Lake*	East	North St. Vrain Creek	1931 – reported fishless 1952 – Yellowstone into Boundary Lakes 1964 – cutthroat from Bellevue SFH
23	Ouzel Creek*	East	North St. Vrain Creek	1980 – brook trout removal above Ouzel Falls 1981-1983- restocked greenback from BFTC**
24	Ouzel Lake	East	North St. Vrain Creek	1980 – brook trout removal 1981-1983- restocked greenback from BFTC**
25	Hunters Creek*	East	North St. Vrain Creek	Fish present before 1939 (1 contradicting report), likely due to irrigation work and from St. Vrain 1939 – Yellowstone
26	Pear Lake*	East	North St. Vrain Creek	1988- antimycin hybrid removal 1989-1990 – stocked BFTC**
27	Adams Lake*	West	East Inlet	1925 –native trout State of CO Hatchery, unclear if successful 1942 –Yellowstone
28	Paradise Creek* - unnamed lake b/w boundary Adams lake	West	East Inlet	1925-1947 - Paradise Creek stocked “black spotted” fry Boundary Lake (headwaters of Paradise) outside Park until 1981, stocked with Trappers lake CRC
29	Lake Nanita*	West	North Inlet	1918 – cutthroat, probably Grand Lake SFH 1931- cutthroat trout stocked, cutthroat reported present 1966 - cutthroat
30	North Inlet*	West	North Inlet	1878- diary account of native trout present (but not at altitude of 2002 USFWS sampling) 1937- cutthroat eggs from stated of Colorado in upper North Inlet 1939 – native trout 1940’s – multiple Yellowstone
31	Pettingell Lake*	West	North Inlet	1931- account of cutthroat present 1934- cutthroat Grand Lake Fish Hatchery 1964- cutthroat Bellevue SFH
32	Ptarmigan Creek*	West	North Inlet	1985 – Yellowstone population removed with antimycin, restocked with Colorado River Cutthroat from Williamson Lakes, CA (representing Trapper’s Lake
33	Columbine Creek*	West	Lower Colorado River	1915- 10,000 cutthroat, likely Yellowstone 1930’s – Hatchery cutthroat and Yellowstone unclear if these were in the area currently containing cutthroat
34	Baker Creek	West	Upper Colorado River	Forest Service, Above Grand Ditch
35	Colorado River (COL–Lulu city 2002) (CO - 2000)	West	Upper Colorado River	1931 – 1960 – cutthroat and rainbow stocked
36	Onahu Creek*	West	Upper Colorado River	Julian Lake stocked possibly before 1931 1934 – Julian Lake cutthroat from state of Colorado – possible downstream into Onahu 1940’s- Yellowstone 1959 – cutthroat from Rifle SFH
37	Timber Lake*	West	Upper Colorado River	1979 – non-native cutthroat removal antimycin, restocked with Colorado River cutthroat from Clinton Gulch

\* Listed by USFWS (Chris Kennedy) as top 30 priority population

\*\* BFTC – Bozeman Fish Technology Center were derived from Como Creek fish

**Table 2.** Populations are listed with US FWS and CU Boulder sample label designation, water drainage, and proportion of native and non-native (rainbow trout and Yellowstone/Snake River cutthroat trout) mitochondrial DNA present.

	Population	USFWS/CU Sample Label	Water	Rainbow nuclear Present?	Rainbow mtDNA present?	Yellowstone mtDNA present?
1	Arrowhead Lake*	AR	Big Thompson River	NO 0/5	NO 0/5	NO 0/5
2	Fern Lake*	FN	Big Thompson River	NO 0/44	NO 0/35	NO 0/35
3	Forest Canyon*	FC	Big Thompson River	NO 0/31	NO 0/32	NO 0/32
4	Gorge Lakes Stream/ Little Rock Lake*	GG	Big Thompson River	NO 0/26	NO 0/32	NO 0/32
5	Spruce Lake*	SP	Big Thompson River	NO 0/40	NO 0/40	NO 0/38
6	Big Thompson River – North Fork	NF	Big Thompson River	NO 0/15	NO 0/15	NO 0/15
7	Lake Husted	HU	North Fork Big Thompson River		NO 0/32	NO 0/32
8	Lake Louise*	LU	North Fork Big Thompson River	NO 0/46	NO 0/62	NO 0/62
9	Upper West Creek	WU	North Fork Big Thompson River	NO 0/42	NO 0/42	NO 0/42
10	South Fork Cache la Poudre River	Bean Museum # 131818 – 131824 GBC SF SF CDOW	Cache la Poudre River	NO 0/28	NO 0/26	NO 0/27
11	Hague Creek	Hague	Cache la Poudre River	NO 0/16	NO 0/16	NO 0/16
12	Caddis Lake*	CA	Fall River	NO 0/31	NO 0/32	NO 0/32
13	Lawn Lake*	LN Lawn	Fall River (above RR)	NO 0/44	NO 0/31	NO 0/32
14	Roaring River*	RR	Fall River	NO 0/30	NO 0/28	<b>YES</b> 3/30 3%
15	Ypsilon Lake*	YP	Fall River	NO 0/37	NO 0/38	NO 0/38
16	Bear Lake*	BR	Glacier Creek	NO	NO	NO

			0/20	0/20	0/20	
17	Dream Lake*	DM	Glacier Creek	NO 0/29	NO 0/32	<b>YES</b> 10/32 31%
18	The Loch	LV	Glacier Creek	NO 0/14	NO 0/14	<b>YES</b> 2/14 14%
19	Cony Creek* Below barrier	CN Cony	North St. Vrain Creek	<b>YES</b> 1175/4802** 24%	<b>YES</b> 153/343 45%	<b>YES</b> 1/156 1%
20	Cony Creek* Above barrier	Cony	North St. Vrain Creek	NO 0/1360	NO 0/80	<b>YES</b> 7/68 10%
21	Hunters Creek*	HN	North St. Vrain Creek	NO 0/27	NO 0/29	NO 0/28
22	Lower Hutcheson Lake*	LH	North St. Vrain Creek	NO 0/29	NO 0/21	<b>YES</b> 2/23 9%
23	Middle Hutcheson*	MH	North St. Vrain Creek	NO 0/32	NO 0/32	<b>YES</b> 4/64 6%
24	Upper Hutcheson Lake*	UH	North St. Vrain Creek	NO 0/28	NO 0/36	<b>YES</b> 11/43 26%
25	Ouzel Creek*	OZ	North St. Vrain Creek	NO 0/29	NO 0/33	NO 0/30
26	Ouzel Lake	OL	North St. Vrain Creek	NO 0/10	NO 0/10	NO 0/10
27	Pear Lake*	PR	North St. Vrain Creek	NO 0/12	NO 0/12	NO 0/12
28	Adams Lake*	AD	East Inlet	NO 0/38	NO 0/41	NO 0/41
29	Paradise Creek* - unnamed lake b/w Boundary & Adams lake	PDU	East Inlet	NO 0/30	NO 0/31	<b>YES</b> 12/32 38%
30	Lake Nanita*	NN	North Inlet	NO 0/54	NO 0/61	NO 0/61
31	North Inlet*	NI	North Inlet	NO 0/32	NO 0/32	<b>YES</b> 4/40 10%
32	Pettingell Lake*	PET	North Inlet	NO 0/34	NO 0/34	<b>YES</b> 1/34 3%
33	Ptarmigan Creek*	PT	North Inlet	NO 0/15	NO 0/15	NO 0/15
34	Columbine Creek*	CC	Lower Colorado River	NO 0/35	NO 0/32	NO 0/32

35	Baker Creek	BK	Upper Colorado River	NO 0/15	NO 0/14	NO 0/14
36	Colorado River	COL 2002 CO 2000	Upper Colorado River	NO 0/22	NO 0/27	<b>YES</b> 14/34 41%
37	Onahu Creek*	ON	Upper Colorado River	NO 0/11	NO 0/11	NO 0/11
38	Timber Lake*	TM	Upper Colorado River	NO 0/19	NO 0/19	NO 0/19

\*\* Cony Creek population is part of Jessica Metcalf's dissertation work on the hybrid dynamics between cutthroat trout and rainbow trout. The rainbow nuclear ratio in the below barrier population represents 7 nuclear markers times 343 individuals.

**Table 3**

**a.** Average heterogosity ( $H_o$ ) based on eleven loci for three putative historic populations (Como, Apache, and Graneros) and one population with a history of past introductions (CC = Columbine Creek).

	Como	Apache	CC	Graneros
$H_o$	0.41	0.45	0.67	0.50

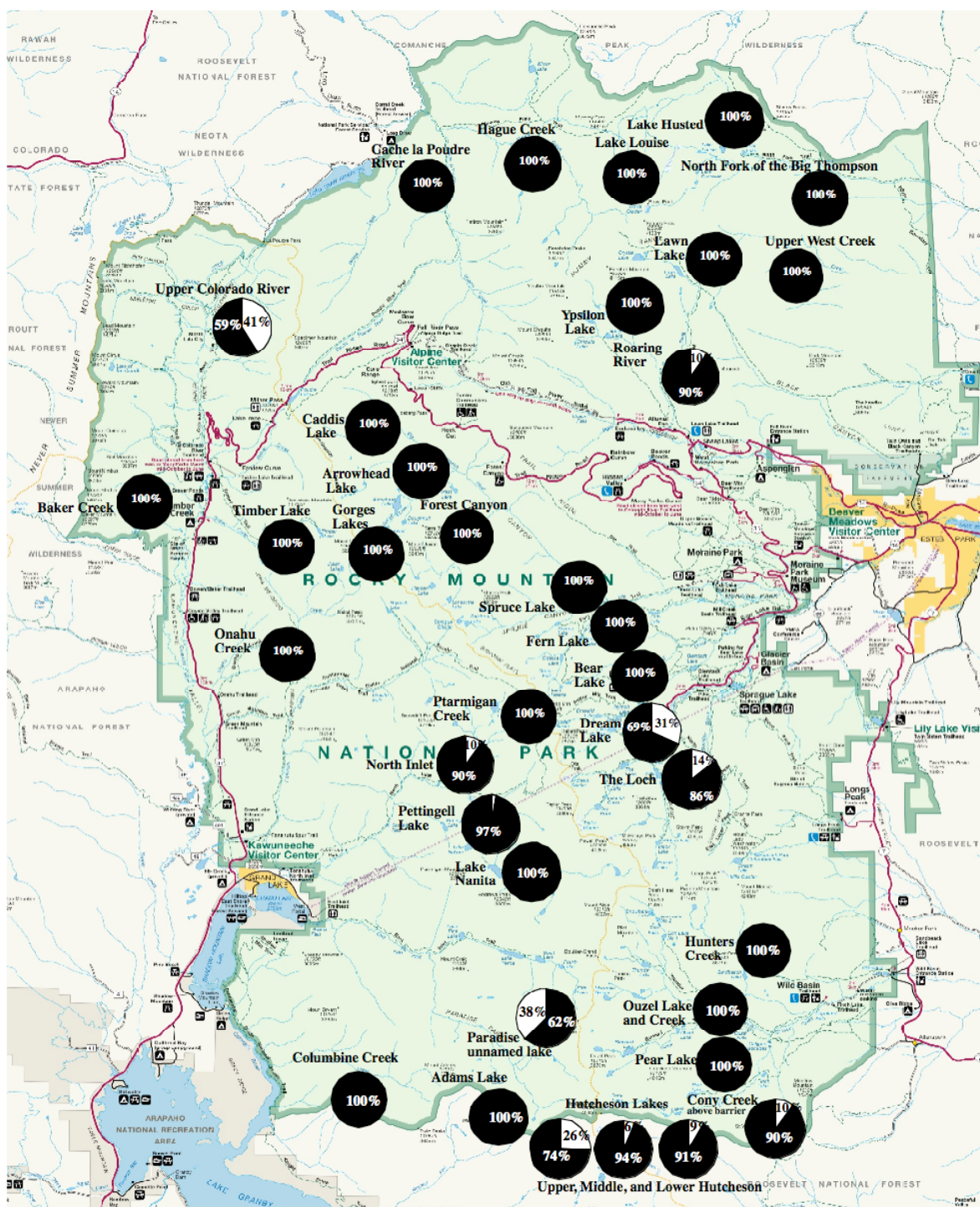
**b.** Average heterozygosity for ten populations based on three microsatellites. Including two putative historic populations (Como, Apache) and 8 populations with a history of past introductions (CC = Columbine Creek, RC = Roaring Creek, HN = Hunters Creek, NI = North Inlet, PET = Pettingal, MH = Middle Hutcheson, N = Nanita, and UH = Upper Hutcheson).

	Como	Apache	CC	RC	HN	NI	PET	MH	N	UH
$H_o$	0.52	0.55	0.77	0.60	0.55	0.70	0.45	0.57	0.52	0.58

**Table 4.** Fst values (lower triangle) and significance (upper triangle) for ten populations based on 3 microsatellites. All populations showed significant differentiation except for Roaring Creek and Pettingall.

	Como	Apache	CC	RC	HN	NI	PET	MH	NN	UH
Como		+	+	+	+	+	+	+	+	+
Apache	0.370		+	+	+	+	+	+	+	+
Columbine	0.296	0.184		+	+	+	+	+	+	+
Roaring Creek	0.351	0.062	0.149		+	+	-	+	+	+
Hunters Creek	0.390	0.140	0.161	0.065		+	+	+	+	+
North Inlet	0.298	0.089	0.101	0.021	0.137		+	+	+	+
Pettingal	0.390	0.104	0.169	0.008	0.050	0.092		+	+	+
Middle Hutcheson	0.368	0.102	0.157	0.055	0.139	0.054	0.094		+	+
Nanita	0.387	0.111	0.183	0.010	0.126	0.048	0.035	0.062		+
Upper Hutcheson	0.377	0.061	0.165	0.030	0.147	0.044	0.070	0.017	0.033	





**Figure 1.** Percentage of native cutthroat (black) mitochondria DNA versus Yellowstone/Snake River cutthroat (white) mitochondrial DNA detected in each surveyed population.

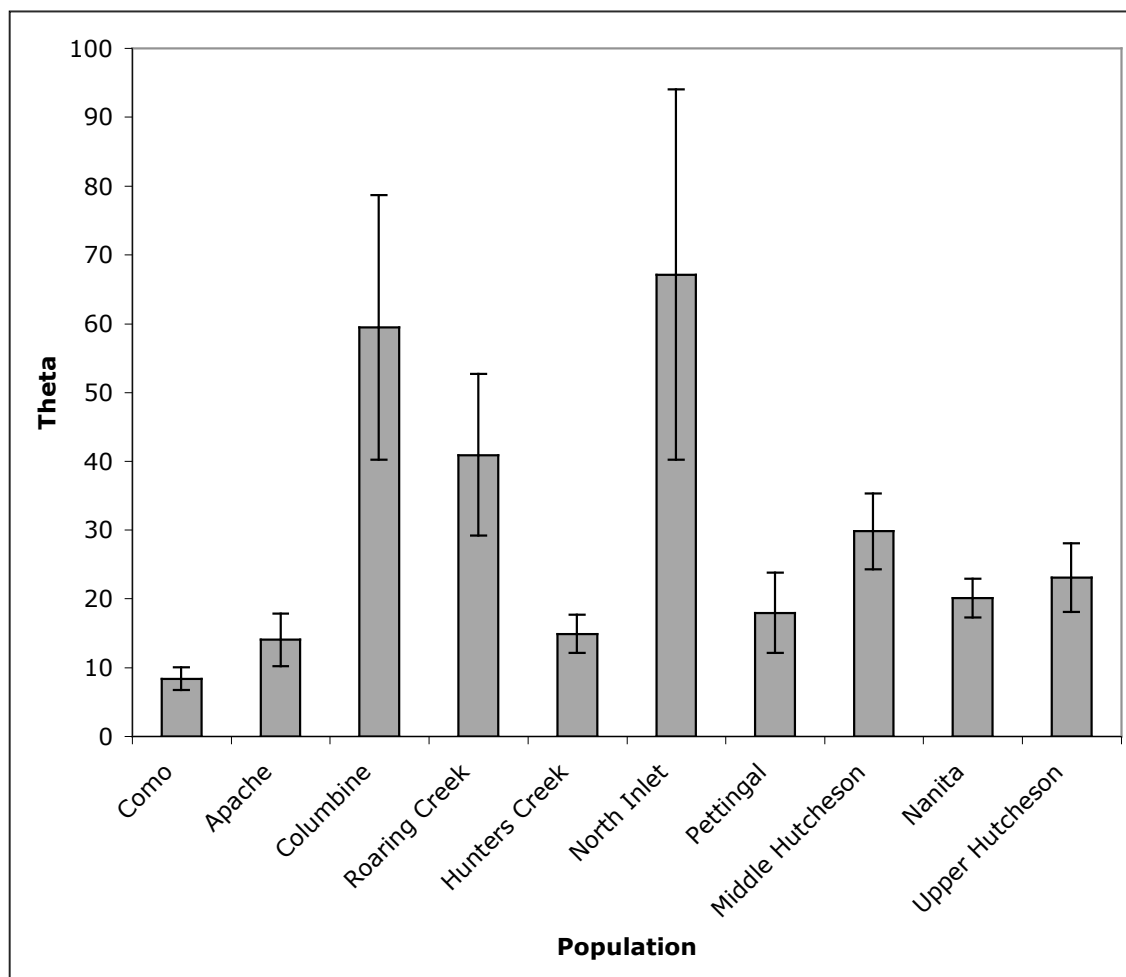


Figure 2. Theta ( $4Ne\mu$ ) values with standard deviations for ten populations based on three microsatellite loci. Note that theta values for the putative historic populations (Como and Apache) are less than for all restoration populations.

Appendix. Proportion of non-native cutthroat trout mtDNA genes (and the standard error) in Greenback and Colorado River cutthroat trout populations with evidence for non-native genes.

<b>Population</b>	<b>Proportion Non-native</b>	<b>Standard Error</b>
Roaring River	0.10	0.05
Dream Lake	0.31	0.08
The Loch	0.14	0.09
Cony Creek (above barrier)	0.10	0.04
Lower Hutcheson	0.09	0.06
Middle Hutcheson	0.06	0.03
Upper Hutcheson	0.26	0.02
Paradise Creek	0.38	0.09
North Inlet	0.10	0.05
Pettingell Lake	0.03	0.03
Colorado River	0.41	0.08

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