Clear Creek Dam Fish Passage Assessment
Third Annual Progress Report

U.S. Fish and Wildlife Service
Mid-Columbia River Fishery Resource Office
Yakima Sub-Office

U.S. Bureau of Reclamation
Columbia-Cascades Area Office

April 28, 2015

Authors:
Jeff A. Thomas (USFWS)
Patrick A. Monk (USFWS)

This page intentionally blank

Foreword

The Clear Creek Dam Fish Passage Assessment is a cooperative investigation being conducted by staff from the U.S. Fish and Wildlife Service Mid-Columbia River Fishery Resource Office (Yakima Sub-Office), the U.S. Bureau of Reclamation's Columbia-Cascades Area Office, and Region 3 of the Washington Department of Fish and Wildlife (WDFW). Planning for this study began in early 2012 and the assessment will continue through 2015. This is the third and final annual progress report which will be prepared during the course of this study. A final report will be submitted during the first half of 2016.

The first annual progress report was produced in April, 2013 and contains an abundance of background information. It also described the methods which are being employed in this investigation. This report will focus on the expansion of the study during its third year (2014), modifications made in response to things learned during the first two years, and results from year three of the assessment. The three sections which follow directly below also appeared in the first annual report. They are repeated in this report due to their importance in framing the study.

Study History and Funding

The USFWS Mid-Columbia River Fishery Resource Office (Yakima Sub-office) began submitting study proposals to investigate fish passage conditions at Clear Creek Dam in 2008. These proposals were submitted annually and sought funding through various sources for money that was limited and for which competition was heavy. The proposal did not receive funding for four consecutive years. Concurrently, Reclamation's Yakima Field Office (YFO) was seeking agency funding targeted for various ESA-related activities. In late 2011, they learned that funding was available to initiate this study and approached the USFWS about collaborating on the effort. Biologists with Region 3 of WDFW had been supportive of the proposed study from its inception. When informed that funding had been acquired to initiate the assessment, the Regional Office offered staff time and materials. The Washington Department of Ecology provided supplemental funding to WDFW to modify the fish trap used in the study. In addition to the initial funding secured in 2011, Reclamation's Yakima River Basin Water Enhancement Project (YRBWEP) has contributed significantly towards the assessment. The study is being managed by the USFWS.

Study Goal

The ultimate goal of this study is to ensure that the population of bull trout (Salvelinus confluentus) which spawns in the North Fork (NF) Tieton River can successfully reach spawning habitat in the river above Clear Creek Dam. It is not believed this population currently has such access on a consistent basis. Not only is this a current problem for this population but the severity of it may increase in the future. Climate change models developed for the Pacific Northwest are consistent in predicting warmer winters and decreased snowpack. It is essential that cold-water species such as bull trout have access to habitat at higher elevations if their populations are to persist. The data we obtain will enable us to advise the YFO on operations at Clear Creek Dam that will facilitate adult bull trout migration past the structure or lead to construction of new passage facilities.

Study Objectives

The three primary objectives of this investigation are: 1) to determine when NF Tieton River bull trout attempt to migrate upstream past Clear Creek Dam; 2) to assess their success at doing so under various hydrologic conditions; and 3) to determine post-spawn migration timing and the extent to which the population uses Clear Lake. There are also several ancillary objectives which will add to the limited body of knowledge available for this population. The accomplishment of these should help fish managers prescribe appropriate actions to ensure the long-term health and persistence of the NF Tieton population. The ancillary objectives include determining spawning frequency, collecting genetic information, and estimating the effective population size.

Study Expansion and Modifications

Additional Antennae and Power Supply

As was described in the second annual report, three PIT tag interrogation sites were operating in 2013. These were located at the exit/entry portal of the pool-and-weir fish ladder (upper ladder), across the lower spillway channel at its downstream terminus, and in the NF Tieton River 0.75 mile upstream of Clear Lake. A fourth antenna located at the top of the spillway channel which spanned the channel directly above the concrete weir on the spillway crest was in place but never operated successfully in 2013

The lower spillway antenna, which had broken during high flows in late-September 2013, was replaced on April 29-30, 2014. Unfortunately, about two weeks later the antenna was broken again. This time it was damaged by an animal, most likely a beaver. After waiting out the spring runoff and the arrival of new materials the antenna was reconstructed on July 9 utilizing a modified “beaver resistant” design which will be described below.

A new antenna was installed on April 29, 2014 in the pool-and-weir fish ladder seven weirs down from the top of the ladder (three up from the bottom). The objectives for this site are to provide confirmation that any bull trout detected at the top of the ladder (potentially leaving Clear Lake) actually continued downstream, to provide the ability to cross-check the detection efficiency of the lower spillway antenna, and to provide information on travel time for fish ascending the ladder.

A new antenna was also installed across the upper spillway. This installation was completed on September 3, 2014 and employed a new design in attempt to rectify the continuous difficulties we have encountered related to detection efficiency at this site.

A temporary PIT tag interrogation site was installed about 40 meters upstream of the trap on September 12, 2014. The purpose of this antenna was primarily to ascertain if previously tagged bull trout approaching the trap would shy away from it.

Antenna Construction and Installation

The new antennas in the lower ladder and just above the trap, as well as the two reconstructed antennas in the spillway channel, were constructed from General Cable Carol Super-VU Tron Supreme power cable, 12 gage with 4 strands.

In order to work in the lower spillway channel the lake elevation was dropped below the spillway crest (3011.0 feet) and the ladder was boarded up, dewatering the channel. Fourteen new stainless steel anchor bolts with eye nuts attached were installed in the bedrock to augment those previously installed. A 3/8 inch diameter non-stretch marine rope was strung through the eyelets, looped back on the right bank, and stretched tightly with turnbuckles installed on the left bank. Rather than stringing the antenna wire through the eyelets, the wire was affixed to the rope using over 100 heavy duty cable ties. We believed this would prevent the wire from wearing through where it contacted the eyelets which we suspected to be the reason the antenna failed the previous September. The installation was completed on April 30. However, the antenna quit operating just a little over two weeks later (May 16) and it was subsequently discovered that an animal, probably a beaver, had gnawed through both the rope and the wire (Figure 1). Obviously, this was completely unexpected. We now had to come up with a new design that would not only be durable enough to withstand extreme hydraulic conditions but gnawing animals as well. We ordered materials and waited out the spring runoff. On July 9 the antenna was reinstalled, stringing the rope and antenna wire through semi-flexible 1.5-inch HDPE pipe that was custom cut and fit between the anchors (Figure 2).

The antenna wire in the lower ladder was affixed to the weir using conduit anchors and run up the ten-foot face of the left bank cliff. The configuration of the antenna was an elongated oval loop running across the weir just under the weir opening (Figure 3). This antenna was powered by two six-volt batteries which had to be exchanged weekly.

A new upper spillway antenna was installed on September 3. It was not imperative for this site to operate as early as the others because it was only useful for monitoring bull trout emigrating from Clear Lake which they would not be doing, if at all, until after the spawning period in late-September. Since this antenna has been problematic since early 2013 we abandoned the swim-through configuration and employed a flat-plate design identical to those installed on the lower spillway and the NF Tieton River (Figure 4).

The temporary antenna installed upstream of the trap on September 12 was a flat-plate design which spanned the channel. It was powered by a single 12-volt battery which had to be periodically exchanged. It was recharged near the trap base camp with a solar panel.

The operational details for these antennas as well as those on the upper ladder and the NF Tieton River will be described in the *Antenna Performance* section later in this document.

Trap Modifications

There were no further modifications to the trap for 2014. It did require a few repairs after the complete washout in 2013 but they were minor. All repairs were done by WDFW's Region 3 screen shop.

Trap Location

The trap was installed at the same location on the NF Tieton River in 2014 as it was the last two years. This location was approximately 6.5 miles upstream of Clear Lake.

As was the case the previous two years we were unable to take road vehicles to the trap base camp at the end of FS 1207 due to concerns about the flood-damaged bridge at Miriam Creek. Once again, we utilized ATVs to transport equipment and personnel to and from the camp.

Figure 1. Damage inflicted on the lower spillway antenna by a suspected beaver in May 2014.

Figure 2. Reinstalled "beaver resistant" lower spillway antenna (July 9, 2014)

Figure 3. Installation of lower ladder antenna on April 29, 2014

Figure 4. Newly designed flat-plate antenna installed on the upper spillway on September 3, 2014.

Water Temperature Monitoring

Data loggers were deployed at the upper ladder and lower spillway PIT tag interrogation sites on April 29, 2014; the logger at the lower spillway site was removed in June because the water temperature recorded there was essentially identical to that at the upper ladder. Loggers were deployed in the outlet channel of Clear Creek Dam and at the NF site on May 1. The data loggers used were Onset Hobo® Water Temp Pro v2 (#U22-001). Data were uploaded using a hobo shuttle every two weeks.

2014 Trapping and Tagging

Environmental Conditions

Except for one brief period, environmental conditions during the trapping period (September 9-30) were generally stable, much different than the previous year. Air temperatures were comfortable during the day and did not reach freezing temperatures at night except for a brief cold snap which occurred September 13-14. Water temperatures ranged from 3.8°C (experienced during this cold snap) to 10.5°C with an average diel water temperature over the period of 7.8°C. Unlike 2013, there was little significant rainfall except for a three-day period from September 22-24.

Hydrologic conditions over the first ten days were stable with no rapid fluctuation in river stage, either up or down. River stage ranged from one-half inch above to 1.9 inches below that which occurred on September 9. Water clarity over this period was generally good although slightly turbid at times. The river began to rise on September 18 indicating that rain was likely falling higher in the watershed but this increase in stage did not exceed four inches until September 24 when intermittent light rains turned steady and the stage rose over six inches in the early morning hours. Streamflow diminished fairly quickly following this rise and by the next afternoon the stage was back within the range experienced the first ten days of the trapping period. Another significant rise in stage (four inches) did not occur until the last day the trap was operated. With the higher flows came turbid conditions, which persisted from September 22-26.

Trapping

The trap was assembled on September 9, 2014 (the same date as last year). Hydrologic conditions were excellent for trap assembly with the river running low and clear. Assembly went smoothly and the trap was fully operational by 3:00 PM. It was operated until the early morning hours of September 30 (20.5 days). The trap was checked periodically over the course of the day and routine maintenance was performed. This maintenance included cleaning the weir panels of leaves and other small organic debris which seemed to be accumulating with greater regularity during normal operations then in either of the first two years of the study. One person was usually present at the camp to monitor the trap during daylight hours; two were present at night. At night the trap was checked between 9:00 and 10:00 PM and again at least once between 1:00 and 4:00 AM. The first check after sunrise occurred between 6:00 and 7:00 AM. During the checks bull trout were sometimes observed directly below the weir, obviously seeking to migrate upstream. These fish could often be captured with a dip-net for processing but this was not possible in all cases.

The trapping operation progressed without complications except for a 2.5 day period extending from the early morning hours of September 24 through about noon on September 26. The river had risen nine inches over the previous nine hours and during the trap check at 4:00 AM on September 24 it was discovered that two weir panels on the right bank had buckled under the pressure of accumulated organic debris and considerable force was being exerted on other weir panels. The crew quickly began removing pickets to relieve the pressure. It was still raining at daylight so more pickets were removed, which saved the weir and trap but rendered it useless for trapping fish. The trap was not reassembled until the rain completely ceased and the stage had receded to a safe level for in-river work on the morning of September 26.

The weirs were slightly compromised by another, less severe, event not long after midnight on September 30. The equipment was scheduled for removal later that day so no attempt was made to address the situation. The weir panels did not appear to be in jeopardy of collapsing which proved to be a correct assessment.

Data Collection and PIT tagging

All bull trout which were not recaptures were worked immediately after being removed from the trap. Recaptures, identified by scanning each fish with an Oregon RFID® portable PIT tag reader, were immediately released either upstream or downstream of the trap depending on sex (see below). Captured fish were netted out of the trap using a long-handled dip net. The untagged fish were transferred to an 80-quart cooler where they were anesthetized. The anesthesia used was tricaine-s (i.e., MS-222) mixed at a 50mg/L concentration with river water. Since MS-222 is acidic, buffer ($NaHCO_3$, i.e., baking soda) was added to the solution to raise the pH back to the baseline level. The pH was measured using an Oakton® pH Testr 20. To ensure the consistency and safety of the solution the cooler was pre-marked to hold 25 liters of water and the amounts of MS-222 (1.25 grams) and buffer (57 grams) needed were premeasured. Solutions were discarded away from the stream after each tagging session.

The fish were measured for total length (TL), sexed, and a small tissue sample was taken from the anal fin and preserved in 70% isopropyl alcohol for genetic analysis. A scalpel was used to make a one-half inch vertical incision just posterior and ventral to the pectoral musculature near the end of the pectoral fin. This incision penetrated only the epidermal layer under which a half-duplex PIT (HDX PIT) tag was horizontally inserted. We used 23 mm x 3.65 mm tags (manufactured by Texas Instruments, Inc.) operating on the 134.2 kHz radio frequency identification standard for animal tagging. The tag was gently pushed in between muscle and skin towards the tail of the fish until barely visible, at which point a cocktail straw was used to implant it about one inch further. This surgical procedure was fairly simple and did not require any sutures. After being placed in the anesthetic solution, full anesthetization usually occurred within 7-10 minutes. The time required to work each fish was between 5-7 minutes. No complications were encountered during HDX PIT tag implantation.

After completing the tag implantation the reader was used to scan the tag number and fish were placed in 6-inch diameter PVC flow-thru recovery tubes. These were secured in the channel where a light current existed with the head of the fish oriented upstream. Once placed in the holding tubes all of the bull trout were fully recovered within 15-20 minutes and released downstream if they were females. Males, which are capable of spawning again, were released upstream of the trap.

As noted above not all of the bull trout captured were trapped. Some were dip-netted directly below the weir. The disposition of these fish was the same as for those that were trapped.

2014 Hook and Line Sampling

It was confirmed in 2013 that bull trout were present in the stilling basin directly below Clear Creek Dam in late summer; in 2014 we sought to capture some of these fish to obtain genetic samples and implant PIT tags. Our objectives were to determine the genetic origin of these bull trout and to see if any would attempt to migrate up the spillway channel. Attempts to angle for bull trout began on May 1 and continued throughout the summer when hydraulic conditions below the dam would allow. No fish were captured in May; for June and much of July conditions were not conducive to angling below the dam. Finally, in late July two bull trout were caught. On July 31 we returned to the site and successfully angled for bull trout. The weather was clear and the water temperature was 10.0°C. Bull trout were caught using large lures or flies with single barbless hooks. Heavy fishing line was used to insure that fish were landed quickly without a protracted struggle. The data collection and PIT tagging procedure was identical to that employed at the trap.

Results

Trapping

A total of 13 adult bull trout were captured at the trap in 2014. Only five of these had not been previously tagged and one was recaptured (netted directly downstream of the weir) two days after it had been tagged. Two of the remaining four "new" fish were not trapped; they were captured with a dip-net directly downstream of the weir.

All of the remaining seven bull trout captured in 2014 were recaptures. Two had been tagged in 2012 with one of these recaptured a second time. These 2012 fish were netted downstream of the weir. Four of the recaptured bull trout had been tagged in 2013 with three captured in the trap and one netted downstream.

Overall, fewer bull trout were captured than we anticipated would be. The number of redds found in the NF Tieton River and a primary spawning tributary (19) was greater than it had been since 2009. However, in 2014 stable hydrologic conditions were interrupted by a high flow event which produced good conditions for migration, during which the trap was not operating for over two days when pickets had been removed. It seems likely that an opportunity to capture emigrating bull trout was missed during this event.

All but one of the 13 adult bull trout captured at the trap in 2013 were captured at night. Nine were found between 9:00 and 11:00 PM, three between 1:30 and 5:30 AM, and one at 7:00 AM. Fish were captured between September 10 and September 28.

The only other fish species captured were two mountain whitefish (*Prosopium williamsoni*) and the only other animals found in the trap were toads on a few occasions.

Hook and Line Sampling

Twelve adult bull trout were caught while angling directly below Clear Creek Dam on July 31, 2014. These included five males and seven females. All but one of these fish, a female tagged in 2012, had not been encountered previously during the study. These fish were caught over the course of just three hours between 9:30 AM and 12:30 PM. It was evident that we could have continued to catch bull trout if we had so desired. Adult bull trout were obviously present in large numbers.

PIT Tagging

Five adult bull trout captured at the trap were implanted with HDX PIT tags in 2014. One was tagged the first full day the trap was operating (September 10) and the other four on separate days from September 22-28. These fish ranged in size from 49.5-68.5 cm (TL); three were males and two were females. The average total length of the bull trout tagged in 2014 was 57.5 cm, slightly larger than the 14 tagged in 2013 (56 cm) and slightly smaller than the 10 tagged in 2012 (59 cm). The total number of bull trout tagged at the trap in the first three years of study now stands at 29 (14 males and 15 females). A list of the fish tagged in 2014 along with relevant information about each is presented in Table 1 below.

Table 1. Adult bull trout captured and PIT tagged at the trap on the NF Tieton River in September, 2014.

Date captured	Time	Sex	Length (cm)	DNA code	PIT tag code	Tagger
10-Sep	10:30 PM	Male	60	14FF12	180597192	J. Thomas
22-Sep	9:15 PM	Male	68.5	14FF13	180597448	J. Thomas
23-Sep	9:15 PM	Female	49.5	14FF14	180597198	J. Thomas
26-Sep	9:00 PM	Female	53	14FF15	180597277	P. Monk
28-Sep	9:00 PM	Male	56.5	14FF16	180597232	P. Monk

Ten adult bull trout caught below the dam were PIT tagged; a genetic sample was taken from another but this fish was not tagged as it was bleeding slightly from the mouth so it was immediately released after a fin clip was obtained. These fish ranged in size from 44-62 cm (TL) (average: 52.3 cm); five were males and six were females. A list of the fish tagged in July, 2014 below Clear Creek Dam along with relevant information about each is presented in Table 2 below.

Table 2. Adult bull trout captured and PIT tagged below Clear Creek Dam in July, 2014.

Date captured	Sex	Length (cm)	DNA code	PIT tag code	Tagger
31-July	Female	50	14FF1	180597411	P. Monk
31-July	Male	53.5	14FF2	180597353	P. Monk
31-July	Male	58	14FF3	Not tagged	NA
31-July	Male	59	14FF4	180597437	J. Thomas
31-July	Female	46.5	14FF5	180597276	J. Thomas
31-July	Female	51	14FF6	180597473	R. Randall
31-July	Male	48	14FF7	180597402	J. Thomas
31-July	Male	62	14FF8	180597231	P. Monk
31-July	Female	44	14FF9	180597283	R. Randall
31-July	Female	48.5	14FF10	180597332	J. Thomas
31-July	Female	54.5	14FF11	180597237	J. Thomas

Antenna Performance

Upper Spillway

The new flat-plate antenna at this PIT tag interrogation site operated without interruption from mid-day on September 3 until about noon on October 8. It operated at an effective amperage (EA) we were satisfied with based on past experience. However, we were not completely satisfied with the read range we observed which was between 8-12 inches when fully tuned. This range would be capable of detecting a tag within the water column between the stream bed and a couple of inches above the spillway apron but it seemed unlikely that it would at higher lake levels likely to occur later in the fall. We never got a chance to find out. After noon on October 8 the data from the reader indicated erratic readings of the timer-tag. This probably indicated the antenna was out of tune but it went undetected until October 16 when we uploaded data at the site. These data indicated that the reader had stopped due to low voltage two days previous. The data from the adjacent upper ladder reader also showed a low voltage stoppage for the same period. It was clear that the joint power source for these two antennas (two 12-volt batteries recharged with solar panels) was insufficient to power both with shorter autumn days and increasing cloud cover so we turned the upper spillway antenna off. As it turned out, the batteries were about to expire. We replaced the batteries two weeks later (see Upper Ladder section below) but decided not to reactivate the upper spillway reader. The antenna wire runs 85 feet across the spillway crest, loops and comes back. The power required to run an antenna of this length (in combination with the upper ladder antenna) appears to be in excess of what can be reasonably supplied late in the fall. Since we did not intend to keep this antenna operating for much longer in 2014 we opted to shut it down for the season.

We will activate the upper spillway antenna again in late August of 2015 but are not optimistic the antenna will remain operational well into the fall when bull trout might still be seeking to leave Clear Lake. However, if we can keep the lower ladder and lower spillway sites operating into November, 2015, detection of emigrating bull trout will still be possible.

Upper Ladder

The upper ladder PIT tag interrogation site (referred to in previous annual reports as just “the ladder” since the lower ladder antenna had not been installed) was operating normally on January 1, 2014. This is the one site that we do not decommission for the winter so it had been operating almost all of the previous year as well. This is a very reliable antenna which consistently has a read range over 36 inches. The site operated without complications until the afternoon of October 13 (288 days). For the next two weeks it operated intermittently, down due to low voltage a total of 103 hours. Since the upper spillway antenna had been turned off we suspected that our batteries had reached their life expectancy so they were replaced on October 28. The antenna operated without interruption until December 17 (50.5 days). For the last two weeks of the year there were intermittent power outages totaling 135 hours. It is evident that the short days of late December coupled with persistent cloud cover will result in some power outages even with fresh batteries. Fortunately, migratory fish are not known to move much during this time period. All things considered the upper ladder PIT tag interrogation site operated for just over 355 days in 2014, 97.3% of the time.

Lower Spillway

The lower spillway PIT tag interrogation site operated continuously for 16.5 days from April 30 through May 16. Due to extreme hydrologic conditions we were unable to reinstall the new “beaver resistant” antenna until July 9, 54.5 days later. This was extremely unfortunate as this time period missed was viewed as a potential prime migratory window.

After reinstallation the antenna operated continuously until October 22 (105 days) with a read range of 16-18 inches. After this date intermittent power outages began to occur despite the fact that we had installed an additional solar panel at the site. Between October 22 and 26 the antenna was down a total of approximately 52 hours. It had rained steadily for much of this time period, elevating spillway flows to extremely high levels. During a site visit on October 28, when flows had receded, it was discovered that the rope the antenna wire was affixed to, but not the antenna wire, was severed. While the antenna was technically still operating the read range was greatly reduced. The wire remained intact until the site was decommissioned on November 13 but with the low read range and frequent intermittent power outages it is extremely doubtful the antenna was functioning properly past October 26.

This antenna will be crucial to our work in 2015. It is imperative that we have it continuously operating from early spring until at least mid-November. In April, 2015 it will once again be reinstalled employing the same design but affixed to a heavier marine rope. New batteries will be installed with three solar panels used to charge them late in the season. We will have one more important thing working in our favor. Since last year the Yakima Field Office (USBR) has automated the gates of Clear Creek Dam which will enable them to control lake levels in real time and eliminate the uncontrolled high flows down the spillway which can damage the antenna.

Lower Ladder

Following installation on May 14 this antenna operated without interruption until the early morning hours of July 14 (60 days). Following a 17-hour low voltage interruption it then ran uninterrupted for another 30 days. The read range for the antenna was good at 20-24 inches. After mid-August we had some problems maintaining the power supply for the lower ladder antenna. Batteries had to be manually exchanged and battery life was somewhat erratic, resulting in intermittent stoppages. However, during the 88 days between August 15 and November 10 (when the site was decommissioned) the antenna was operating 86 percent of the time.

The lower ladder antenna will be equipped with a solar power source to charge the batteries in 2015. The site will be activated the third week of April.

North Fork Tieton River

The NF Tieton PIT tag interrogation site was activated on April 30, 2014. Just two days later it shut down due to an expired battery which was not discovered for four days. After the batteries were replaced the antenna ran uninterrupted until June 21 (45.5 days) when another battery went bad a very short time after the weekly battery exchange. The site was down for another seven days but once the problem was corrected, operated uninterrupted until it was decommissioned for the winter on November 13 (136 days). Between April 30 and November 13 the NF Tieton site was operating 93.3 percent of the time.

Water Temperatures

There were no apparent problems when water temperature data were uploaded from the loggers to the shuttle every two weeks in 2014. However there was a big problem when the data were downloaded. Although the loggers had been deployed at the end of April, the data prior to July 30 were unavailable either from the shuttle or the loggers themselves. We have no idea what occurred but this was a setback because we are particularly interested in what occurs in the spillway channel compared to the outlet channel before July 30. This will not happen in 2015. We have purchased new loggers and a shuttle; the data will be downloaded from the shuttle and examined every two weeks beginning with deployment in April. The following is a description of the water temperature data we were able to obtain in 2014.

The mean daily temperature in the spillway channel exceeded 18°C for eight days between July 30 and October 28; on 13 additional days it exceeded 17°C. Not until August 31 did average daily water temperatures drop below 15°C (the temperature believed to limit bull trout distribution) where they remained through the period (Figure 5). Water temperatures in the outlet channel were much cooler, dropping steadily after August 13 and averaging just 11.3°C daily through August 31. The lowest average daily temperature reached in the spillway, 10.2°C on October 15, was hit in the outlet channel on September 5. Clearly at some point, probably by mid-July, high water temperatures in the spillway channel could deter bull trout from attempting to migrate up it.

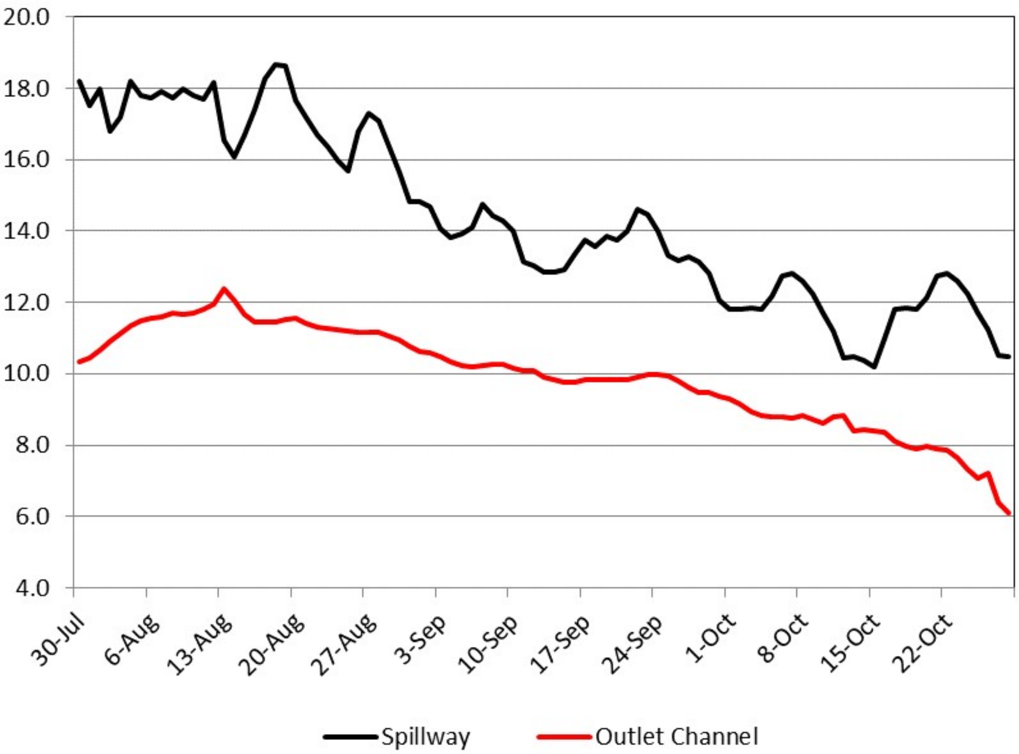

Figure 5. Average daily water temperatures (°C) for 2014 in the spillway and outlet channels of Clear Creek Dam on the North Fork Tieton River.

Between July 30 and October 28 average daily water temperatures in the North Fork Tieton River ranged from 4.7-11.9°C with the highest temperatures ($\geq$11°C) generally occurring the middle two weeks of August. After September 1 average daily temperatures were below 10°C for all but one day.

2014 PIT Tag Detections

Twenty-four of the 39 adult bull trout tagged in the first three years of the study were detected at least once in 2014. Twenty-three of these were among the 29 tagged at the trap. These included five of the 10 fish tagged in 2012, 13 of the 14 tagged in 2013, and all five of the bull trout tagged in 2014. Only one of the 10 bull trout tagged below Clear Creek Dam was subsequently detected. Detection details follow.

Bull trout tagged in 2012

Five bull trout tagged in 2012 were not detected, or otherwise re-encountered (i.e., recaptured) in 2014. Two of these have never been detected after tagging. The other three were last detected in 2013; all at the NF Tieton PIT tag interrogation site in August or September. The detection record for the other five fish tagged in 2012 is presented in Table 3 below.

Table 3. 2014 detections of adult bull trout tagged in 2012.

Date	Tag Number	Sex	Site	Year Tagged
7-July	180597181	F	NF Tieton	2012
23-Jul	Same fish		NF Tieton	
25-Jul	Same fish		NF Tieton	
15-Jul	180597295	M	NF Tieton	2012
3-Oct	Same fish		NF Tieton	
1-Jul	180597382	M	NF Tieton	2012
25-Sep	Same fish		Trap	
13-July	180597290	M	NF Tieton	2012
13-Sep	Same fish	M	Trap	
24-Sep	Same fish		Trap	
4-Nov	Same fish		Lower Ladder	
26-Aug	180597363	F	Lower Spillway	2012
27-Aug	Same fish		Lower Ladder	
27-Aug	Same fish		Upper Ladder	

Four of these bull trout were detected at the NF Tieton site in July, obviously migrating up the river. One was not subsequently detected, one was later detected at the same site on October 3 and one was detected multiple times near the end of September at the PIT tag interrogation site just upstream of the trap. The fourth bull trout detected at the NF Tieton site in July (tag #180597290) was recaptured twice (once below the weir and once in the trap), was detected multiple times just upstream of the trap in late September, and was last detected at the lower ladder site on November 4. The last bull trout identified in the table (tag #180597363) was our most detected fish in 2013, a distinction this female retained in 2014. She was caught below the dam on July 31 and began ascending the spillway channel on August 26, exiting the upper ladder the next day. On September 10 she was detected at the NF Tieton site then captured directly downstream of the weir three days later and released upstream. Twelve days hence she was detected upstream of the trap, at the NF Tieton site two days after that, and was last detected at the upper ladder site on October 22.

Table 4. 2014 detections of adult bull trout tagged in 2012.

Date	Tag Number	Sex	Site	Year Tagged
19-June	180597446	F	NF Tieton	2013
13-Sep	Same fish		Trap	
16-Sep	Same fish		NF Tieton	
10-July	180597185	F	NF Tieton	2013
6-Sep	Same fish		NF Tieton	
30-Oct	Same fish		Upper Ladder	
15-July	180597211	F	NF Tieton	2013
15-Sep	Same fish		Trap	
17-Sep	Same fish		Trap	
25-Sep	Same fish		Trap	
1-Oct	Same fish		NF Tieton	
7-July	180597244	F	NF Tieton	2013
12-Sep	Same fish		NF Tieton	
7-July	180597257	F	NF Tieton	2013
18-Sep	Same fish		Trap	
1-Oct	Same fish		NF Tieton	
9-July	180597333	F	NF Tieton	2013
18-Sep	Same fish		NF Tieton	
1-July	180597348	F	NF Tieton	2013
18-Sep	Same fish		Trap	
25-Sep	Same fish		Trap	
26-Sep	Same fish		NF Tieton	
13-July	180597420	M	NF Tieton	2013
20-Sep	Same fish		Trap	

Date	Tag Number	Sex	Site	Year Tagged
27-Sep	Same fish		Trap	
2-Oct	Same fish		NF Tieton	
29-July	180597426	M	NF Tieton	2013
20-Sep	Same fish		Trap	
29-Sep	Same fish		NF Tieton	
16-July	180597450	M	NF Tieton	2013
20-Sep	Same fish		Trap	
21-Sep	Same fish		NF Tieton	
15-July	180597467	M	NF Tieton	2013
11-Sep	Same fish		Trap	
26-Sep	Same fish		Trap	
5-Oct	Same fish		NF Tieton	
5-Aug	180597493	M	NF Tieton	2013
18-Sep	Same fish		Trap	
21-Sep	Same fish		NF Tieton	
12-Sep	180597311	F	Lower Spillway	2013
17-Sep	Same fish		Lower Spillway	
24-Sep	Same fish		Lower Spillway	
25-Sep	Same fish		Lower Spillway	

Bull trout tagged in 2013

The 2014 detection log for the bull trout tagged in 2013 is presented in Table 4 below. Only one of the 14 bull trout tagged in 2013 went undetected in 2014. This female (tag #180597354) has not been detected or recaptured since it was detected leaving the NF Tieton River in late September, 2013. One of the remaining 13 bull trout (tag #180597311) did not make it up the river in 2014. She was detected at the lower spillway PIT tag interrogation site in late September, 2013 indicating she had left Clear Lake; in 2014 she was detected four times from

September 12-25 at the same site attempting to ascend the spillway channel, apparently without success as she was not detected leaving the upper ladder.

The other 12 bull trout tagged in 2013 were detected at the NF Tieton site between June 19 and August 5. Nine of these were later detected just upstream of the trap and two others (which had not been detected there) were recaptured in the trap. All twelve were detected at the NF Tieton site between September 6 and October 5. One of the twelve (tag # 180597185) was later detected at the upper ladder site on October 30.

Bull trout tagged in 2014

Two of the five bull trout tagged at the trap in 2014, both males, were released upstream so they could possibly spawn again. Both were detected one-to-two weeks later at the PIT tag interrogation site upstream of the trap. Another fish, a female, was also detected there just a couple of days after she was tagged. Although she had been released downstream of the trap she had evidently migrated back upstream when weir pickets were removed during the high flow event mentioned previously. All five of the bull trout tagged in 2014 were later detected at the NF Tieton site between September 28 and October 3. One (tag #180597192) was detected in the lower ladder on November 4. Only one of the bull trout tagged below the dam was later detected. This male (tag #180597353) was detected multiple times at the lower spillway site on October 10-11 and again on October 18.Table 5. 2014 detections of adult bull trout tagged in 2013.

Genetic Analyses

Forty genetic samples collected from adult bull trout from 2012 through 2014 were sent to the WDFW Molecular Genetics Lab for analysis. The analysis revealed that thirty-four of these fish were pure bull trout from the North Fork Tieton River population based on the genetic baseline established from previous years sampling. Six were bull/brook trout hybrids, all apparently first generation. All of these hybrids were captured at the trap (three in 2012, two in 2013, and one in 2014). None varied in appearance from the pure bull trout we captured which is surprising. One of the bull trout sampled in 2014 (sample #14FF0012) was genetically identical to a fish sampled in 2012 (sample #12AG0025) indicating they were full siblings. Another identically matched a juvenile NF Tieton bull trout sampled in 2010 and thus was likely the same fish.

None of the eleven fish sampled below Clear Creek Dam were hybrids. However, one fish caught there was a recapture (tag #180597363, a female tagged in 2012) which turned out to be a hybrid (note: this fish is included in the total hybrid number above). The complete genetic report is included in Appendix A.

Effective Population Size

Because of the inherent unpredictability of nature, the conservation of species depends on protecting genetic diversity. When diversity is lost, genetic combinations that ensure survival in variable environments may be lost as well (Rieman and McIntyre 1993; Rieman and Allendorf 2001). Genetic variation will be lost through time in isolated populations, and this loss occurs more quickly in small populations than in large ones. Loss of genetic variation can influence the dynamics and persistence of populations through at least three mechanisms: inbreeding depression, loss of phenotypic variation and plasticity, and loss of evolutionary potential (Allendorf and Ryman 2002). Both theory and empirical evidence clearly indicate the populations that are small and isolated will eventually lose genetic variation and have an increased probability of extirpation (Frankham 1996; Wofford et al. 2005; Whiteley et al. 2010).

The implication is that some minimum number of organisms and effective interactions ar enecessary to maintain genetic diversity and ensure the persistence of a population. Soulé (1987) asserted that the scientific community should provide guidance for the public so conservation programs could proceed. In 1980, he proposed the “50/500” rule (Soulé 1980). That is, in a completely closed population, an effective population size (Ne) of 50 is needed to prevent excessive rates of inbreeding and 500 are needed to maintain genetic variation.

Following the “50/500” rule, Rieman and Allendorf (2001) used VORTEX (Miller and Lacy 1999), a generalized, age-structured, simulations model, to relate Ne to adult numbers under a range of life histories and other conditions characteristic of bull trout populations. Their most realistic estimates of Ne were between 0.5 and 1.0 times the mean number of adults spawning annually. Therefore, a cautious interpretation of their results would be that an average of 100 (i.e., 100 x 0.5 = 50) adults spawning each year would be required to minimize risks of inbreeding in a population and 1,000 (i.e., 1,000 x 0.5 = 500) would be necessary to maintain genetic variation indefinitely.

Effective population size (Ne) was calculated by the WDFW Genetics Lab. The statistic was calculated for each of the collection years using the pairwise sibship method implemented in the program COLONY (Wang 2004). In the pairwise sibship method, the program uses maximum likelihood to estimate whether a pair of samples are full-sibs, half-sibs, or unrelated. Then it calculates the effective number of parents that gave rise to the collection. The program assumes that the collection is a single age class and the bull trout collected in a single year might include multiple age classes. Therefore the estimate should be treated cautiously.

The effective population sizes calculated for the three collection years were fairly consistent: 2012 (Ne = 18, 95% CI 8-58), 2013 (Ne = 23, 95% CI 12-56), and 2014 (Ne = 21, 95% CI = 10-49). Because only a subset of fish may spawn in a given year and reproductive success is unequal, the effective population size is generally smaller than the census size.

Discussion

To restate the objectives of this investigation, the three primary objectives are: 1) to determine when NF Tieton River bull trout attempt to migrate upstream past Clear Creek Dam; 2) to assess their success at doing so under various hydrologic conditions; and 3) to determine post-spawn migration timing and the extent to which the population uses Clear Lake. There are also several ancillary objectives which will add to the limited body of knowledge available for this population. They include determining spawning frequency, collecting genetic samples, and estimating the effective population size. After three years of effort, a review of the progress made in addressing these objectives is appropriate.

In considering the first primary objective it is necessary to first examine part of the third- the extent to which the population uses Clear Lake. At the inception of this investigation it was assumed that this population primarily resided in Rimrock Reservoir downstream of Clear Creek Dam. It appears that this assumption may not have been entirely correct. Only four of the 29 adult bull trout PIT tagged up the NF Tieton River are positively confirmed to have left Clear Lake. A female tagged in 2012 (tag #180597363), while never positively detected leaving the lake in 2012 or 2013, was detected successfully ascending the spillway channel in both 2013 and 2014 (this fish was also captured below Clear Creek Dam in 2014). Interestingly, her genetic analysis revealed she is a hybrid. A male tagged in 2012 (tag #180597290) was confirmed to have left Clear Lake in November, 2014. A female tagged in 2013 (tag #180597311) was confirmed to have left Clear Lake that year and was subsequently detected unsuccessfully attempting to return in September, 2014. The fourth fish which definitely left the lake was a male tagged in 2014 (tag #180597192) who was traveling with his genetically identical brother (tag #180597290, see above).

Eighteen of the 24 bull trout tagged at the trap in 2012 and 2013 apparently never left Clear Lake as they have not been detected either leaving or coming back. Yet all 18 of these fish were detected up the river in either or both 2013 and 2014, and many were recaptured at the trap. (Note: two of the bull trout tagged in 2012 and 2013 have not been detected or recaptured after tagging and one was last detected leaving the NF Tieton River five days after it was tagged in 2013). It might seem reasonable to conclude that a strong majority of this adfluvial population resides full-time in Clear Lake when not on a spawning run. But this conclusion is confounded by the fact that all eleven adult bull trout sampled below Clear Creek Dam in July, 2014 were genetically identified as pure NF Tieton bull trout; a twelfth was the recaptured hybrid discussed above. That is a significant number of adult bull trout that may be unable to migrate up the spillway channel given the apparently small size of this population. Only one tried that we know of and this male's numerous unsuccessful attempts to ascend the channel occurred in mid-October.

At this time it is impossible to evaluate the progress in reaching the first primary objective of the assessment mostly due to a small sample size. As noted above, we have only detected three fish actually attempting to migrate up the spillway channel. One (the hybrid) was successful twice, once in late July, 2013 and once in late August, 2014. With extremely warm water temperatures occurring on both occasions, this hardly seems like an optimal time to migrate. The other two failed, one in mid-to-late September and the other in mid-October, 2014 although water temperatures were suitable (<15°C) on both occasions. It should be noted that both of these fish, had they been successful, would have arrived in the spawning area of the NF Tieton River well after the majority, if not all, of the spawning activity had occurred. We do not know if others also failed, perhaps earlier than July, because the lower spillway PIT tag interrogation site was not installed until July 9 in 2013 and was down between May 16 and July 9, 2014. It will be a definite priority in 2015 to have the lower spillway site up and running in late April to fill this data gap.

Small sample size also precludes a determination of the hydrological conditions which best facilitate passage up the spillway channel. Even a small change in lake elevation (e.g. 0.1 foot) results in a significant difference in spillway discharge and thus passage conditions. After observing the spillway for three years we have seen a wide range of flow conditions and have developed a reasonable idea of those we feel provide the best opportunity for successful passage up the steep lower segment of the channel. These conditions appear to be when the lake elevation is between 3011.2 and 3011.4 feet (mean sea level). The lake was between these levels both times when successful passage up the spillway occurred. However, it was also between these levels the two times it did not. Typically lake levels have significantly exceeded this range from early May through June which we believe may be an important immigration period for NF Tieton bull trout. Over the winter Reclamation's Yakima Field Office has automated the gates at Clear Creek Dam. For the first time since this study began they will have the ability to control lake levels and provide spillway flow conditions more conducive to bull trout passage during the late spring. With the number of NF Tieton bull trout we know are still downstream from the dam (those PIT tagged below the dam last July) and those previously tagged (which may have left Clear Lake, we are hopeful that we will be able to determine whether or not it is feasible to provide effective bull trout passage up the spillway through flow manipulation and if so, what lake levels would provide the desired flows.

The peak spawning activity appears to occur in the NF Tieton River the first three weeks of September. Considering the data from both 2013 and 2014, the earliest date we detected adult bull trout returning to the river, as determined at the NF Tieton PIT tag interrogation site, was June 19 and the latest was September 10. Ninety percent of returning fish were detected between July 1 and August 10 (N=21) (Note: The NF Tieton site was not installed until August 1 in 2013). Once they have spawned, individuals from this population do not appear to linger in the river for long. The earliest date we detected adult bull trout leaving the river was September 6 and the latest was October 13. Ninety-three percent of emigrating fish were detected between September 15 and October 5 (N=41).

Twenty of the 24 bull trout tagged in either 2012 or 2013 ascended the NF Tieton River in successive years. Five of the ten tagged in 2012 were up the river three consecutive years;

three returned the year after they were tagged but were not detected in 2014. Twelve of the 14 fish tagged in 2013 were detected up the river in 2014. The confirmed presence of all of these fish does not guarantee they reproduced but it might be assumed that it is the reason they were there.

An estimate of the effective size (Ne) of this population of bull trout was presented above. Effective population size is a theoretical construct and does not represent the actual size of the population such as would be derived from a census. Nevertheless, it is valid indicator of a population's status with respect to genetic diversity. One can see from the numbers presented that this population is relatively small and likely at risk of loss of genetic variation with annual Ne values ranging from 18-21 individuals and confidence intervals ranging from 8-58 for the three years combined. We are working on a population size estimation for the NF Tieton River bull trout population, which will be presented in the final report for this assessment.

2015 Activities

We will not be trapping any more bull trout on the NF Tieton River in 2015. Our original intent was to run the trap for three consecutive years and given that we only trapped five new fish in 2014 with eight recaptures of fish previously tagged, we feel we have adequately sampled this population. We will be conducting additional hook-and-line sampling below Clear Creek Dam in July with a goal of PIT tagging ten additional adult bull trout and obtaining genetic samples. Had not all, or at least most, of the fish we captured in 2014 belonged to the NF Tieton population we probably would not be repeating the effort. But there is a chance we may be tracking these fish past 2015, the scheduled termination date for this assessment, and tagging more fish would be a worthwhile endeavor. The analysis of additional genetic samples will also add to our knowledge concerning the size of this population and if, as was previously thought, a large number of individuals from it reside in Rimrock Reservoir.

In addition to the PIT interrogation sites we employed in 2014, another will be installed in the outlet channel of the dam. This site will be helpful in determining whether bull trout are drawn to the colder water released from the dam, when this occurs, and if bull trout drawn there are inclined to leave and attempt migration up the spillway channel.

References

Allendorf, F.W., N. Ryman. 2002. The role of genetics in population viability analysis. . *Population Viability Analysis.* D. R. M. S.R. Beissinger. Chicago, University of Chicago Press.

Frankham, R. 1996. "Relationship in genetic variation to population size in wildlife." *Conservation Biology* **10**(6): 1500-1508.

Miller, P.S and R.C Lacey. 1999. VORTEX Version 8 Users Manual. A stochastic simulation of the simulation process. IUCN/SSC Conservation Breeding Specialist Group. Apple Valley, Minnesota.

Rieman, B. E. and F. W. Allendorf. 2001. "Effective Population Size and Genetic Conservation Criteria for Bull Trout." *N orth American Journal of Fisheries Management* 21: 756-764.

Rieman, B. E. and J. D. McIntyre. 1993. Demographic and habitat requirements for conservation of bull trout. Ogden, UT (324 25th St. Ogden 84401), U.S. Dept. of Agriculture, Forest Service, Intermountain Research Station.

Soulé, R. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. In: Soulé, M.E.; Wilcox, B.A., eds. Conservation biology: an evolutionary-ecological perspective. Sunderland, MA: Sinauer Associates: 151-169.

Soulé, R. 1987. Where do we go from here? In: Soulé, M.E., ed. Viable populations for conservation. Cambridge, England: Cambridge University Press: 175-183.

Wang, J. 2004. Sibship Reconstruction From Genetic Data With Typing Errors. Genetics 166: 1963-1979.

Whiteley, A. R., K. Hastings, et al. 2010. "Genetic variation and effective population size in isolated populations of coastal cutthroat trout " *Conservation Genetics* 11(5): 1929-1943.

Wofford, J. E. B., R. E. Gresswell, et al. 2005. "Influence of Barriers to Movement on Within-Watershed Genetic Variation of Coastal Cutthroat Trout." *Ecological Applications* **15**(2): 628-637.

Acknowledgements

The third year of the Clear Creek Dam fish passage assessment was successful not only because of the dedicated efforts of staff from the USFWS and USBR but also because of the contributions of the individuals and organizations acknowledged here.

John Easterbrooks and Eric Anderson from the Washington Department of Fish and Wildlife were once again responsible for providing the ATVs necessary to travel to and from the trap site. Both also worked on trap installation and removal, and put in several days as part of the crew manning the trap. We would also like to thank Cassandra Anderson from the 2014 Bull Trout Task Force who for the second year provided valuable assistance manning the trap.

We would like to thank the Washington Department of Fish and Wildlife's Molecular Genetic Lab, particularly Maureen Small and Sarah Bell, for their excellent work in conducting the genetic analysis.

Finally we would like to acknowledge Reclamation's Yakima Field Office, the Columbia River Fisheries Enhancement Group, and the U.S. Forest Service (Naches Ranger Station). Their cooperation with various aspects of this study was greatly appreciated.

Appendix A

2015 Genetic Report for Bull Trout Sampled in the

North Fork Tieton River

This page intentionally blank

2015 Clear Creek Dam Bull Trout Passage Assessment

USFWS

Mid-Columbia River Fishery Resource Office

Yakima Sub-Office

February 2016 - Final Report

Maureen P. Small[1], Jeff Thomas[2], and Sarah Bell[1]

[1]WDFW Molecular Genetics Lab, Conservation Unit, 1111 Washington St. SE, Olympia, WA 98501

[2]USFWS Mid-Columbia River Fishery Resource Office, 1917 Marsh Road, Yakima, WA 98901

Background & Project Justification

The Clear Creek Dam Bull Trout Passage Assessment is a cooperative investigation being conducted by staff from the U.S. Fish and Wildlife Service's Mid-Columbia Fishery Resource Office (Yakima Sub-Office), the U.S. Bureau of Reclamation's Columbia-Cascades Area Office, and the Washington Department of Fish and Wildlife (Region 3). The study stream is the North Fork Tieton River located in the Yakima Basin (WRIA 38). Planning for this study began in early 2012 and the assessment will continue through 2015.

The local population of bull trout spawning in the North Fork Tieton River was unrecognized until 2004 when biologists observed juvenile fish (obtaining genetic samples from some) and documented spawning activity for the first time during a comprehensive fish census (USFWS 2005). This census occurred above Clear Lake, a relatively small (4,400 acre-feet) impoundment formed when Clear Creek Dam was constructed on the North Fork Tieton River (hereafter North Fork) in 1914. Clear Creek Dam is located slightly less than one kilometer above Rimrock Lake, a much larger (198,000 acre-feet) reservoir impounded by Tieton Dam in 1925. Both of these dams were constructed by the U.S. Bureau of Reclamation (BOR) and are operated by the agency's Yakima Field Office (YFO). There are two additional bull trout populations found above Tieton Dam which spawn in separate tributaries of Rimrock Lake, South Fork Tieton River and Indian Creek. All three of these bull trout populations display the adfluvial life history type and adults from all three populations reside in Rimrock Lake when not spawning or migrating. Prior to impoundment they were fluvial or resident populations because there were no lakes (reservoirs) in the Tieton basin before the dams.

Clear Creek and Tieton dams were constructed without fish passage facilities. Consequently, upon their completion anadromous salmonids were excluded from habitat upstream and resident fish populations above the dams were isolated. While Tieton Dam remains impassable, two fish ladders were constructed in the bedrock spillway channel of Clear Creek Dam in 1992. The lower "ladder" is actually a series of four denil ladders interspaced with resting pools located on the right bank of the spillway. The slopes of the four ladder sections range from 12.5 to 45 percent. The upper ladder is a pool-and-weir design constructed on the left side of the spillway channel. It contains 11 weirs with a two-foot hydraulic drop from weir to weir. The pool-and-weir ladder appears to be functional but this is not the case with the denil ladder. The denil ladder is considered too steep and does not meet accepted passage criteria (USBR 2004) so it may have never passed fish with much success. Currently, it does not appear to be usable for fish passage as it has not been maintained in years, is completely clogged with gravel, and is otherwise in disrepair.

The ultimate goal of this study is to ensure that this population of bull trout can successfully reach its spawning habitat in the North Fork Tieton above Clear Creek Dam. It is believed that this population currently has such access only under rare flow conditions. Not only is this a current problem for this population but the severity of it may increase in the future. Climate change models developed for the Pacific Northwest are consistent in predicting warmer winters and decreased snowpack. It is essential that cold-water species such as bull trout have access to habitat at higher elevations if their populations are to persist. The data we obtain should enable us to advise the YFO on operations at Clear Creek Dam that will facilitate adult bull trout migration past the structure. There is a strong possibility that the results of this study will lead to a decision to construct passage facilities at the dam or rebuild the facilities in the spillway channel.

The three primary objectives are of this investigation are: 1) to determine when North Fork Tieton River bull trout attempt to migrate upstream past Clear Creek Dam; 2) to assess their migration success under various hydrologic conditions; and 3) determine post-spawn migration timing and the extent to which the population uses Clear Lake. In order to track the movements of adult bull trout, fish are captured in a picket-weir box trap as they migrate downstream after spawning and surgically implanted with passive integrated transponder (PIT) tags. The fish are trapped and tagged on the North Fork about five miles above Clear Lake. After release the tagged fish can be detected at any of several locations where detection antenna arrays have been installed. The ancillary objectives include: determine spawning frequency, collect genetic samples to augment the genetic baseline, and estimate effective population size. The primary and ancillary information should help fish managers develop actions to ensure the population's long-term health and persistence. This report addresses the ancillary objectives.

WDFW Genetics Lab Activities

This stage of the project seeks to better characterize the North Fork Tieton River bull trout population for the genetic baseline. The baseline will be used in future work described above. For the baseline work, tissue samples were collected from bull trout captured in the North Fork Tieton River (see Table 1) in 2015 (N=14). North Fork Tieton fish were compared to bull trout collected in the North Fork Tieton in previous years (Table 1), and to other Tieton basin populations, and examined for introgression by brook trout planted in the tributary, that have naturalized.

For each tissue sample (fin clips) DNA was extracted using silica membrane based kits (Macherey-Nagel) following the manufacturers protocol. Microsatellite alleles were amplified by PCR (polymerase chain reaction) using fluorescently end-labeled primers with poly-adenylated tails to stabilize the reactions and some primers were labeled with fluorescent vector tails (see Table 2). PCR conditions are given for the loci in Table 2. PCRs were conducted using an M-J Research PTC-200 thermal cycler or an Applied Biosystems 9700 duel block thermal cycler, with a simple thermal profile consisting of: initial denature at 95º C for 3 min., followed by 4 cycles of denature at 95º C for 30 sec., anneal for 30 sec at a touchdown temperature starting at 60°C decreasing 1°C each cycle, extend at 72º C for 1 min., followed by 36 cycles of denature at 95°C for 30 sec, anneal at 50°C for 30 sec and then extend at 72°C for 1 min (40 cycles total), final extension at 72º C for 10 min (see Table 2). PCR products were visualized using an ABI-3730 DNA Analyzer with internal size standards (GS500LIZ 3730) and GeneMapper 3.7 software. Alleles were binned and named to AFTC standardized nomenclature using GeneMapper 3.7 software.

We used GENETIX 4.03 (Belkhir et al. 2002) to graphically view the genetic variation among among collections in three-dimensional space. GENETIX performs a factorial correspondence analysis, in which composite axes are generated from the combination of allele frequencies that describes the most genetic variation among individual fish. The program generates a plot of individuals in three dimensions according to their genotype. Individuals that are genetically similar plot near each other and individuals that are genetically different plot distantly from each other. Individuals with mixed ancestry (hybrids) plot between clusters.

We used a Bayesian clustering analysis implemented in STRUCTURE 2.2 (Pritchard et al. 2000) to estimate individual and population ancestry, and to identify possible hybrids. We included all collections listed in Table 1, and set the number of clusters or possible populations at 2 – 8. The brook trout were included in the analysis to identify any hybrids between brook and bull trout. STRUCTURE sorts individuals (or portions of individuals if they are hybrids) into a number of hypothetical population clusters (K), minimizing Hardy-Weinberg disequilibrium and linkage disequilibrium in the clusters. These clusters are composed of individuals with common ancestry shared through contemporary or recent gene flow. Bull trout populations are generally spatially segregated and natal fidelity is high such that contemporary gene flow is minimal and all members of a population (or closely related populations) would occupy the same cluster. Because the analysis may find multiple solutions, we conducted analyses in 5 independent runs that allowed admixture with 50,000 burn-ins (a randomization procedure that prevents starting conditions from influencing outcomes) and 200,000 iterations and observed solution stabilities at each K. For each run, the program outputs a likelihood value for the number of clusters, given the data. When the likelihood value reaches a maximum or asymptote the program has resolved the identifiable genetic groups in the data set.

Effective number of breeders was calculated for each of the collection years using the pairwise sibship method implemented in the program COLONY (Wang 2004). In the pairwise sibship method, the program uses maximum likelihood to estimate whether a pair of samples are full-sibs, half-sibs, or unrelated. Then it calculates the effective number of parents that gave rise to the collection. The program assumes that the collection is a single age class and the bull trout collected in a single year might include multiple age classes or might be offspring of multiple aged individuals. In species with overlapping generations the effective population size is greater than the effective number of breeders calculated with a linkage disequilibrium method (Waples et al. 2014) and the estimate should be treated cautiously.

Results

The factorial correspondence analysis suggested seven individuals collected in the North Fork Tieton River were bull trout-brook trout hybrids (Figure 1): these samples plotted in the space between the bull trout and brook trout clusters (12AG0010, 12AG0026, 12AG0030, 13HJ0008, 13HJ0013, 14FF0016, and 15HG0005). In the STRUCTURE analysis these seven fish had roughly equal ancestry in the North Fork Tieton and brook trout clusters (Figure 2a and Figure 2b), indicating that they were bull/brook trout hybrids (Table 3). Because the ancestry values were roughly equal for membership in the bull trout and brook trout clusters, we suspect these were first generation hybrids. The STRUCTURE analysis also suggested that one fish collected in the North Fork Tieton originated in the South Fork Tieton (15HG0008) and another fish originated in Indian Creek (15HG0011). Two of the fish previously collected in the North Fork Tieton appeared to have mixed North Fork Tieton-Indian Creek ancestry (01AAC0012 and 10NE0026, see Appendix I). Two fish previously collected in South Fork Tieton (96GS0311 and 96GS0321) appeared to have mixed North Fork Tieton-South Fork Tieton ancestry, suggesting that North Fork Tieton fish occasionally spawned with South Fork Tieton fish in the South Fork Tieton. Also, two fish previously collected in Indian Creek had mixed North Fork Tieton-Indian Creek ancestry (96GT0803 and 11LJ0021), suggesting that North Fork Tieton fish occasionally spawned with Indian Creek fish in Indian Creek.

Seventy-five of the fish collected in the North Fork Tieton had over 90% ancestry in the North Fork Tieton population and will be added to the genetic baseline for North Fork Tieton. One fish collected in 2014 was resample of a fish collected in 2010 (10NE0010 matched 14FF0014). This fish was a juvenile (130mm) when previously sampled. Another bull trout sampled in 2014 (14FF0012) was likely a full sibling of an adult male (12AG0025) captured and PIT tagged in 2012 because they had identical genotypes. This was not a resample of 12AG0025 because that fish has been detected several times in the system and 12AG0025 has a different tag number from 14FF0012. The effective number of breeders calculated for the four collection years were fairly consistent: 2012 (Ne = 18, 95%CI 8-58), 2013 (Ne = 23, 95%CI 12-56), 2014 (Ne = 21, 95%CI = 10-49), and 2015 (Ne = 18, 95%CI = 9-45). Because only a subset of fish may spawn in a given year and reproductive success is unequal, the effective number of breeders calculated is generally smaller than the census size. Further, in species with overlapping year classes, the effective number of breeders is usually less than the effective population size – in simulations for brook trout the effective number of breeders calculated using a linkage disequilibrium method was roughly half the effective population size (Waples et al. 2014). Because bull trout are similar to brook trout with regard to age of maturity and iteroparity, effective number of breeders calculated for bull trout might be similarly expanded to estimate the effective population size and census size would also be larger than effective population size.

References

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 2001. *genetix* 4.02, *logiciel sous Windows* TM *pour la genetique des populations*. Laboratoire Genone, Populations, Interactions: CNRS UMR 5000, Universite de Montpellier II, Montpellier, France.

Pritchard J, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945 - 959.

USBR (2004). Draft Predesign Memorandum. Clear Creek Dam Fish Passage Facilities. June 2004.

USFWS (2005). Fish Census of the North Fork Tieton River, Yakima River Basin, Washington. Final report, U.S. Fish and Wildlife Service, Mid-Columbia River Fishery Resource Office.

Wang J. 2004. Sibship Reconstruction From Genetic Data With Typing Errors. Genetics 166: 1963-1979.

Waples RS, Antao T, Luikart G. 2014. Effects of overlapping generations on linkage disequilibrium estimates of effective population size. Genetics 114.

Table 1. List of samples genotyped for the study (contemporary) and archived baseline collections used for comparisons.

	Contemporary Samples		
Type	**WDFW Code**	**Location**	**N**
Bull trout	15HG	North Fork Tieton	14
	14FF	North Fork Tieton	16
	13HG	North Fork Tieton	14
	12AG	North Fork Tieton	10
	Archived Samples in baseline		
	WDFW Code	Population	N
Bull trout	96GS	SF Tieton	48
	98JY	SF Tieton	15
	01AAB	SF Tieton	13
	02NQ	SF Tieton	8
	03GG	SF Tieton	8
	03AAA	SF Tieton	7
	03AAB	SF Tieton	2
	08KI	SF Tieton	2
	03GG	North Fork Tieton	3
	01AAC	North Fork Tieton	1
	05NN	North Fork Tieton	5
	08MY	North Fork Tieton	1
	10NE	North Fork Tieton	27
	12AG	North Fork Tieton	10
	13HG	North Fork Tieton	14
	14FF	North Fork Tieton	16

Type	WDFW Code	Location	N
	96GT	Indian	49
	01AAC	Indian	28
	03AAA	Indian	7
	03AAB	Indian	4
	03GG	Indian	12
	11LJ	Indian	37
Brook trout	09ID, 09IE, 09IF	Lake Shannon Brook Trout	32

Table 2. List of microsatellite loci and PCR conditions. Poly-a tails (+a) were added to DNA primers to stabilize PCR reactions. Where label vectors (V) were added to primers, their concentration is also provided. Final $MgCl_2$ concentration was 1.5 mM for all reactions.

Multiplex	Primer/Vector	Primer/Vector Conc (mM)	Dye
Sco-E	Omm-1128 +a	0.15	vic
	Sco-105 +a	0.08	ned
Sco-I	Sco-218 V1+a	0.16	none
	V1	0.08	vic
	Sco-202 V2+a	0.13	none
	V2	0.06	6fam
	Sco-200 V4+a	0.21	none
	V4	0.1	pet
	Sco-220 V3+a	0.12	none
	V3	0.06	ned
Sco-J	Sco-216 V2+a	0.16	none
	V2	0.08	6fam
	Sco-215 V4+a	0.12	none
	V4	0.06	pet
Sco-K	Sco-109 +a	0.25	6fam
	Sfo-18 V3 +a	0.16	none
	V3	0.08	ned
	Smm-22 V4 +a	0.16	none
	V4	0.08	pet
Sco-L	Sco-106 +a	0.14	6fam
	Sco-102 +a	0.07	vic
	Omm-1130 +a	0.14	ned
Sco-M	Sco-212 V2 +a	0.16	none
	V2	0.08	6fam
	Sco-107 +a	0.12	ned

Table 3. Biological data and genotypic information for bull trout collected in the North Fork Tieton River 2012, 2013, 2014, and 2015. The STRUCTURE cluster membership values following the assignments are plotted in Figure 2a and Figure 2b.

		Structure clusters									
WDFWI	**Assign**	**NFTiet**	**SFTiet**	**Indi**	**Broo**	**Date**	**Stream**	**Vial #**	**FL:**	**Sex**	**Match**
15HG00	15NFTiet	0.993	0.002	0.00	0.00	7/2/2015	NF	15HG	570	Male	
15HG00	15NFTiet	0.993	0.002	0.00	0.00	7/2/2015	NF	15HG	510	Female	
15HG00	15NFTiet	0.989	0.004	0.00	0.00	7/2/2015	NF	15HG	650	Female	
15HG00	15NFTiet	0.966	0.004	0.02	0.00	7/2/2015	NF	15HG	635	Male	
15HG00	hybrid	0.720	0.003	0.00	0.27	7/2/2015	NF	15HG	610	Female	
15HG00	15NFTiet	0.987	0.007	0.00	0.00	7/2/2015	NF	15HG	585	Female	
15HG00	15NFTiet	0.991	0.002	0.00	0.00	7/2/2015	NF	15HG	675	Male	
15HG00	15NFTiet	0.024	0.962	0.00	0.00	7/2/2015	NF	15HG	380	Unkno	
15HG00	15NFTiet	0.990	0.004	0.00	0.00	7/2/2015	NF	15HG	620	Male	
15HG00	15NFTiet	0.988	0.003	0.00	0.00	7/2/2015	NF	15HG	510	Female	
15HG00	15NFTiet	0.086	0.038	0.87	0.00	7/2/2015	NF	15HG	635	Female	
15HG00	15NFTiet	0.992	0.002	0.00	0.00	7/2/2015	NF	15HG	585	Female	
15HG00	15NFTiet	0.992	0.003	0.00	0.00	7/2/2015	NF	15HG	560	Female	
15HG00	15NFTiet	0.991	0.003	0.00	0.00	7/2/2015	NF	15HG	510	Female	
14FF00	NFTieton	0.990	0.003	0.00	0.00	7/31/2014	NF	14FF1	500	Female	
14FF00	NFTieton	0.937	0.003	0.05	0.00	7/31/2014	NF	14FF2	535	Male	
14FF00	NFTieton	0.984	0.003	0.00	0.00	7/31/2014	NF	14FF3	580	Male	
14FF00	NFTieton	0.990	0.003	0.00	0.00	7/31/2014	NF	14FF4	590	Male	
14FF00	NFTieton	0.961	0.022	0.01	0.00	7/31/2014	NF	14FF5	465	Female	
14FF00	NFTieton	0.958	0.006	0.02	0.00	7/31/2014	NF	14FF6	510	Female	
14FF00	NFTieton	0.988	0.004	0.00	0.00	7/31/2014	NF	14FF7	480	Male	
14FF00	NFTieton	0.989	0.005	0.00	0.00	7/31/2014	NF	14FF8	620	Male	
14FF00	NFTieton	0.992	0.002	0.00	0.00	7/31/2014	NF	14FF9	440	Female	
14FF00	NFTieton	0.916	0.021	0.06	0.00	7/31/2014	NF	14FF1	485	Female	
14FF00	NFTieton	0.971	0.006	0.01	0.00	7/31/2014	NF	14FF1	545	Female	
14FF00	NFTieton	0.992	0.002	0.00	0.00	9/10/2014	NF	14FF1	600	Male	12AG00
14FF00	NFTieton	0.988	0.005	0.00	0.00	9/22/2014	NF	14FF1	685	Male	
14FF00	NFTieton	0.993	0.003	0.00	0.00	9/23/2014	NF	14FF1	495	Female	10NE00
14FF00	NFTieton	0.959	0.011	0.01	0.01	9/26/2014	NF	14FF1	530	Female	
14FF00	hybrid	0.606	0.002	0.00	0.38	9/28/2014	NF	14FF1	565	Male	
13HJ00	NFTieton	0.989	0.003	0.00	0.00	9/13/2013	NF	13HJ1	435	Female	
13HJ00	NFTieton	0.987	0.004	0.00	0.00	9/13/2013	NF	13HJ2	475	Male	

WDFWI	Assign	NFTiet	SFTiet	Indi	Broo	Date	Stream	Vial #	FL:	Sex	Match
13HJ00	NFTieton	0.992	0.002	0.00	0.00	9/17/2013	NF	13HJ3	590	Female	
13HJ00	NFTieton	0.985	0.004	0.00	0.00	9/18/2013	NF	13HJ4	590	Male	
13HJ00	NFTieton	0.990	0.003	0.00	0.00	9/18/2013	NF	13HJ5	680	Female	
13HJ00	NFTieton	0.986	0.003	0.00	0.00	9/18/2013	NF	13HJ6	550	Female	
13HJ00	NFTieton	0.900	0.092	0.00	0.00	9/19/2013	NF	13HJ7	680	Female	
13HJ00	hybrid	0.543	0.003	0.00	0.44	9/22/2013	NF	13HJ8	590	Female	
13HJ00	NFTieton	0.992	0.002	0.00	0.00	9/23/2013	NF	13HJ9	490	Female	
13HJ00	NFTieton	0.993	0.002	0.00	0.00	9/24/2013	NF	13HJ1	755	Female	
13HJ00	NFTieton	0.992	0.003	0.00	0.00	9/24/2013	NF	13HJ1	495	Male	
13HJ00	NFTieton	0.884	0.006	0.10	0.00	9/25/2013	NF	13HJ1	450	Male	
13HJ00	hybrid	0.556	0.002	0.00	0.43	9/26/2013	NF	13HJ1	540	Female	
13HJ00	NFTieton	0.990	0.003	0.00	0.00	9/26/2013	NF	13HJ1	490	Male	
12AG00	hybrid	0.597	0.002	0.00	0.39	9/24/2012	NF	12AG	465	Female	
12AG00	NFTieton	0.990	0.003	0.00	0.00	9/24/2012	NF	12AG	705	Male	
12AG00	NFTieton	0.992	0.002	0.00	0.00	9/30/2012	NF	12AG	550	Male	
12AG00	NFTieton	0.992	0.002	0.00	0.00	9/26/2012	NF	12AG	820	Male	
12AG00	NFTieton	0.992	0.002	0.00	0.00	9/19/2012	NF	12AG	490	Male	
12AG00	hybrid	0.565	0.004	0.00	0.42	9/29/2012	NF	12AG	570	Female	
12AG00	hybrid	0.651	0.002	0.00	0.34	9/19/2012	NF	12AG	600	Female	
12AG00	NFTieton	0.992	0.002	0.00	0.00	9/19/2012	NF	12AG	700	Female	
12AG00	NFTieton	0.991	0.002	0.00	0.00	9/19/2012	NF	12AG	480	Male	
12AG00	NFTieton	0.980	0.002	0.01	0.00	9/29/2012	NF	12AG	525	Male	

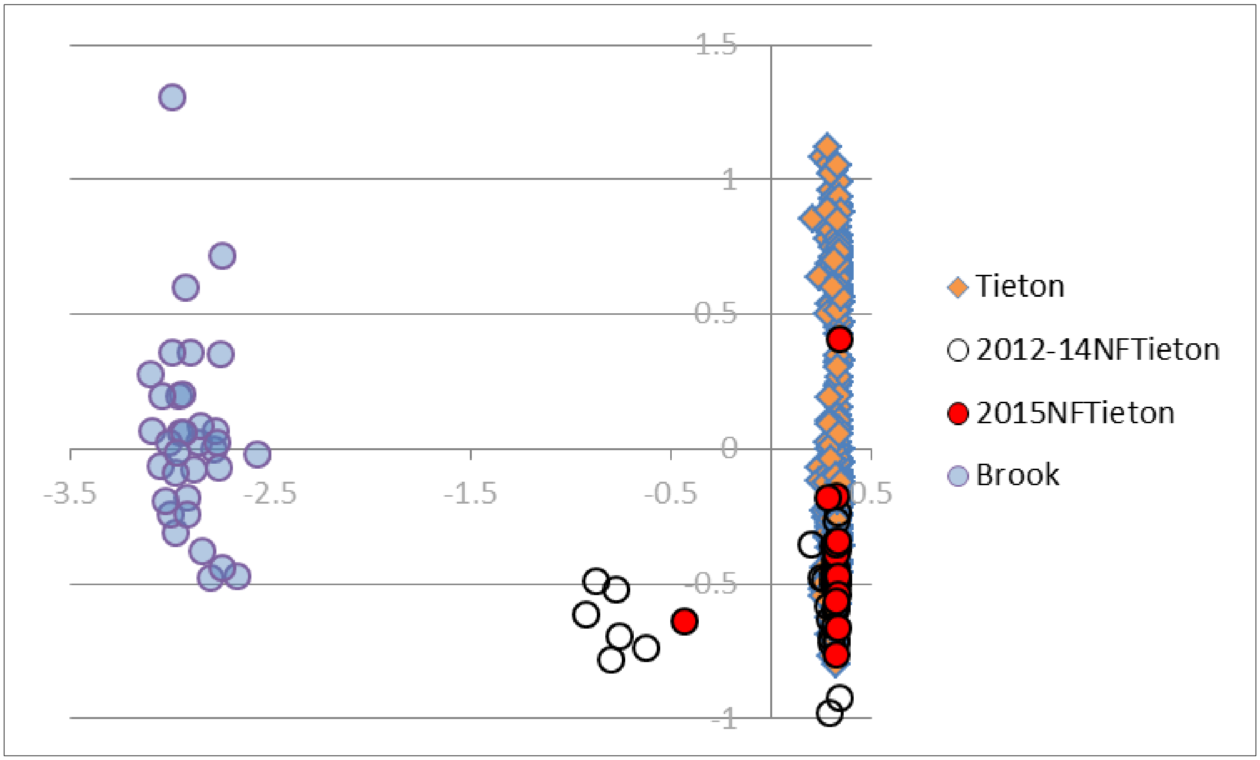

Figure 1. Factorial correspondence analysis plot: individual samples are plotted in a two dimensional genetic space that encompasses the maximum amount of genetic variation in the data set.

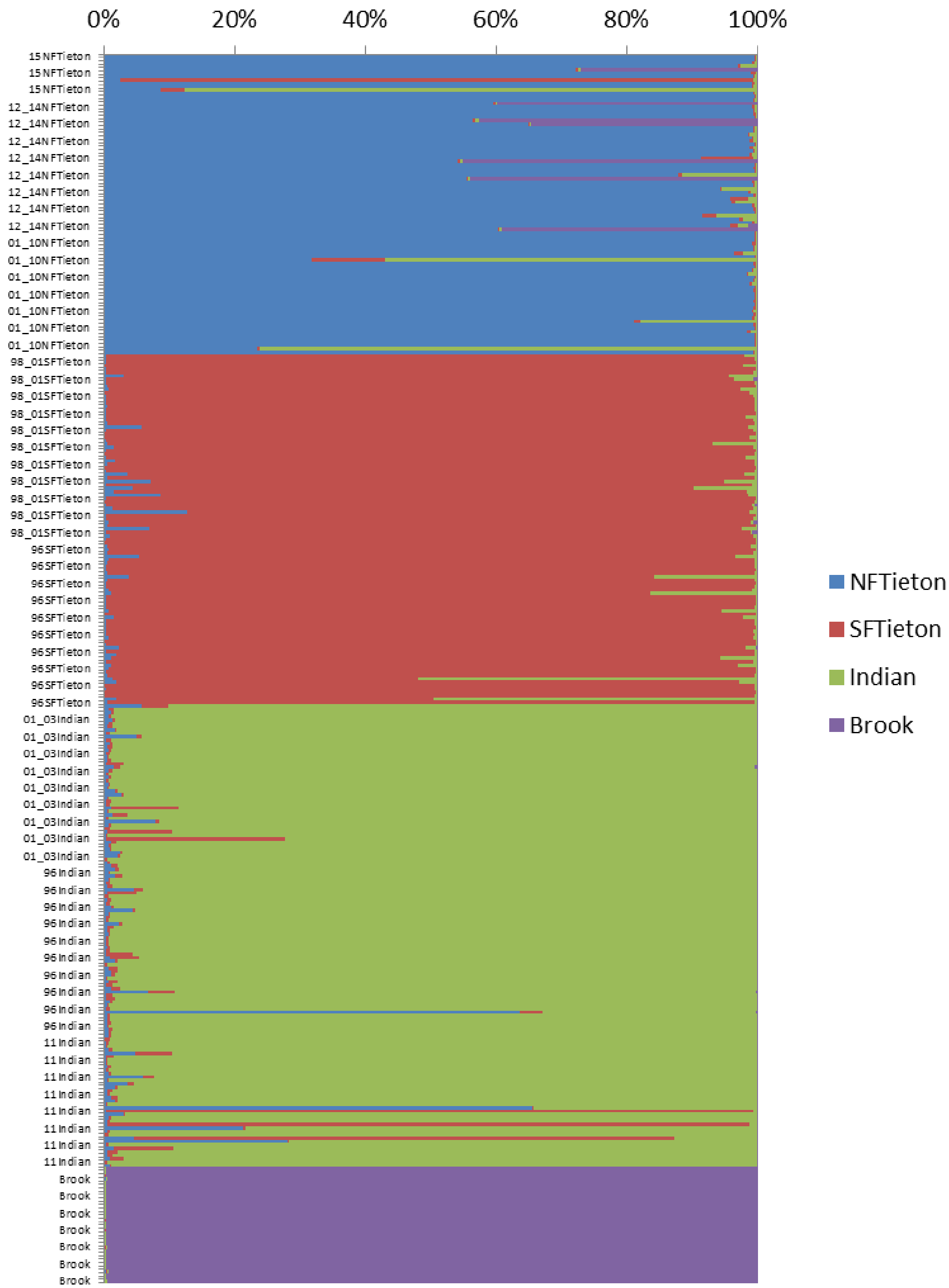

Figure 2a. Plot of ancestry values from STRCTURE for samples analyzed in 2015 (top of plot) in comparison to the Yakima bull trout genetic baseline in the Tieton basin. Brook trout were included in analysis to assess possible hybridization. Each baseline sample has a color associated with their gene pool. Single ancestry individuals have a single color from a single gene pool and mixed ancestry individuals have two or more colors from multiple gene pools.

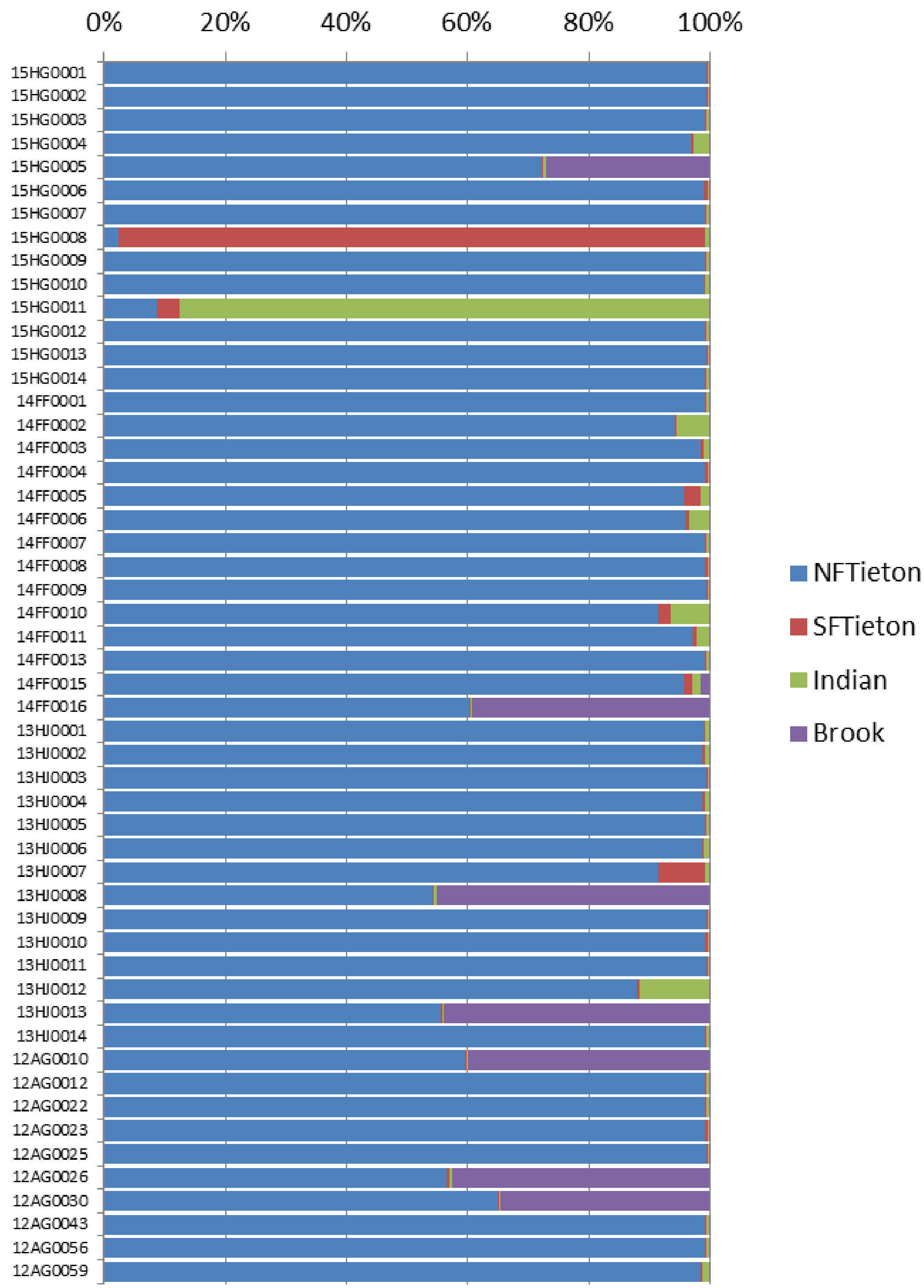

Figure 2b. STRUCTURE plot detail of the Yakima bull trout samples analyzed in 2015 (samples analyzed in fall 2015 at top). Individuals with roughly equal bull and brook trout ancestry (half blue and half purple) were likely first generation hybrids.

Appendix I. List of Tieton basin bull trout from the Yakima bull trout baseline and their cluster membership values from STRUCTURE (see Figure 2a).

		Structure Clusters									
Fish ID	**Collection**	**NFTiet**	**SFTiet**	**Indi**	**Bro**	**Fish ID**	**Collection**	**NFTiet**	**SFTiet**	**Indi**	**Bro**
01AAC0	01_10NFTi	0.315	0.111	0.56	0.00	03GG004	01_03Ind	0.057	0.042	0.89	0.00
03GG009	01_10NFTi	0.990	0.003	0.00	0.00	03GG002	01_03Ind	0.008	0.007	0.98	0.00
03GG011	01_10NFTi	0.948	0.013	0.02	0.00	03GG004	01_03Ind	0.012	0.003	0.98	0.00
03GG011	01_10NFTi	0.994	0.002	0.00	0.00	03GG004	01_03Ind	0.008	0.004	0.98	0.00
05NN000	01_10NFTi	0.992	0.003	0.00	0.00	03GG008	01_03Ind	0.013	0.005	0.98	0.00
05NN000	01_10NFTi	0.992	0.002	0.00	0.00	03GG008	01_03Ind	0.008	0.006	0.98	0.00
05NN000	01_10NFTi	0.993	0.002	0.00	0.00	03GG008	01_03Ind	0.005	0.009	0.97	0.00
05NN000	01_10NFTi	0.989	0.005	0.00	0.00	01AAC0	01_03Ind	0.017	0.003	0.97	0.00
05NN000	01_10NFTi	0.990	0.002	0.00	0.00	01AAC0	01_03Ind	0.003	0.007	0.97	0.00
08MY00	01_10NFTi	0.991	0.004	0.00	0.00	01AAC0	01_03Ind	0.050	0.008	0.93	0.00
10NE000	01_10NFTi	0.993	0.003	0.00	0.00	01AAC0	01_03Ind	0.004	0.007	0.98	0.00
10NE000	01_10NFTi	0.988	0.003	0.00	0.00	01AAC0	01_03Ind	0.009	0.005	0.98	0.00
10NE000	01_10NFTi	0.981	0.002	0.01	0.00	01AAC0	01_03Ind	0.006	0.008	0.98	0.00
10NE000	01_10NFTi	0.993	0.002	0.00	0.00	01AAC0	01_03Ind	0.008	0.003	0.98	0.00
10NE000	01_10NFTi	0.991	0.002	0.00	0.00	01AAC0	01_03Ind	0.004	0.005	0.98	0.00
10NE000	01_10NFTi	0.986	0.002	0.00	0.00	01AAC0	01_03Ind	0.006	0.002	0.98	0.00
10NE000	01_10NFTi	0.993	0.002	0.00	0.00	01AAC0	01_03Ind	0.005	0.006	0.98	0.00
10NE000	01_10NFTi	0.992	0.003	0.00	0.00	01AAC0	01_03Ind	0.004	0.027	0.96	0.00
10NE000	01_10NFTi	0.993	0.002	0.00	0.00	01AAC0	01_03Ind	0.016	0.009	0.96	0.00
10NE001	01_10NFTi	0.993	0.003	0.00	0.00	01AAC0	01_03Ind	0.008	0.005	0.98	0.00
10NE001	01_10NFTi	0.992	0.003	0.00	0.00	01AAC0	01_03Ind	0.003	0.005	0.99	0.00
10NE001	01_10NFTi	0.992	0.003	0.00	0.00	01AAC0	01_03Ind	0.008	0.004	0.98	0.00
10NE001	01_10NFTi	0.991	0.004	0.00	0.00	01AAC0	01_03Ind	0.004	0.003	0.99	0.00
10NE001	01_10NFTi	0.988	0.002	0.00	0.00	01AAC0	01_03Ind	0.007	0.003	0.98	0.00
10NE001	01_10NFTi	0.990	0.004	0.00	0.00	01AAC0	01_03Ind	0.005	0.003	0.99	0.00
10NE001	01_10NFTi	0.989	0.004	0.00	0.00	01AAC0	01_03Ind	0.017	0.004	0.97	0.00
10NE001	01_10NFTi	0.803	0.009	0.17	0.00	01AAC0	01_03Ind	0.027	0.003	0.96	0.00
10NE001	01_10NFTi	0.992	0.003	0.00	0.00	01AAC0	01_03Ind	0.005	0.003	0.98	0.00
10NE001	01_10NFTi	0.993	0.002	0.00	0.00	01AAC0	01_03Ind	0.004	0.008	0.98	0.00
10NE002	01_10NFTi	0.979	0.006	0.01	0.00	01AAC0	01_03Ind	0.004	0.005	0.98	0.00
10NE002	01_10NFTi	0.992	0.002	0.00	0.00	01AAC0	01_03Ind	0.012	0.101	0.88	0.00
10NE002	01_10NFTi	0.992	0.002	0.00	0.00	01AAC0	01_03Ind	0.005	0.003	0.98	0.00
10NE002	01_10NFTi	0.993	0.003	0.00	0.00	01AAC0	01_03Ind	0.013	0.023	0.96	0.00
10NE002	01_10NFTi	0.993	0.002	0.00	0.00	01AAC0	01_03Ind	0.003	0.005	0.99	0.00

Fish ID	Collection	NFTiet	SFTiet	Indi	Bro	Fish ID	Collection	NFTiet	SFTiet	Indi	Bro
10NE002	01_10NFTi	0.233	0.004	0.75	0.00	03AAB0	01_03Ind	0.079	0.005	0.90	0.00
10NE002	01_10NFTi	0.991	0.002	0.00	0.00	03AAB0	01_03Ind	0.007	0.005	0.98	0.00
01AAB0	98_01SFTie	0.004	0.973	0.01	0.00	03AAB0	01_03Ind	0.002	0.008	0.98	0.00
01AAB0	98_01SFTie	0.003	0.988	0.00	0.00	03AAB0	01_03Ind	0.006	0.098	0.89	0.00
01AAB0	98_01SFTie	0.003	0.992	0.00	0.00	03AAA0	01_03Ind	0.004	0.002	0.99	0.00
01AAB0	98_01SFTie	0.002	0.972	0.02	0.00	03AAA0	01_03Ind	0.003	0.273	0.71	0.00
01AAB0	98_01SFTie	0.003	0.992	0.00	0.00	03AAA0	01_03Ind	0.011	0.009	0.97	0.00
01AAB0	98_01SFTie	0.004	0.987	0.00	0.00	03AAA0	01_03Ind	0.008	0.004	0.98	0.00
01AAB0	98_01SFTie	0.030	0.922	0.04	0.00	03AAA0	01_03Ind	0.009	0.003	0.97	0.00
01AAB0	98_01SFTie	0.004	0.957	0.02	0.00	03AAA0	01_03Ind	0.025	0.004	0.96	0.00
01AAB0	98_01SFTie	0.003	0.990	0.00	0.00	03AAA0	01_03Ind	0.022	0.003	0.97	0.00
01AAB0	98_01SFTie	0.005	0.989	0.00	0.00	03GG011	01_03Ind	0.002	0.003	0.99	0.00
01AAB0	98_01SFTie	0.007	0.963	0.02	0.00	03GG011	01_03Ind	0.006	0.004	0.98	0.00
01AAB0	98_01SFTie	0.002	0.941	0.01	0.00	03GG011	01_03Ind	0.012	0.009	0.97	0.00
01AAB0	98_01SFTie	0.003	0.989	0.00	0.00	03GG012	01_03Ind	0.017	0.005	0.97	0.00
03GG001	98_01SFTie	0.003	0.990	0.00	0.00	96GT006	96Indian	0.007	0.002	0.98	0.00
03GG001	98_01SFTie	0.004	0.989	0.00	0.00	96GT012	96Indian	0.018	0.011	0.96	0.00
03GG008	98_01SFTie	0.005	0.988	0.00	0.00	96GT014	96Indian	0.007	0.003	0.98	0.00
03GG008	98_01SFTie	0.003	0.990	0.00	0.00	96GT014	96Indian	0.003	0.007	0.96	0.00
03GG009	98_01SFTie	0.003	0.990	0.00	0.00	96GT015	96Indian	0.006	0.007	0.98	0.00
03GG009	98_01SFTie	0.003	0.976	0.01	0.00	96GT015	96Indian	0.046	0.013	0.93	0.00
08KI000	98_01SFTie	0.004	0.969	0.00	0.00	96GT015	96Indian	0.006	0.044	0.94	0.00
08KI000	98_01SFTie	0.006	0.987	0.00	0.00	96GT015	96Indian	0.002	0.005	0.99	0.00
03GG002	98_01SFTie	0.057	0.928	0.01	0.00	96GT015	96Indian	0.005	0.006	0.98	0.00
98JY000	98_01SFTie	0.003	0.984	0.00	0.00	96GT016	96Indian	0.004	0.005	0.98	0.00
98JY000	98_01SFTie	0.002	0.992	0.00	0.00	96GT016	96Indian	0.012	0.003	0.98	0.00
98JY000	98_01SFTie	0.002	0.982	0.01	0.00	96GT016	96Indian	0.045	0.003	0.95	0.00
98JY000	98_01SFTie	0.003	0.992	0.00	0.00	96GT016	96Indian	0.007	0.003	0.98	0.00
98JY000	98_01SFTie	0.005	0.915	0.06	0.00	96GT016	96Indian	0.004	0.006	0.98	0.00
98JY000	98_01SFTie	0.015	0.975	0.00	0.00	96GT017	96Indian	0.003	0.004	0.99	0.00
98JY000	98_01SFTie	0.003	0.992	0.00	0.00	96GT018	96Indian	0.023	0.005	0.96	0.00
98JY000	98_01SFTie	0.002	0.989	0.00	0.00	96GT018	96Indian	0.005	0.010	0.97	0.00
98JY001	98_01SFTie	0.003	0.964	0.01	0.00	96GT019	96Indian	0.006	0.004	0.98	0.00
98JY001	98_01SFTie	0.017	0.973	0.00	0.00	96GT071	96Indian	0.007	0.003	0.98	0.00
98JY001	98_01SFTie	0.006	0.984	0.00	0.00	96GT072	96Indian	0.004	0.003	0.98	0.00

Fish ID	Collection	NFTiet	SFTiet	Indi	Bro	Fish ID	Collection	NFTiet	SFTiet	Indi	Bro
98JY001	98_01SFTie	0.002	0.992	0.00	0.00	96GT073	96Indian	0.004	0.003	0.94	0.00
98JY001	98_01SFTie	0.004	0.989	0.00	0.00	96GT074	96Indian	0.004	0.003	0.99	0.00
98JY001	98_01SFTie	0.036	0.940	0.02	0.00	96GT075	96Indian	0.005	0.005	0.98	0.00
03AAB0	98_01SFTie	0.006	0.987	0.00	0.00	96GT075	96Indian	0.004	0.006	0.98	0.00
03AAB0	98_01SFTie	0.072	0.874	0.05	0.00	96GT075	96Indian	0.003	0.042	0.95	0.00
03AAA0	98_01SFTie	0.003	0.986	0.00	0.00	96GT075	96Indian	0.009	0.045	0.94	0.00
03AAA0	98_01SFTie	0.045	0.853	0.09	0.00	96GT075	96Indian	0.018	0.003	0.97	0.00
03AAA0	98_01SFTie	0.016	0.964	0.01	0.00	96GT076	96Indian	0.002	0.003	0.99	0.00
03AAA0	98_01SFTie	0.086	0.895	0.01	0.00	96GT076	96Indian	0.008	0.013	0.97	0.00
03AAA0	98_01SFTie	0.003	0.992	0.00	0.00	96GT077	96Indian	0.012	0.009	0.97	0.00
03AAA0	98_01SFTie	0.002	0.992	0.00	0.00	96GT077	96Indian	0.012	0.005	0.97	0.00
03AAA0	98_01SFTie	0.004	0.984	0.00	0.00	96GT077	96Indian	0.003	0.003	0.99	0.00
03GG011	98_01SFTie	0.014	0.971	0.00	0.00	96GT077	96Indian	0.007	0.014	0.97	0.00
02NQ000	98_01SFTie	0.127	0.854	0.01	0.00	96GT077	96Indian	0.003	0.011	0.97	0.00
02NQ000	98_01SFTie	0.004	0.990	0.00	0.00	96GT078	96Indian	0.011	0.014	0.97	0.00
02NQ000	98_01SFTie	0.003	0.986	0.00	0.00	96GT078	96Indian	0.066	0.041	0.87	0.00
02NQ000	98_01SFTie	0.007	0.980	0.00	0.00	96GT079	96Indian	0.003	0.010	0.98	0.00
02NQ000	98_01SFTie	0.006	0.988	0.00	0.00	96GT079	96Indian	0.003	0.014	0.97	0.00
02NQ000	98_01SFTie	0.069	0.905	0.02	0.00	96GT079	96Indian	0.009	0.004	0.98	0.00
02NQ000	98_01SFTie	0.003	0.982	0.00	0.00	96GT079	96Indian	0.006	0.002	0.98	0.00
02NQ000	98_01SFTie	0.010	0.981	0.00	0.00	96GT080	96Indian	0.004	0.005	0.98	0.00
96GS021	96SFTieton	0.003	0.992	0.00	0.00	96GT080	96Indian	0.634	0.035	0.32	0.00
96GS021	96SFTieton	0.002	0.993	0.00	0.00	96GT080	96Indian	0.005	0.004	0.98	0.00
96GS022	96SFTieton	0.006	0.981	0.00	0.00	96GT080	96Indian	0.006	0.003	0.98	0.00
96GS022	96SFTieton	0.008	0.984	0.00	0.00	96GT080	96Indian	0.006	0.005	0.98	0.00
96GS022	96SFTieton	0.006	0.985	0.00	0.00	96GT080	96Indian	0.005	0.003	0.98	0.00
96GS022	96SFTieton	0.054	0.899	0.03	0.00	96GT080	96Indian	0.008	0.006	0.98	0.00
96GS023	96SFTieton	0.008	0.982	0.00	0.00	96GT080	96Indian	0.007	0.004	0.98	0.00
96GS023	96SFTieton	0.005	0.988	0.00	0.00	96GT081	96Indian	0.007	0.005	0.98	0.00
96GS023	96SFTieton	0.003	0.986	0.00	0.00	96GT085	96Indian	0.002	0.008	0.98	0.00
96GS023	96SFTieton	0.004	0.989	0.00	0.00	11LJ0001	11Indian	0.004	0.003	0.98	0.00
96GS024	96SFTieton	0.005	0.988	0.00	0.00	11LJ0002	11Indian	0.003	0.003	0.99	0.00
96GS024	96SFTieton	0.039	0.800	0.15	0.00	11LJ0003	11Indian	0.008	0.006	0.98	0.00
96GS024	96SFTieton	0.005	0.986	0.00	0.00	11LJ0004	11Indian	0.049	0.055	0.89	0.00
96GS024	96SFTieton	0.003	0.991	0.00	0.00	11LJ0005	11Indian	0.004	0.011	0.98	0.00

Fish ID	Collection	NFTiet	SFTiet	Indi	Bro	Fish ID	Collection	NFTiet	SFTiet	Indi	Bro
96GS025	96SFTieton	0.003	0.991	0.00	0.00	11LJ0006	11Indian	0.003	0.003	0.99	0.00
96GS025	96SFTieton	0.007	0.982	0.00	0.00	11LJ0007	11Indian	0.003	0.003	0.99	0.00
96GS025	96SFTieton	0.012	0.820	0.16	0.00	11LJ0008	11Indian	0.006	0.005	0.98	0.00
96GS026	96SFTieton	0.003	0.991	0.00	0.00	11LJ0009	11Indian	0.004	0.004	0.98	0.00
96GS026	96SFTieton	0.004	0.991	0.00	0.00	11LJ0010	11Indian	0.007	0.004	0.98	0.00
96GS026	96SFTieton	0.004	0.989	0.00	0.00	11LJ0011	11Indian	0.059	0.018	0.92	0.00
96GS026	96SFTieton	0.003	0.989	0.00	0.00	11LJ0012	11Indian	0.006	0.002	0.98	0.00
96GS027	96SFTieton	0.007	0.934	0.05	0.00	11LJ0013	11Indian	0.037	0.009	0.95	0.00
96GS028	96SFTieton	0.002	0.993	0.00	0.00	11LJ0014	11Indian	0.017	0.004	0.97	0.00
96GS028	96SFTieton	0.016	0.961	0.02	0.00	11LJ0015	11Indian	0.006	0.007	0.98	0.00
96GS028	96SFTieton	0.003	0.990	0.00	0.00	11LJ0017	11Indian	0.006	0.004	0.98	0.00
96GS028	96SFTieton	0.003	0.990	0.00	0.00	11LJ0018	11Indian	0.012	0.009	0.96	0.00
96GS028	96SFTieton	0.003	0.992	0.00	0.00	11LJ0019	11Indian	0.018	0.003	0.97	0.00
96GS029	96SFTieton	0.003	0.988	0.00	0.00	11LJ0020	11Indian	0.003	0.003	0.99	0.00
96GS029	96SFTieton	0.003	0.987	0.00	0.00	11LJ0021	11Indian	0.654	0.002	0.34	0.00
96GS029	96SFTieton	0.007	0.982	0.00	0.00	11LJ0022	11Indian	0.003	0.958	0.00	0.00
96GS029	96SFTieton	0.002	0.993	0.00	0.00	11LJ0023	11Indian	0.030	0.003	0.96	0.00
96GS029	96SFTieton	0.003	0.991	0.00	0.00	11LJ0024	11Indian	0.007	0.005	0.97	0.00
96GS029	96SFTieton	0.023	0.952	0.01	0.00	11LJ0025	11Indian	0.005	0.005	0.98	0.00
96GS029	96SFTieton	0.003	0.990	0.00	0.00	11LJ0026	11Indian	0.005	0.979	0.01	0.00
96GS029	96SFTieton	0.020	0.968	0.00	0.00	11LJ0027	11Indian	0.212	0.004	0.78	0.00
96GS030	96SFTieton	0.011	0.922	0.05	0.00	11LJ0028	11Indian	0.006	0.004	0.98	0.00
96GS030	96SFTieton	0.004	0.986	0.00	0.00	11LJ0029	11Indian	0.002	0.005	0.98	0.00
96GS030	96SFTieton	0.011	0.956	0.02	0.00	11LJ0030	11Indian	0.046	0.821	0.12	0.00
96GS030	96SFTieton	0.007	0.987	0.00	0.00	11LJ0031	11Indian	0.280	0.003	0.71	0.00
96GS030	96SFTieton	0.003	0.991	0.00	0.00	11LJ0032	11Indian	0.004	0.004	0.98	0.00
96GS031	96SFTieton	0.005	0.987	0.00	0.00	11LJ0033	11Indian	0.015	0.091	0.88	0.00
96GS031	96SFTieton	0.013	0.464	0.51	0.00	11LJ0034	11Indian	0.005	0.016	0.97	0.00
96GS031	96SFTieton	0.016	0.831	0.02	0.00	11LJ0035	11Indian	0.006	0.007	0.98	0.00
96GS031	96SFTieton	0.002	0.991	0.00	0.00	11LJ0036	11Indian	0.010	0.021	0.96	0.00
96GS031	96SFTieton	0.003	0.991	0.00	0.00	11LJ0037	11Indian	0.002	0.003	0.99	0.00
96GS031	96SFTieton	0.002	0.994	0.00	0.00	11LJ0038	11Indian	0.009	0.002	0.98	0.00
96GS032	96SFTieton	0.002	0.992	0.00	0.00						
96GS032	96SFTieton	0.020	0.483	0.49	0.00						
96GS032	96SFTieton	0.006	0.987	0.00	0.00						