

Yellowstone Cutthroat Trout Survey, Monitoring, and Restoration - 2006



Final report

by

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ACKNOWLEDGEMENTS

The Henry's Fork Foundation (HFF) fisheries crew in the summer of 2006 was composed of Michael Wilson (crew leader), Steve Rood (R. A. James Greene Scholarship/Colgate University), Jon McKibben (Don C. Byers Memorial Scholarship/Brigham Young University-Idaho), and Ashley Donohoe, Eric Gagne, and John Bovay (A. Paul Knight Memorial Scholarships/Washington and Lee University); they collected most of the survey and monitoring field data contained herein.

Lee Mabey (Caribou-Targhee National Forest; CTNF) was instrumental in making this project possible; including equipment procurement, logistical support, and field assistance. Dan Garren (Idaho Department of Fish and Game; IDFG) led the Yellowstone cutthroat trout restoration work for the Sawtell Creeks and the electrofishing survey of the Camas Creek mainstem. Trapper Bradshaw and Justin Smith (IDFG) assisted with the Sawtell Creek restoration project and Jo Cobb and Kori Blakely (IDFG) assisted with the Camas Creek survey work. Darren Rhea and Eric Gardenunio (Wyoming Department of Game and Fish) assisted with the Boone Creek surveys.

Pat Koelsch (Bureau of Land Management) coordinated the Medicine Lodge drainage monitoring work and provided guidance in the field. Corey Lyman and Brad Higginson (CTNF) assisted with the Fall River surveys in Yellowstone National Park. Bryan Jones (BYU-Idaho) assisted with the Fall River and Wyoming Creek surveys. Joseph Dryden and Spencer Graham (BYU-Idaho) assisted with the Wyoming Creek survey.

Matt Campbell of the IDFG Eagle Fish Genetics Lab completed the genetic analysis of all putative YCT samples. Steve Trafton and Joan Rice of HFF provided project support.

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Cover photo: Yellowstone cutthroat trout from Tygee Creek are released into Sawtell Creek as part of the restoration project, August 3, 2006.

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EXECUTIVE SUMMARY

Stream surveys, stream monitoring, and Yellowstone cutthroat trout (YCT) restoration took place in the Henrys Fork and Sinks drainages in 2006. Surveys and monitoring were conducted by electrofishing to provide data on YCT distribution, abundance, and genetic status.

Thirty-seven sites were surveyed on 22 streams in the Henrys Fork and two sites were surveyed on Camas Creek in the Sinks Drainage in 2006. In the Henrys Fork Drainage, twenty-three (62%) of the sites had water, and fourteen sites (38%) were dry. Of the sites with water; 10 (43%) had no trout, 6 (26%) had brook trout only, 2 (9%) had cutthroat trout only, 2 (9%) had rainbow trout only, 1 (4%) had cutthroat trout and hybrids, 1 (4%) had rainbow trout only, and 1 (4%) was not electrofished. In the Sinks Drainage, brown trout were found at one site and no trout in the other site.

Genetic analyses were completed on 155 Yellowstone cutthroat trout from three streams in the Henrys Fork: Jackass Creek, Wyoming Creek, Tygee Creek; and two streams in the Sink drainages: Middle Dry Creek and East Fork Rattlesnake Creek. No hybridization or introgression was found in the fish from Jackass Creek, but both Wyoming Creek and Rattlesnake Creek had hybridization and introgression. Yellowstone cutthroat trout from all five streams had the same haplotype (Hap12); it was fixed in Tygee, Jackass, and East Fork Rattlesnake creeks, and was the dominant haplotype observed in Wyoming and Middle Dry creeks. The haplotypes observed do not provide evidence for, or against, the currently accepted theory that Yellowstone cutthroat trout are native to the Sinks Drainage.

The only abundance data collected from a genetically pure Yellowstone cutthroat trout population in 2006 was at Jackass Creek. An abundance of 12 YCT > 100 mm/100 m of stream length was estimated; this is similar to abundance estimates from past fisheries surveys of this creek.

Monitoring of BLM sites in 2006 found significant declines in fish numbers and species presence compared to previous surveys, including the absence of YCT from three sites and decreases in two others. Similarly, rainbow trout were absent from six sites. YCT only were found within two sites, but it is likely that these fish are hybridized or introgressed with rainbow trout.

Yellowstone cutthroat trout were restored to two forks of Sawtell Creek for a total of about 8.5 km of stream length. This successful project will serve as a template for the restoration of Yellowstone cutthroat trout into other headwater streams in the Henrys Fork and Sinks drainages.

INTRODUCTION

Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*, YCT) evolved as the only trout in the Snake River above Shoshone Falls and the Yellowstone River upstream of the Tongue River (Behnke 1992). The distribution and abundance of this cutthroat trout subspecies has declined; extirpation or introgressive hybridization of YCT has occurred in over 75% of their historical stream habitat (May et al. 2003). In 1998, YCT were petitioned for listing as “threatened” under the Endangered Species Act. The U.S. Fish and Wildlife Service (USFWS) issued a 90-day finding of “not warranted” in 2001 (USFWS 2001) and a full status review finding of “not warranted” in 2006 (USFWS 2006). YCT are considered a “Sensitive Species” or “Species of Special Concern” by the U.S. Forest Service, the American Fisheries Society, and in all states (Idaho, Wyoming, Montana, Utah, and Nevada) that they inhabit.

The distribution of YCT in the Henrys Fork and the Sinks drainages has declined to a greater extent than that seen throughout most other drainages of the subspecies’ range. Through 1999, Yellowstone cutthroat trout were found in about 17% of total fish-bearing habitat surveyed in the Henrys Fork (excluding the Teton River drainage) and only 3% contained cutthroat trout isolated from other nonnative salmonids, such as rainbow trout (*O. mykiss*) and brook trout (*Salvelinus fontinalis*; Jaeger et al. 2000). Meyer and Lamansky (2003) stated that only 12 streams in the Henrys Fork drainage are currently known to contain Yellowstone cutthroat trout. In the Sinks drainages, cutthroat trout inhabited 43% of fish bearing habitat surveyed and were the only trout present in 19% of fish-bearing habitat (Jaeger et al. 2000). However, the degree of isolation of cutthroat trout from other salmonids in the Sinks drainage is largely unknown, therefore these populations may be at high risk of invasion (Jaeger et al. 2000).

The Henry's Fork Foundation (HFF) has recently compiled all fisheries survey data collected by the Caribou-Targhee National Forest (CTNF), the Idaho Department of Fish and Game (IDFG), the Bureau of Land Management (BLM), the Wyoming Game and Fish Department (WGFD), and HFF in the Henrys Fork and Sinks drainages from 1996 to 2004. Preliminary results of the YCT Status Summary indicate that the distribution of the cutthroat trout in Henrys Fork and Sinks drainages has continued to decline in recent years. Rainbow trout or hybrids (*O. clarki bouvieri* x *O. mykiss*) have been found during the past five years in what were assumed to be YCT-only populations. These recent findings highlight the need to complete surveys on the streams in which YCT remain, and to strengthen our knowledge and monitoring of existing YCT populations. Similarly, the need for more rigorous study and definitive descriptions of YCT in the Henrys Fork and Sinks drainages was noted following fisheries surveys throughout the upper Snake River Basin in Idaho (Meyer and Lamansky 2003).

Limited genetic information has been collected for YCT populations in the Henrys Fork and Sinks drainages, and it was recommended that all remaining isolated cutthroat trout populations be genetically evaluated (Jaeger et al. 2000). The need for testing of YCT populations is evident by the increasing invasion of rainbow trout, which has potentially increased the rate of hybridization and introgression of YCT populations. Small, isolated populations may also have limited genetic diversity because of the effects of genetic drift.

In addition, it may be possible to determine the relatedness of populations by looking at the haplotype frequencies present in populations. For example, the Beaver-Camas and Medicine Lodge watersheds are thought to be within the YCT historic range (Gammet 2003), but genetic proof of this relationship is not available. We were interested in whether comparisons of haplotype frequencies among YCT populations in the Henrys Fork Drainage and Sinks Drainage would provide evidence to support that the Sinks Drainage is part of the historic YCT range.

The decline in YCT distribution and abundance has prompted agencies and organizations to pursue restoration efforts in the Henrys Fork and Sinks drainages. One of these restoration efforts is at Sawtell Creek, which contains two headwater stream branches in the northern part of the Henrys Fork Drainage. Brook trout were chemically removed from these two stream branches in 2003 and the piscicide treatment was repeated in 2004. In addition, habitat restoration work had been ongoing in preparation for YCT reintroduction in 2006.

The objectives of the YCT Survey, Monitoring, and Restoration Project are: 1) complete fisheries surveys on the remaining unsurveyed streams that may support YCT in the Henrys Fork and Sinks drainages; 2) resurvey eight fisheries monitoring sites (BLM) in the Medicine Lodge watershed; and 3) restore YCT to the branches of Sawtell Creek.

METHODS

Stream Survey

Stream lengths selected for sampling were identified from the YCT Status Summary. Past survey sites were plotted on a 1:100,000 Geographic Information Systems (GIS) streams layer. These data were used to classify stream lengths as unsurveyed or surveyed. Unsurveyed stream lengths, or those that had limited snorkeling data, were the focus for survey in 2006.

Survey sites were selected and surveyed according to the methodology that was developed by agencies and organizations in 2005 (De Rito and Emery-Miller 2006). Sampling sites were selected within identified stream lengths using a random-systematic method and GIS measurement tools. The initial downstream sampling site was randomly selected within the first two kilometers of the unsurveyed stream length and then successive upstream sites were located every two kilometers systematically for the extent of the stream length. If the stream was fewer than four kilometers in length then the midpoint was selected as the sampling site. For every three sampling sites, one site was selected randomly for three-pass electrofishing and the remaining two sites were selected as one-pass electrofishing sites. If only a single site was located on a stream, then it was typically a three-pass electrofishing site. All sampling sites were assigned Global Positioning System (GPS) coordinates (Universal Transverse Mercator (UTM), North American Datum 1927) using www.topozone.com.

The UTM coordinates and handheld GPS units were used in the field to locate stream sampling sites. If no water was found at a site then no additional data, besides pictures, were collected. If a site had water, then a 100-m length of stream was delineated with a measuring tape. Block nets were set up across the stream at the beginning and end of the site. One or two backpack electrofishing units (Smith-Root Model 15-D) were used to sample fish within a site. The survey of the mainstem of Camas Creek was conducted with a canoe equipped for electrofishing, because the creek was too large to efficiently sample with backpack electrofishing gear.

At multiple-pass sites, electrofishing passes were conducted until fewer than 40 % of trout from the previous pass were captured. No additional passes were made if no fish were collected. Population estimates and 95% confidence intervals were calculated with MicroFish (2005). The fork length of captured fish was measured to the nearest millimeter.

Genetic samples were collected from all fish of the genus *Oncorhynchus* (cutthroat trout, rainbow trout and suspected hybrids) encountered at a site. If few *Oncorhynchus* were captured within a 100-m site then spot electrofishing above and below the site typically occurred to capture additional fish. Larger fish were preferred for genetic sampling, because they were less likely to be siblings. The adipose fin was clipped on fish > 150 mm, whereas a pelvic fin was clipped on fish < 150 mm. Fin clips were stored in individual vials of ethanol alcohol and the putative species labeled. In addition, existing samples of Yellowstone cutthroat trout from three populations in the Henrys Fork and Sinks drainages were utilized for genetic testing.

Genetic samples were analyzed at the IDFG Eagle Fish Genetics Lab. Individual samples were genetically identified by screening with six nuclear DNA markers (diagnostic between Yellowstone cutthroat trout and rainbow trout). Samples were also analyzed using mtDNA sequencing analysis to determine degree of similarity among populations in the Henrys Fork and Sinks drainages (see Appendix A: Campbell 2008)

Habitat measurements were made within sampling sites upon completion of electrofishing. The wetted width was measured to the decimeter and depth to the centimeter at four transects at 0, 33, 66, and 100 m within the site and averaged. The number of pools and large woody debris pieces (singles and aggregates) were counted within the site. The percentage of stable banks was estimated along with substrate composition within riffles and pool tailouts. Comments regarding habitat quality, morphological characteristics, restoration opportunities, etc. were recorded. Photographs were taken at the beginning and end of a site (looking upstream and downstream), and of any interesting or representative features.

Stream Monitoring

BLM established eight fisheries monitoring sites on Horse, Indian, Irving, and Middle creeks in the Medicine Lodge watershed in 1997, 1999, or 2001. Sites were about 100 m in length (range 93 m to 132 m) and two-pass electrofishing was conducted at all sites. Population estimates were calculated for all trout > 100 mm in length (BLM unpublished data). All sites contained trout at the time of initial survey. Six sites contained cutthroat trout and rainbow trout/hybrids; two of these sites also had brook trout. Two sites had no cutthroat trout, but had rainbow trout/hybrids and brook trout.

In 2006, HFF resurveyed the above BLM sites. Fish and habitat sampling and analysis were similar to that described in the Henrys Fork Drainage. Multiple-pass depletion electrofishing was conducted at all sites, except Upper Middle Creek where only single-pass electrofishing was conducted. Fish population estimates and species composition are compared between the original surveys of these monitoring sites and those completed in 2006.

Cutthroat Trout Restoration

The north and south branches of Sawtell Creek are located along the east slope of Sawtell Mountain (Figure 1). The creeks do not connect to one another and are no longer connected to the Henrys Lake Outlet because of irrigation diversion and natural dewatering. There are about 5 km of perennial water flow in the south branch and about 3.5 km of perennial water flow in the north branch. In 2003 and 2004, the creeks were treated with piscicide to remove brook trout populations during a cooperative effort by the IDFG, CTNF, and HFF. Electrofishing was conducted by HFF on about 2.2 km of the south branch of Sawtell Creek during 2006 to confirm that brook trout were no longer present in the creek, prior to the reintroduction of YCT.

Habitat restoration work took place on the two creek branches to improve pond and stream habitat during 2003 to 2006 in preparation for YCT reintroduction. The habitat restoration work was led by the CTNF. HFF served as the fiduciary sponsor of the project. Two former irrigation ponds (about 6 acres total) were rehabilitated on the lower ends of both creek branches to improve fish habitat. This work involved dredging, outlet reconstruction, and stabilization of these ponds with a backhoe. Also, about 671 m of jack fence was built around the North Sawtell pond to exclude cattle. Natural stream channel conditions were restored above and below the ponds in areas that had been previously ditched.

Source populations of YCT for the 2006 restoration of Sawtell Creek were identified by the following criteria; 1) the populations contained only YCT and had no known, or suspected, rainbow trout introgression; 2) populations were from stream residents in small creeks, similar to the branches of Sawtell Creek; and 3) populations were geographically near Sawtell Creek, to account for similar genetic structuring and to facilitate translocation of fish.

RESULTS

Stream Survey

Thirty-seven sites were surveyed on 22 streams in the Henrys Fork watershed within the Caribou-Targhee National Forest (N = 24 sites on 16 streams), Yellowstone National Park (N = 7 sites on 2 streams), or private land (N = 6 sites on 4 streams) between June 29, 2006 and November 1, 2006 (Figure 1).

Twenty-three (62%) of the sites had water, whereas fourteen sites (38%) were dry. Of the sites with water; 10 (43%) had no trout, 6 (26%) had brook trout only, 2 (9%) had cutthroat trout only, 2 (9%) had rainbow trout only, 1 (4%) had both cutthroat trout and hybrids (cutthroat trout x rainbow trout), 1 (4%) had rainbow trout only, and 1 (4%) was not surveyed on Willow Creek, because it was located in a pond.

Cutthroat trout were sampled for genetic testing from Jackass Creek, Wyoming Creek, and the Fall River (along with hybrids) near the Grassy Creek confluence in Yellowstone National Park. Fall River fish were not genetically tested because it was apparent from phenotypic identification that hybrids were present and this population was likely introgressed. Genetic analyses were completed on 155 Yellowstone cutthroat trout from three streams in the Henrys Fork drainage: Jackass, and Wyoming, Tygee creeks; and two streams in the Sinks drainages: Middle Dry and East Fork Rattlesnake (Figures 1 and 2; see Appendix A: Campbell 2008). No hybrids or introgression were found in the fish from Jackass Creek, but both Wyoming Creek and Rattlesnake Creek had hybridization and introgression with rainbow trout. Over half (11/21) of the Wyoming Creek fish were post F1 hybrids, but the level of introgression was fairly low (~ 5 %). Analyses from East Fork Rattlesnake Creek produced some conflicting results, but there does appear to be some hybridization with rainbow trout (see Appendix A: Campbell 2008). Hybridization or introgression was previously found within fish from Middle Dry Creek, but not Tygee Creek.

Yellowstone cutthroat trout from all five streams had the same haplotype (Hap12); it was fixed (the only haplotype) in Tygee, Jackass, and East Fork Rattlesnake creeks, and it was the dominant haplotype observed in Wyoming and Middle Dry creeks (see Appendix A: Campbell 2008). A single fish from both Wyoming Creek and Middle Dry Creeks had haplotypes that were most similar to rainbow trout (Middle Dry Creek) or westslope cutthroat trout (Wyoming Creek; see Appendix A: Campbell 2008).

Yellowstone cutthroat trout abundance was 12 fish > 100 mm/100 m of stream length in Jackass Creek; the only abundance data collected on a YCT population in 2006. No YCT were found during the survey of Camas Creek, with only brown trout present in one site and no trout in the other. Stream habitat was measured at 16 sites on 8 streams. Individual stream survey reports are in Appendix B (separate document).

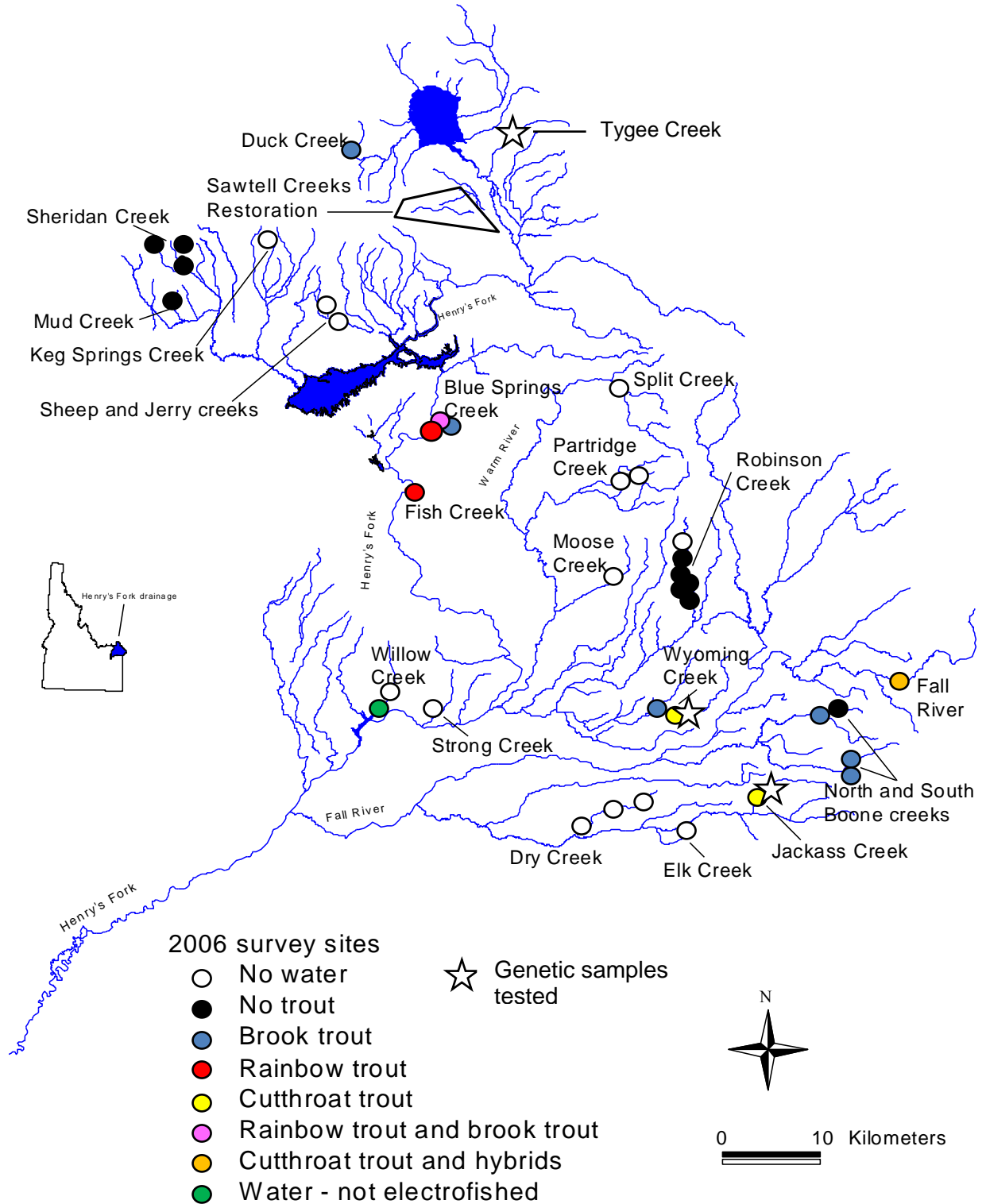
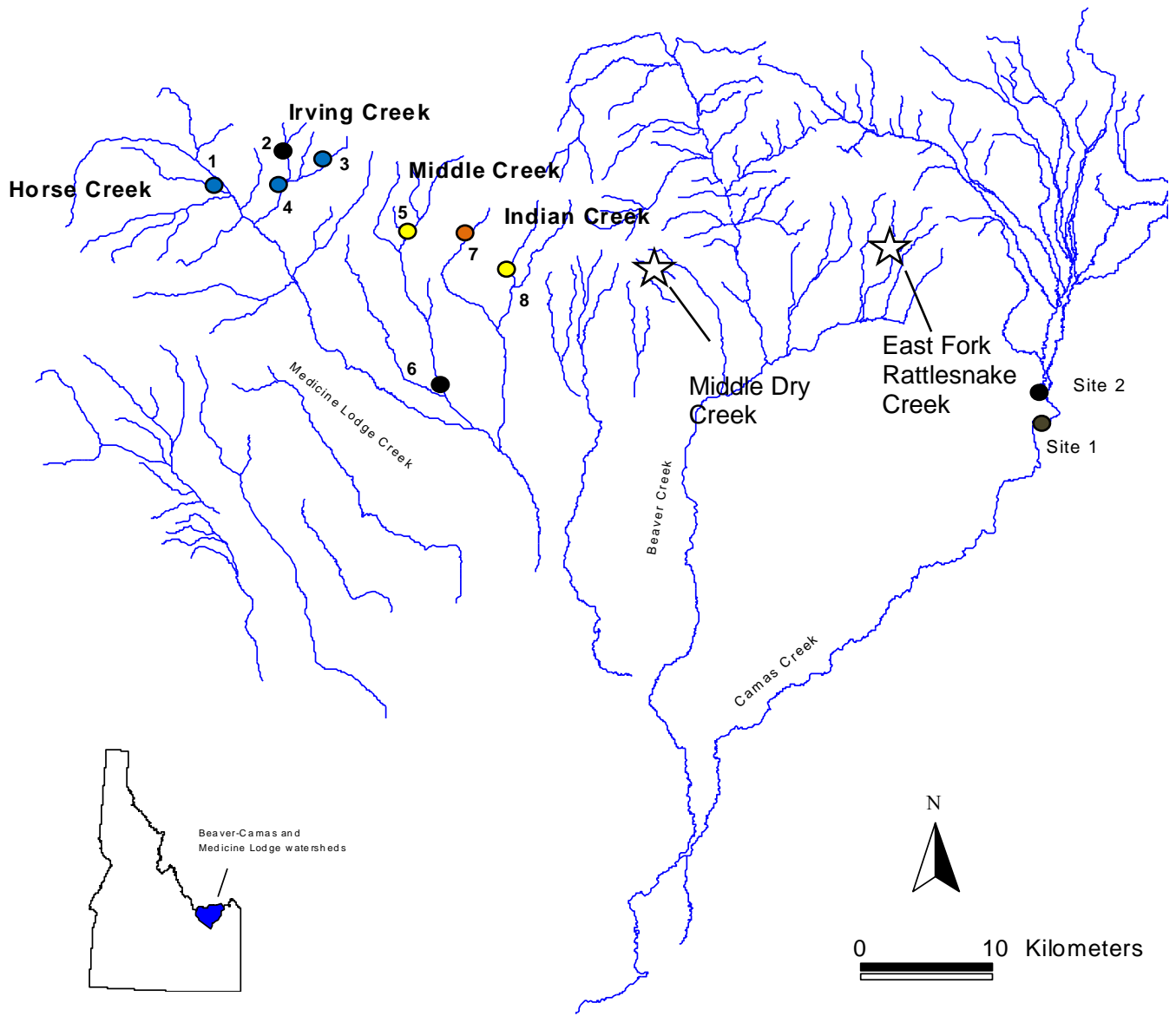


Figure 1. Water and trout presence/absence at 37 surveyed sites on 22 streams in the Henry's Fork drainage in 2006. Yellowstone cutthroat trout genetic samples were tested from three populations in the Henry's Fork drainage. Yellowstone cutthroat trout were restored into a total of about 8.5 km of the north and south branches of Sawtell Creek in 2006.



Trout Presence

- Brook trout
 - Cutthroat trout
 - Brown trout
 - Cutthroat trout and rainbow trout/hybrids
 - No trout
- ☆ Genetic samples tested

Figure 2. Trout presence at 8 monitoring sites in the Medicine Lodge watershed and 2 survey sites on Camas Creek in 2006. Yellowstone cutthroat trout genetic samples were tested from two populations in the Sinks drainage.

Stream Monitoring

The eight BLM monitoring sites within the Medicine Lodge watershed were resurveyed between 24 June and 27 June 2006. Six sites contained trout; two of these sites had putative YCT only and one had YCT with rainbow trout/hybrids (Figure 2). Three sites had brook trout only.

Significant differences in fish numbers and species present were found in all eight sites, compared to previous surveys. YCT were no longer found in three sites; both forks of Irving Creek and lower Middle Creek (Figure 3). YCT decreased in two sites; upper Middle Creek (- 94%) and West Fork Indian Creek (- 78%). YCT increased by 7% in East Fork Indian Creek, where they were the only species found in 2006. Six sites that had rainbow trout in 1997/2001 had no rainbow trout in 2006 (Figure 3). Rainbow trout were not previously found in West Fork Indian Creek, but were found in 2006. No hybrids were found in 2006 within six sites that previously had hybrids. The number of hybrids in the West Fork Indian Creek increased slightly. All three sites on Irving Creek and the site on Horse Creek had fewer brook trout (decrease of 55% -100%).

Stream habitat was measured at eight sites within four streams. The average wetted width of sites surveyed was 2.1 m (range 0.70 m to 4.15 m). Dominant substrates from stream sites were gravels (n=6), cobble (n=1) and fines (n=1). Seven sites had fewer than 95% stable banks (range 20% to 80%). Cattle grazing and evidence of a recent fire event was noted at Lower Middle Creek and a “gully washer” event was noted for the Upper Middle Creek. Individual stream monitoring reports are contained in Appendix C (separate document).

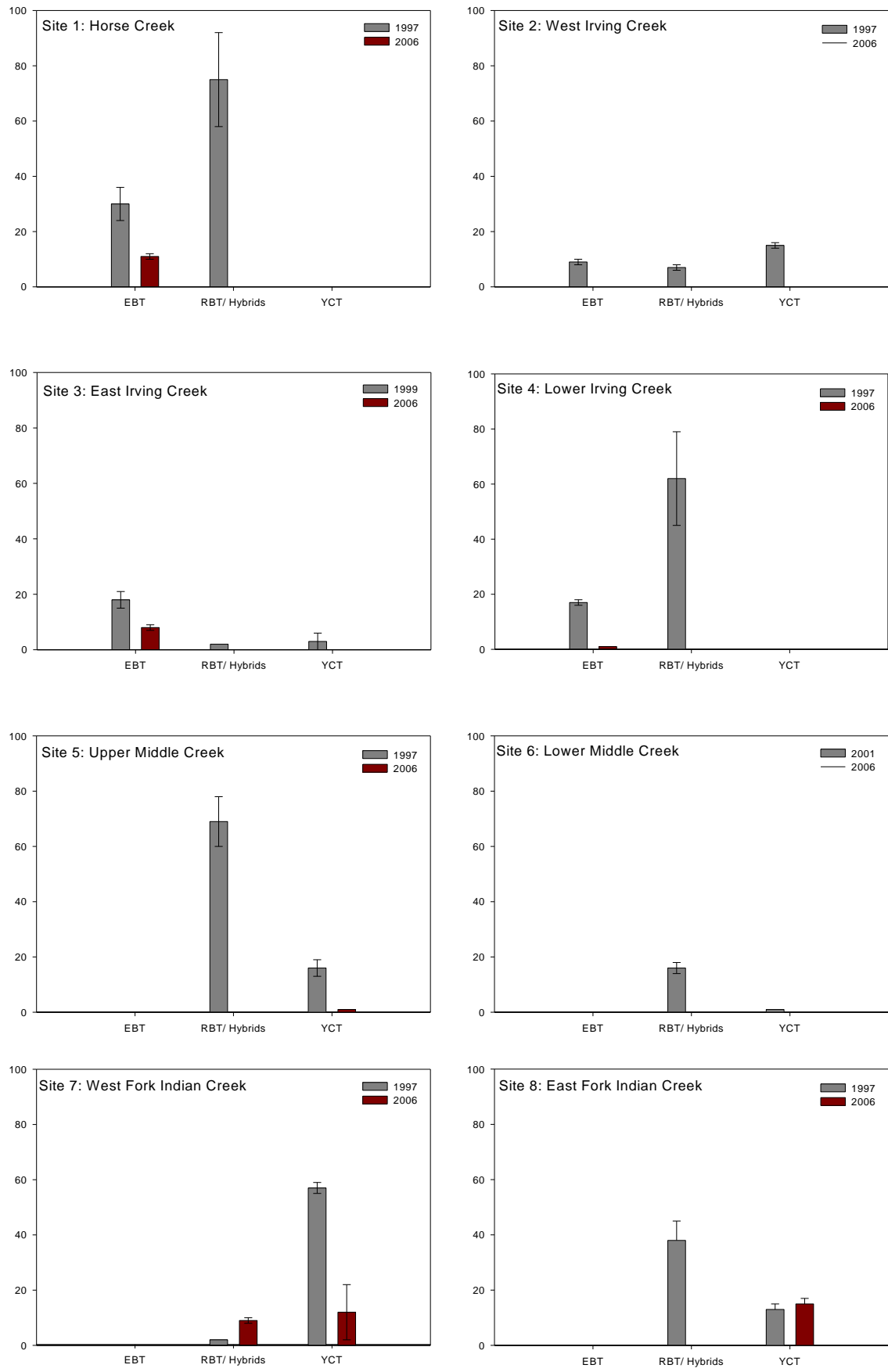


Figure 3. Population estimates of trout >100 mm at time of initial survey (grey) and 2006 (red) at eight sites on four streams in the Medicine Lodge drainage.

Cutthroat Trout Restoration

No brook trout were found in South Sawtell Creek during the survey on August 2, 2006. On August 3 & 4, 2006, a total of over 700 Yellowstone Cutthroat trout were captured from Tygee Creek (~ 550) and Corral Creek (~ 150) and then introduced into North and South Sawtell creeks (Figure 1). Fish were captured from source creeks by backpack electrofishing and then placed into an oxygenated holding tank within the bed of a pickup truck to be transported to the Sawtell Creeks. Fish were shuttled from the pickup truck to the creeks by means of an all terrain vehicle (ATV). Fish were then carried in 5-gallon buckets from the ATV to release sites along about 2 km of each creek.

DISCUSSION

Stream surveys conducted in 2006 have further refined our knowledge of trout distribution in the Henrys Fork and Sinks drainages. The majority of surveys sites were within heretofore mostly unsurveyed stream lengths. A number of these sites contained no water (intermittent) or no trout; such findings are important for further refining our understanding of hydrology and trout distribution. Brook, rainbow, and brown trout (introduced species) were found in the majority of sites with trout. Yellowstone cutthroat trout were not found in any previously unsurveyed stream length, but they were sampled from two previously known isolated populations for genetic testing.

Of the YCT populations tested for hybridization/introgression, only Jackass Creek appears to be a suitable source of genetically pure fish for translocations. However, there are relatively few YCT in Jackass Creek. The density of 12 fish > 100 mm/100 m in 2006 was similar to abundances from past surveys of this creek (Johnson 2003; Meyer and Lamansky 2003) and there are only about 2.5 km of stream length inhabited. This small population is unlikely to be a large source of fish for establishing other populations. YCT in Wyoming and East Fork Rattlesnake creeks have low levels of rainbow trout introgression, but they do meet the state of Idaho criteria of 1- 10% introgression for a conservation population (IDFG 2007) and should be protected.

Genetic analysis provided no indication of the origin timing of YCT in the Sinks Drainage. Yellowstone cutthroat trout in the Sinks and Henrys Fork drainages have the same haplotypes and were not able to be differentiated (Campbell 2008). This similarity could have resulted from a recent founding (in geologic time) by connectivity between the two drainages. Indeed, YCT are able to move from Dry Creek in the Henrys Fork into the Sinks drainages to this day. However, stocking records also indicate that YCT from the Henrys Fork drainage have been recently stocked into the Beaver-Camas drainage (Campbell 2008). The origin timing of YCT in the Sinks Drainage is unable to be resolved with the current genetic analysis techniques.

It appears that there has been an extirpation of an isolated population of YCT in Robinson Creek in Yellowstone National Park. The objective of the visit in 2006 was to take samples from this previously known low density population (Johnson 2003) for genetic testing. However, no fish were found during electrofishing in five sites in 2006

(Figure 1). Robinson Creek was again surveyed in 2007 and no fish were found (HFF unpublished data). This finding further underscores the need to monitor known populations of YCT in these drainages.

No YCT were found within the two survey sites on Camas Creek, but the presence of brown trout in one site may indicate that habitat is suitable for YCT. The IDFG is considering reintroduction of YCT into Camas Creek within the Eighteenmile Ranch in 2009 (Dan Garren, IDFG, personal communication).

Trout diversity and numbers decreased within the BLM monitoring sites in the Medicine Lodge watershed. Trout (various species) were absent from many of the eight sites in 2006, including rainbow trout (n = 6), Yellowstone cutthroat trout (n = 3), and brook (n = 1), where previously they had been found five to nine years ago. The absence of species is concurrent with significant declines of densities of trout of those species present. The two forks of Irving Creek had supported YCT and RBT/hybrids in the late 1990s, but neither of these fishes were found in 2006. The distribution of *Oncorhynchus* species during the late 1990s was likely greater, because of above-average water years, whereas most of the years since have had below-average precipitation. It was thought that low water flows seasonally restricted the use of the West Fork Irving Creek in 2007 (Gourley 2007). Also, drought may be responsible for declines in trout abundance throughout the Medicine Lodge drainage (A. Burgland, BLM, personal communication).

Seasonal differences between the timing of surveys appears to have affected the presence of *Oncorhynchus* species and the abundance of brook trout in 2006. Rainbow trout and hybrids were found in Horse Creek and the Main Irving Creek sites when sampled during September in 1997, but none were found in late July, 2006 (Figure 3). Rainbow trout and hybrids were found in both sites during September of either 2007 or 2008; seasonal differences in water flows and an irrigation diversion near the mouth of Irving Creek likely impede fish movement and fish habitat in these two creeks during the summer (A. Burgland, BLM personal communication). It is also possible that the complete lack of trout in the lower Middle Creek site could be also attributed to the mid-summer survey time in 2006. Survey timing differences are also partly responsible for the apparent declines in brook trout. Young-of-the-year brook trout are smaller in the summer, and therefore not included in population estimates, whereas most brook trout are > 100 mm in the autumn.

Only YCT were found in the Upper Middle Creek and East Fork Indian Creek sites. However, more rainbow trout and hybrids than YCT were present in both sites in 1997 (Figure 3) and rainbow trout and hybrids have recently been found in these streams in areas near the monitoring sites (DeRito and Emery Miller 2006; Gourley 2007). Moreover, rainbow trout and hybrids were found in the East Fork Indian Creek site in 2007, though putative YCT were more abundant (A. Burgland, BLM, personal communication). Rainbow trout introgression/hybridization of YCT is likely widespread throughout both streams. The level of introgression/hybridization, and whether there is any spatial or temporal variation within these populations, is unknown.

Several factors were noted during the survey of the monitoring sites in 2006 that may be affecting fish habitat, such as recent wildfires, “gully washers”, and cattle damaged stream banks. These factors may negatively affect fish habitat and fish densities. These streams are now being closely monitored as grazing permits for these areas have been, or will be, under review (A. Burgland, BLM, personal communication).

The restoration of YCT into Sawtell Creek is a success. In 2007, YCT were found in all survey sites, young-of-the-year YCT were found in lower survey sites, and brook trout were not found in any sites within either branch of Sawtell Creek (unpublished data, IDFG and HFF). This was also the case in 2008 (D. Garren, IDFG, personal communication). The reintroduced YCT are well distributed and are reproducing in both branches of Sawtell Creek.

RECOMMENDATIONS

- The remaining unsurveyed stream length in the Henrys Fork and Sinks drainages should be surveyed.
- The remaining YCT populations in the Henrys Fork and Sinks drainages should be genetically evaluated to determine purity and genetic structure.
- BLM monitoring sites in the Medicine Lodge watershed should be resurveyed at regular intervals, during September.
- The successful YCT restoration in Sawtell Creeks should serve as a template for the other headwater streams in the Sinks and Henrys Fork drainages.
- YCT restoration should continue to be evaluated and implemented for Camas Creek within the Eighteenmile Ranch.

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APPENDIX A. GENETIC RESULTS

Genetic results, Henrys Fork Yellowstone cutthroat samples
Idaho Department of Fish and Game Eagle Fish Genetics Lab



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C.L. "Butch" Otter/ Governor
Cal Groen / Director

March 14, 2008

MEMORANDUM

To: Jim DeRito, Conservation Director, Henrys Fork Foundation
Cc: Dan Schill
From: Matthew Campbell, Idaho Fish and Game, Eagle Fish Genetics Lab
Subject: Genetic results, Henrys Fork Yellowstone cutthroat samples

Jim,

We have completed the genetic analyses on 155 Yellowstone cutthroat trout samples collected from five sample locations in the Henrys Fork and Camas River drainages (Table 1 and Figure 1). Samples from Wyoming Creek, Jackass Creek, and E.F. Rattlesnake Creek were screened for rainbow trout hybridization/introgression with six diagnostic nuclear DNA (nDNA) markers (Occ34, Occ35, Occ36, Occ37, Occ38 and OM55; Ostberg and Rodriguez 2002). These same samples were also examined using mtDNA sequencing analysis of 616 b.p. within the ND2 gene region. In a previous study, examining the origin of Yellowstone cutthroat trout in the Fall and Bechler Rivers (Campbell and Kozfkay 2008), we used Restriction Fragment Length Polymorphism (RFLP) analysis of the combined ND1 and ND2 mtDNA gene regions. We chose sequencing for this study because the ND2 gene region has recently been sequenced on a number of Yellowstone cutthroat trout populations in Idaho and Wyoming (Campbell et al. 2007; Novak et al. 2005), and direct DNA sequencing can sometimes provide better resolution than RFLP analyses (Bagley et al. 2002). For additional comparison purposes we also sequenced samples of Yellowstone cutthroat trout from Middle Dry Creek (N = 26) and Tyghee Creek (N = 24).

Results (purity)-

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We did not find any evidence of rainbow trout or westslope cutthroat trout hybridization in Jackass Creek (N = 46). Extensive hybridization was detected in Wyoming Creek however, with over half of the samples identified as >F₁ hybrids (11/21 = 52.4%). Despite these high levels of hybridization, rainbow trout introgression was fairly low with only 13 rainbow trout alleles observed out of 252 examined (5.2%). This was due to the fact that all of the hybrids identified contained only 1 or 2 rainbow trout alleles of the 12 alleles examined (indicative of more advanced backcrosses).

The screen for hybridization in E.F. Rattlesnake Creek yielded conflicting results. Of the thirty samples examined, seven were identified as hybrids, but of these, six were identified only at the Occ36 locus. We observed a similar result at this locus for one other Yellowstone cutthroat trout population we have examined (Big Cottonwood Creek, IDFG unpublished data). There are a several possible reasons why we might be observing this differential introgression between loci. Two reasons that I think are more plausible include: 1.) These are related individuals (siblings perhaps) produced by at least one advanced backcross hybrid parent, and 2.) The Occ36 locus is not a fixed diagnostic marker (perhaps the “rbt” allele (A) is naturally present in YCT populations at a low level). Under either scenario, the introgression level observed in these samples is unlikely to accurately describe the population. Since one individual in this sample was identified as a hybrid at both the Occ36 and Occ38 locus, I do think that we have evidence that this population has undergone hybridization with rainbow trout.

Table 1. Sample site, % hybridization, % introgression per site, and total samples screened for hybridization. Haplotypes observed in each site is also shown with total numbers successfully sequenced. Haplotypes not shown can be found in Campbell et al. (2007). Samples from Yellowstone Lake were previously sequenced and are shown for comparison purposes.

Sample Site	Hybridization	Introgression	Total (N) _{Hyb/Int}	HAP08	HAP12	HAP13	HAP19	HAP20	HAP21	HAP22	HAP23	HAP24	WC #19	DC #03	Total (N) _{mtDNA}
Wyoming Creek	52.4%	5.2%	21		19								1		20
Jackass Creek	0	0	46		33										33**
Rattlesnake Creek	N/R*	N/R*	30		29										29
Middle Dry Creek	N/A	N/A	N/A		25									1	26
Tyghee Creek	N/A	N/A	N/A		24										24
YellowstoneLake	N/A	N/A	N/A	5		12	1	1	1	1	1	1			23

*Hybridization and introgression levels were not reported for Rattlesnake Creek (please see text for explanation). **13 samples yielded DNA sequences of too low quality to analyze.

Results (mtDNA diversity)-

Previous sequencing of one sample from Tyghee Creek yielded a haplotype we designated as Hap12 (Campbell et al. 2007). All of the additional samples that we sequenced as part of this study from Tyghee Creek also exhibited Hap12 (Table 1 and Figure 1-Hap12 is shown in Yellow). This same haplotype was fixed in samples from Jackass Creek and Rattlesnake Creek and was the dominant haplotype observed in samples from Wyoming Creek and Middle Dry Creek. The only sample from Middle Dry Creek not exhibiting Hap12 was sample #3. This sample exhibited a haplotype (designated as DC #03, colored red on the map) most similar to one observed in a rainbow trout we had previously sequenced. This finding is not surprising given that hybrids have been previously detected in Middle Dry Creek (Meyer et al. 2006).

The only sample from Wyoming Creek not exhibiting Hap12 was sample #19. This sample exhibited a haplotype (designated as WC #19, colored green on the map) that was very divergent from all other Yellowstone cutthroat trout haplotypes we have observed. Estimates of sequence divergence (data not shown) and a bootstrapped neighbor joining tree (Figure 2) from those estimates, indicated that this haplotype was most similar to one observed from a westslope cutthroat trout we had previously sequenced.

This result, along with the results from the screen for rainbow trout hybridization, was surprising. There apparently is no history of stocking in Wyoming Creek of either rainbow trout or westslope cutthroat trout, and all fish sampled phenotypically looked like Yellowstone cutthroat trout. Our results suggest possible hybridization with both rainbow trout and westslope cutthroat trout, although the genotypes are indicative of advanced backcrossing (>F₁ hybrids X westslope cutthroat trout). Previous research has demonstrated that westslope cutthroat trout with low levels of rainbow trout alleles may be phenotypically indistinguishable from pure westslope cutthroat trout (Leary et al. 1984).

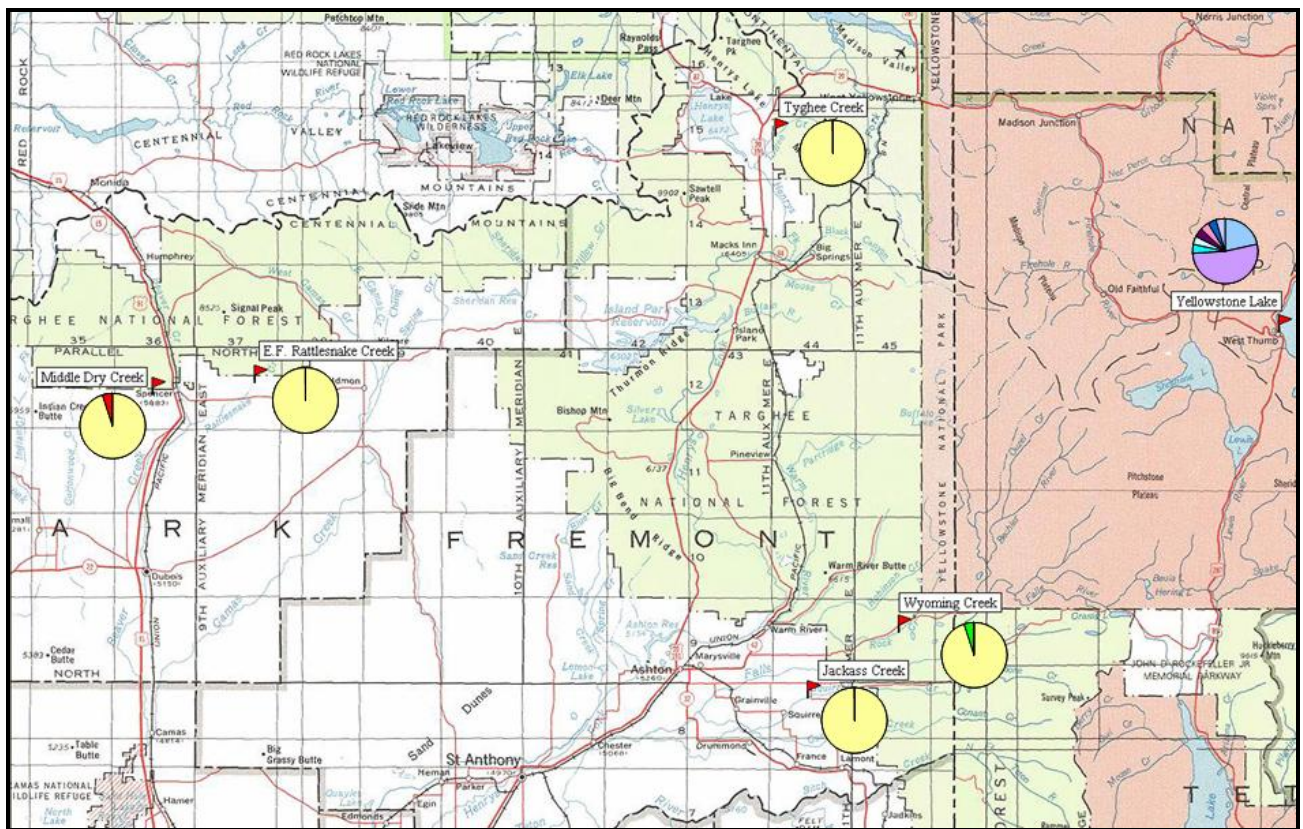


Figure 1. Sampling locations in Camas-Beaver and Henrys Fork drainages and mtDNA haplotype frequencies (represented by pies) observed at each site. Yellow corresponds to hap12, red corresponds to an observed rainbow trout haplotype and green corresponds to an observed westslope cutthroat trout haplotype. Haplotype frequencies observed in Yellowstone Lake are shown for comparison.

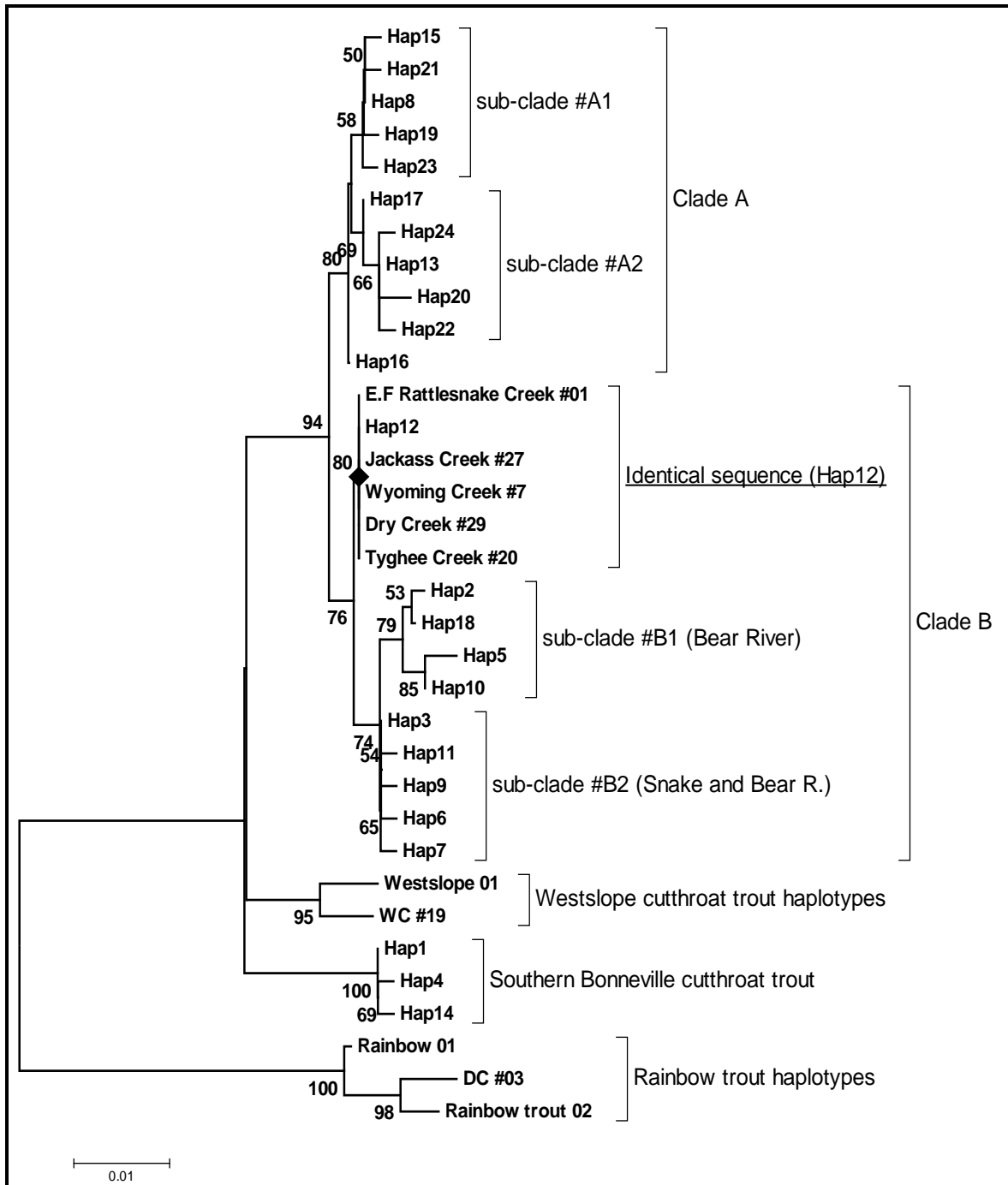


Figure 2. Neighbor-joining dendrogram (Kimura-2 distance model) showing the relationships of the three haplotypes observed in this study (Hap12, DC #03 and WC #19) to 24 haplotypes previously identified in Yellowstone cutthroat trout and Bonneville cutthroat trout. Dendrogram was generated using the software program MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar et al. 2001). Bootstrap values (shown >50) were based on 10,000 replications. For further descriptions of clades and subclades shown, please refer to Campbell et al. (2007).

The predominance of hap12 in all sample areas is consistent with earlier mtDNA RFLP analyses (Campbell et al. 2002; Campbell et al. In press). In these studies we identified a dominant RFLP haplotype that we designated as Haplotype 6 (which corresponds to hap12 in this study). Haplotype 6 is distributed throughout the N.F. Snake River drainage (Teton River, Henrys Fork, and Henrys Fork) and is not found in high frequencies outside the N.F. Snake River, suggesting that it is likely the native haplotype to this area.

It is less clear whether Haplotype 6 (hap12) is native to sample locations within the Beaver-Camas drainage (Middle Dry Creek and E.F. Rattlesnake Creek), that are currently isolated from the Snake River. The Beaver-Camas drainage has been stocked with Yellowstone cutthroat trout from Henrys Lake (IDFG stocking database; available at <http://fishandgame.idaho.gov/fish/stocking/>) and the presence of hap12 within samples from this drainage could be from those past introductions. However, there is geological evidence suggesting that the Beaver-Camas drainage may have had connection with the headwaters of the ancestral Henrys Fork area as recently as several thousand years ago (Link 2003). Under this scenario it would be unlikely for us to see significant genetic divergence between haplotypes over this time period simply due to mutation (Billington and Hebert 1991), and the presence of hap12 may be due to natural founding events.

Please call me if you have any questions or comments.

Sincerely,

Matthew Campbell

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Appendix A. Raw scores

Jackass Creek	Occ34	Occ34	Occ35	Occ35	Occ36	Occ36	Occ37	Occ37	Occ38	Occ38	OM55	OM55	Genotype indicative of:
JC-01	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-02	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-03	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-04	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-05	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-06	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-07	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-08	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-09	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-10	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-11	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-12	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-13	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-14	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-15	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-16	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-17	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-18	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-19	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-20	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-21	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-22	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-23	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-24	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-25	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-26	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-27	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat

JC-28	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-29	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat

Appendix A. Raw scores (continued)

Jackass Creek	Occ34	Occ34	Occ35	Occ35	Occ36	Occ36	Occ37	Occ37	Occ38	Occ38	OM55	OM55	Genotype indicative of:
JC-30	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-31	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-32	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-33	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-34	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-35	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-36	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-37	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-38	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-39	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-40	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-41	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-42	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-44	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-45	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
JC-46	B	B	B	B	B	B	B-1	B-2	B	B	B	B	cutthroat
JC-47	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat
Wyoming Creek	Occ34	Occ34	Occ35	Occ35	Occ36	Occ36	Occ37	Occ37	Occ38	Occ38	OM55	OM55	Genotype indicative of:
WC-01	B	B	B	B	B	B	B-1	B-1	UNK?	B	B	B	cutthroat
WC-02	B	B	B	B	B	B	A	B-1	B	B	B	A	>F ₁ hybrid
WC-03	B	B	B	B	B	B	A	B-1	B	B	B	B	>F ₁ hybrid
WC-04	B	B	B	B	B	B	A	B-1	B	B	B	B	>F ₁ hybrid
WC-05	B	B	B	B	B	B	B-1	B-1	B	B	B	B	cutthroat

