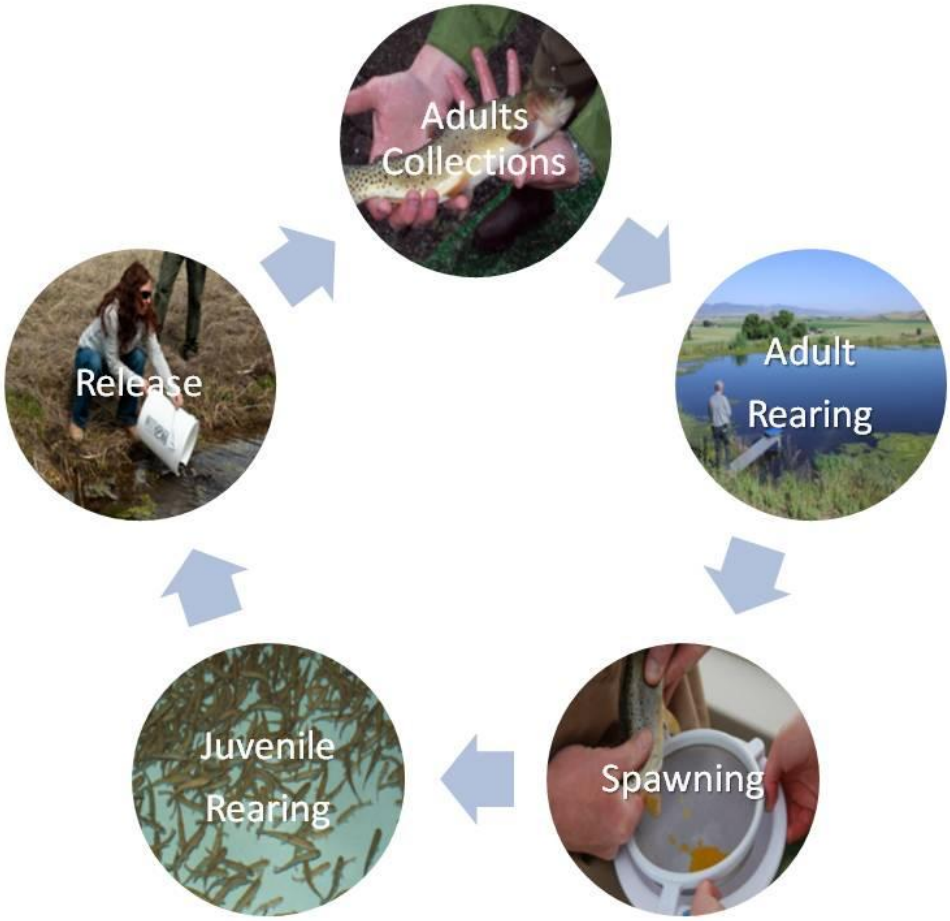
Development of a Bonneville Cutthroat Trout Broodstock Program in the Bear River, Idaho



Prepared by

Idaho Department of Fish and Game

To

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Executive Summary

As part of the mitigation for operating hydroelectric facilities on the Bear River in Idaho, PacifiCorp is funding the development and maintenance of a Bonneville cutthroat trout stocking program. The first three years of funding were defined as the development phase. Future funding will maintain broodstock and pay for rearing and stocking. The Idaho Department of Fish and Game is implementing the program and began collecting potential broodstock in 2007. Because of unique genetic characteristics documented in the population, there are several clades of fish that will be managed separately. Therefore, one stock of cutthroat trout will not be used to supplement all areas of the Bear River in Idaho. Because of past habitat improvements and the number of unoccupied tributaries, the Thatcher Management Area was chosen as the first reach to implement the hatchery program.

To date, 686 sub-adult Bonneville cutthroat trout have been collected from Cottonwood Creek and its tributaries. A small percentage of those fish were genetically classified as hybrids with rainbow trout and were culled from the population. Relatedness analysis was also completed on all of the potential donor stock. Those findings indicated that the donor population contained sufficient genetic diversity to implement a random one-on-one mating strategy. Fish that passed the genetic tests were released into a broodstock rearing pond. To minimize the potential negative impacts of domesticating the stock, only first generation fish will be released. The broodstock population will be maintained by repopulating the brood pond with wild trout from Cottonwood Creek.

The first ripe fish were collected from the broodstock pond in 2010 and 2011. In total, the program has produced over 50,000 eyed eggs. Fish produced in 2010 were reared at the Grace State Fish Hatchery and released in the spring of 2011. About 17,000 cutthroat trout at a mean size of eight inches were stocked. Stocking locations included six tributaries and several sites along the Bear River. In 2011, the program experienced mortalities as fry began feeding. The cause of the mortality was not determined, but a problem with the feed was suspected. As a result of the higher mortality experienced in 2011, despite collecting more eggs, we anticipate about 10,000 fish will be stocked in 2012.

A goal of this program is to release fish in streams that have sufficient habitat to reproduce naturally and eventually hatchery supplementation will no longer be necessary. Once we document success in the Thatcher Management Area, it is anticipated that the program will be expanded to other areas of the Bear River. Based on need and many ongoing successful habitat projects, we anticipate moving the hatchery program to the Nounan Reach of the Bear River above Alexander Reservoir.

Introduction

This document describes the development of conservation hatchery technologies for re-building and re-establishing Bonneville cutthroat trout *Oncorhynchus clarkii utah* (BCT) populations in Idaho. The conservation hatchery program is funded by PacifiCorp's hydropower mitigation program described in the Bear River Settlement Agreement and PacifiCorp's FERC operating license. The program established a three-year development phase followed by maintenance and hatchery supplementation. This report describes the work completed during the three-year development phase. Completed work includes: field collections of donor broodstock, genetic evaluations, spawning protocols, rearing successes, and initial stocking efforts.

BCT are native to the Bonneville basin and are distributed throughout portions of Idaho, Nevada, Utah, and Wyoming (Teuscher and Capurso 2007). Six management units are currently identified in Southeast Idaho reflecting major drainage divides and further separated in the Bear River system by major Bear River Dams (Teuscher and Capurso 2007).

The primary objectives described in the State's management plan for the conservation of BCT are to preserve the genetic integrity of existing populations, maintain the current distribution of the subspecies in Idaho, and to supplement or re-establish populations in areas with low abundance or vacant habitat (IDFG 2007). The initial focus of this program was to develop methods for conservation hatchery program emphasizing the Thatcher Management Area. Many of the tributaries in the Thatcher Area no longer support native BCT. The broodstock program is complimented by numerous habitat improvement projects completed over the past 7-years; making it a logical starting point for the conservation hatchery program.

The IDFG evaluated two approaches for implementing the BCT hatchery program. The broodstock approaches are generally defined as domesticated vs. captive. The domesticated approach replaces adults using offspring from the original donor stock. The captive rearing approach replaces adult broodstock by collecting wild fish annually from the original donor population. A primary goal of this program is to maintain genetic diversity. The best approach for accomplishing that goal depends on the initial genetic structure of the donor stock. If genetic diversity in the original donor stock is poor, a domestic approach can be used to maximize genetic diversity by selecting one-on-one pairings that avoid mating closely related individuals. If the donor stock's genetic structure is diverse, random spawning of captive reared adults is preferable as it maintains local adaptations without compromising genetic diversity.

During the first three years of this program, IDFG completed extensive genetic diversity and relatedness analysis on the Cottonwood Creek population. The genetic results are included at the end of this report as an addendum. Because the donor stock demonstrated excellent genetic diversity, IDFG implemented the captive rearing approach. Only first generation offspring will be produced for stocking. An adult population for spawning will be maintained by annual collections of wild fish. Those fish will be raised in

a pond until mature, spawned, and either sacrificed to complete IDFG required disease testing or released. A maximum of 60 females will be sacrificed annually for disease evaluations.

The Thatcher model for developing and implementing hatchery supplementation will be expanded to other reaches of the Bear River. How quickly the program moves to other population segments depends on the rate at which habitat work and reintroductions succeed at restoring natural self-sustaining populations. Natural populations will be discovered through an existing BCT monitoring program (Teuscher and Capurso 2007). The monitoring program is funded by IDFG and the US Forest Service. Based on existing population data, ongoing habitat work, and the existence of a sound donor population (Eightmile Creek), the Nounan Management Area will be the next area of focus for the broodstock program.

Collecting Wild Broodstock

We selected Cottonwood Creek as the donor tributary for developing the Thatcher Management Area broodstock. Cottonwood Creek has the strongest population of BCT in the Thatcher Management Area (Campbell et al. 2007). To minimize full-sib sampling, numerous sites along Cottonwood Creek were sampled. No more than 30 fish were taken from any given site. Because of low levels of rainbow trout introgression, a visual inspection of each fish was made to remove obvious hybrids. Further culling was completed using genetic analysis.

After collection, potential broodstock were transported to temporary holding containers and individually tagged using Passive Integrated Transponder tags (PIT). A fin clip was taken from each fish and individually labeled using the PIT codes. The fin clips were shipped to IDFG's genetics lab for hybrid and relatedness testing. Once the genetics results were available, hybrid trout were culled from the temporary holding containers. Fish that past the genetic screens, were released in an adult rearing pond located on private land near Grace, Idaho (Figure 1). The same process was replicated in 2008, 2009, and 2011. In total, IDFG collected 686 sub-adult BCT. Table 1 shows total numbers of fish collected by year and their genetic disposition. Allele markers indicated that most of the fish we collected for the broodstock program were characteristic of native BCT (Table 1). Smaller numbers of first generation hybrids (F1) and backcrossed hybrids (>F1 hybrids) were also detected.

For detailed genetic results, see the addendum to this report that describes microsatellite PCR optimization and relatedness estimation for the Cottonwood Creek population. In general, the existing population structure and our collection procedures describe a genetically diverse population. The genetic results support proceeding with a captive rearing program using a one-on-one random mating strategy. That protocol was used in 2011. However, the complete genetic results were not available prior to spawning fish in 2010 and a more rigorous method was used. Those methods are described below.

Table 1. Summary of broodstock collections and their genetic characteristics from Cottonwood Creek. Genetic assignments are shown by number and percent. The varying levels of hybridization are likely a result of location where fish were collected. For example, in 2011, all of the hybrid trout came from the lowest sample location on the creek. In the future, the lower reach will no longer be used as a collection site. The genetic results also provide good rationale for completing a possible stream renovation project in the lowest section of Cottonwood Creek. All of the F1 and >F1 hybrids were culled from the broodstock population.

		Genetic Assignment			% by Assignment		
year	number	BCT	F1	>F1	BCT	F1	>F1
2007	90	87	0	3	96.7	0	3.3
2008	210	173	8	29	82.4	3.8	13.8
2009	193	192	0	1	99.5	0	0.5
2011	193	174	3	16	90.2	1.6	8.3

Figure 1. Adult broodstock pond used for rearing Cottonwood Creek Bonneville cutthroat trout.

2010 Spawning, Incubation, and Rearing

Initial maturation for the first group of broodstock was anticipated spring of 2010. To facilitate collection of the mature BCT, the pond inlet was altered to mimic a natural stream channel. The objective was to create a volitional migration by mature BCT out of the pond. That method worked and eliminated active sampling within the pond that could result in significant stress and mortality. A collection trap was installed in the artificial stream channel (Figure 2). The trap was checked every few days in April and May. Once BCT were observed in the trap, it was checked more frequently. Fish were taken from the trap and transported to a rectangular holding container. The holding container was located at the head of the spring that feeds the pond. The container was partitioned to hold males and females separately.

Figure 2. Attraction channel and trap box used to collect mature BCT that swim out of the pond at maturation.

In 2010, IDFG removed 118 PIT-tagged BCT from the trap box. Of the 118 individuals, 37 were females and 81 were males. Individual fish in the holding tanks were regularly checked for maturation and spawned when ripe. A single female mortality occurred while in holding. The IDFG spawned 36 females and 72 males during seven separate spawning events. Spawning protocols followed the general guidelines currently used by the IDFG Snake River Sockeye Salmon Broodstock Program (Baker et al.

2008). All females were euthanized post spawn for disease testing purposes. Fertilized eggs from each female were held separately until disease results were completed. IDFG culled eggs from diseased females.

Because the future direction (captive rearing or domesticated broodstock) of the Thatcher Management Area was reliant on genetic diversity analysis not completed prior to fish maturing in the spring of 2010, spawning and isolation programs were implemented to facilitate either the domesticated or captive rearing approach. As females were determined ripe, each was given a number based on the order in which spawned (F1 – F36). Prior to fertilization, the ovarian fluid was strained off the eggs and retained for disease testing. The eggs from each female BCT were then divided in half, based on weight in grams, in order to create two exclusive sub-families per female. After division, each sub-family of eggs, along with a portion of the ovarian fluid, was placed into a unique container for fertilization. Individual sub-families of eggs were fertilized with a unique BCT male in a 1% saline solution used as a sperm activator. This process produced 36 family groups, or 72 distinct sub-families (F1A, F1B through F36A, F36B). Individual female eggs were enumerated using the weight of 30 eggs from each female (30 x (total egg weight/30 egg weight) = total eggs).

Eggs were allowed a 20-second fertilization period. This was followed by a one hour water hardening period in unique containers labeled according to sub-family designation. Prior to transport from the spawn site to the Grace State Fish Hatchery (GFH), eggs were transferred from the water hardening containers to individual mesh egg bags labeled according to sub-family designation. The egg bags were then placed into a water filled cooler and transported to GFH.

Upon arrival at GFH, and prior to entry of the incubation building, eggs and bags were disinfected with a 100 parts per million (ppm) iodophor and water solution for 10 minutes (Piper et al. 1982). After disinfection, eggs were poured from the bags into individual plastic mesh egg boxes, labeled by sub-family, and placed into Heath vertical flow incubation trays. Heath trays were supplied with five gallons per minute of GFH spring water, which is a constant 12.2 C.

After a 24 hour period in Heath trays, the eggs were administered a flow through treatment of Formalin at 1667 ppm. It has been shown that Formalin in high concentrations (1600-2000 ppm for 15 minutes) can be used to control fungal infections on trout eggs (Piper et al. 1982).This treatment continued every other day throughout the tender and eyed egg stages of development. Daily Temperature Units (DTUs) were recorded in order to monitor the various development stages of the eggs. The spring water at GFH provides 22 DTUs for development and growth.

GFH staff removed 50 eyed eggs each from 24 randomly selected sub-family groups. These eyed eggs were placed into unique isolation incubation and rearing pots (Figure 3). The isolation pots were necessary to select future broodstock that maximize genetic diversity. However, the isolation pots would only be used for selecting potential broodstock if the domesticated approach was deemed preferable based on genetic diversity analysis. As mentioned above, if the donor population shows adequate genetic diversity, then random mating of wild fish (captive rearing) would be the broodstock approach in future years.

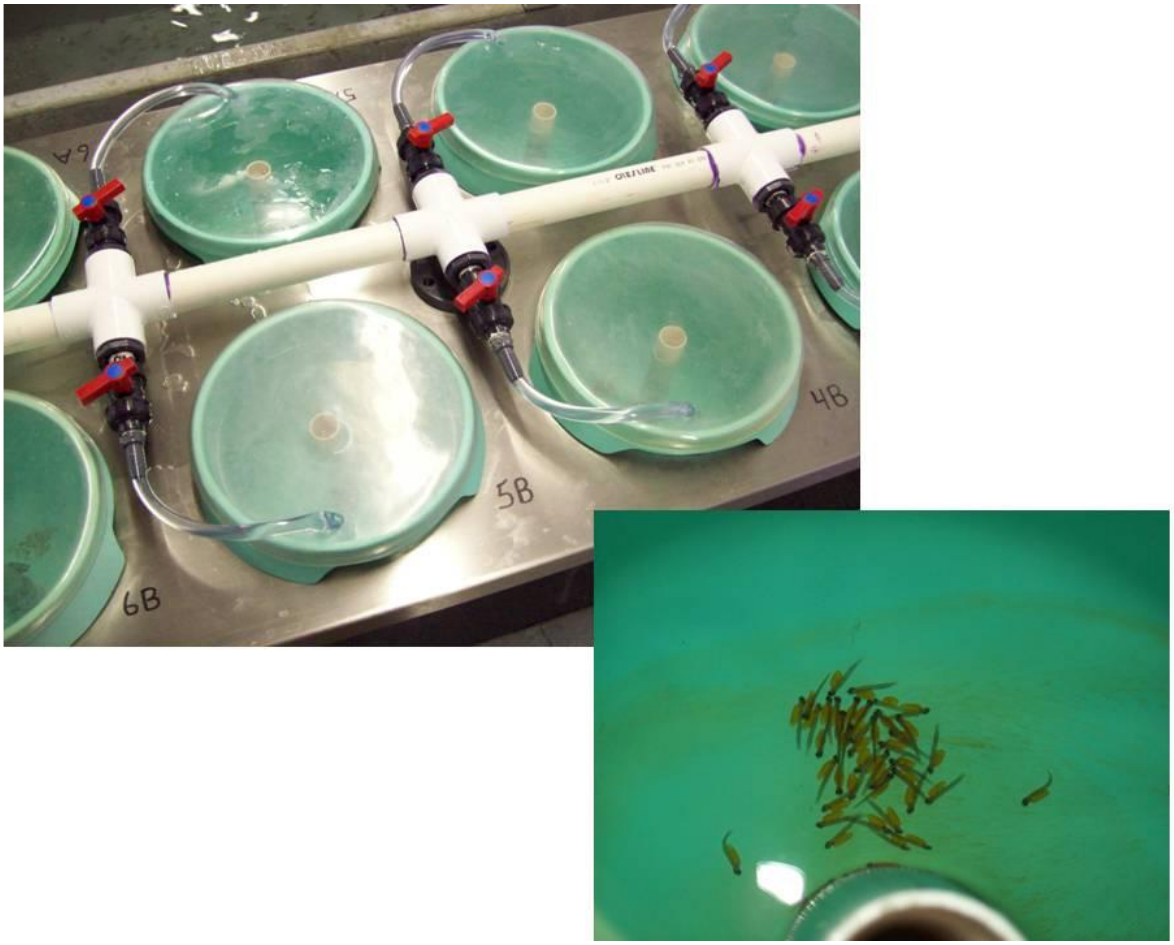

Figure 3. Isolation pots and yoc-sack fry hatching at the Grace State Fish Hatchery. Eyed eggs and fry from each female were held separately in isolation pots until completing disease tests.

Upon hatching, DTUs were recorded. This practice continued until the swim-up fry stage of development. At this stage, the swim-up fry in Heath trays were transferred to indoor concrete vats (3' x 2' x 15') which provide approximately 43 cubic feet of actual rearing space. Fry in the vats and isolation pots were then hand fed, with DTUs recorded for first feeding. As recommended by Kindschi et al. (2009) for cutthroat trout propagation programs, Bio-Oregon Bio-Vita (Longview, Washington) feeds were selected to initiate a feeding regime for Thatcher BCT.

All lots had reached the eyed egg stage of development by 352 DTUs. This parallels the DTU values for the Bear River BCT at Daniels Fish Hatchery in Wyoming. This value of 352 DTUs should be used as the baseline to check for the eyed egg stage in future Bear River BCT culture programs. This will ensure that no unnecessary damage will be done to tender eggs during initial picking, which could yield low eye-up

percentages resulting in decreased overall survival. Eye-up percentage for the 2010 Thatcher MU BCT averaged 88.12% with a range of 13.87% to 100.00% (Table 2).

The first sign of eggs hatching began to occur at 462 DTUs, which was 21 days post fertilization. The hatching stage continued through 528 DTUs, or 24 days post fertilization. Because hatching trout fry should not be exposed to Formalin at high concentrations (Piper et al. 1982), the 1667 ppm flow through treatment ceased at 440 DTUs.

Upon reaching the swim-up fry stage of development, eggs were transferred from incubation trays to concrete vats. The first sign of Thatcher MU BCT reaching this stage occurred at 858 DTUs, or 39 days post fertilization. The swim-up fry stage continued through 924 DTUs, or 42 days post fertilization. Swim-up percentage for the 2010 Thatcher MU BCT was 86%.

When GFH managers determined that approximately 80% of individuals in each lot had reached the swim-up fry stage, hand feeding began. Time of first hand feedings varied between lots, with the first hand feedings occurring from 880 DTUs to 924 DTUs. Fish were started on feed using Bio-Oregon Bio-Vita #0 Mash. Initial feedings were done by sight to ensure minimal waste. Once hand feeding had begun, concrete vats were cleaned daily. During cleaning events, individual mortalities were removed and recorded.

Table 2. Egg production for the 36 females spawned in 2010. Asterisk indicates culled eggs due to disease concerns.

Female	# green eggs	# eyed eggs	% eye-up
1	1,125	1,075	95.56%
2	340	264	77.65%
3	1,100	871	79.18%
4	733	726	99.05%
5	1,625	1,256*	77.29%
6	700	688	98.29%
7	767	689*	89.83%
8	1,006	999*	99.30%
9	1,150	1,143*	99.39%
10	756	720*	95.24%
11	518	497	95.95%
12	1,115	1,036	92.91%
13	990	881	88.99%
14	938	857	91.36%
15	876	324	36.99%
16	483	67	13.87%
17	1,247	1,113	89.25%
18	371	215	57.95%
19	715	680	95.10%
20	716	678	94.69%
21	843	806	95.61%
22	1,142	1,129	98.86%
23	891	876	98.32%
24	436	426	97.71%
25	433	427	98.61%
26	412	404	98.06%
27	638	631	98.90%
28	759	511	67.33%
29	398	350	87.94%
30	377	367	97.35%
31	36	36	100.00%
32	495	424	85.66%
33	427	424	99.30%
34	462	351	75.97%
35	771	755	97.92%
36	677	628	92.76%
Totals	26,468	23,324	88.12%
Total minus culls		18,517	

2011 Hatchery Production and Stocking Summary

In 2011, a total of 81 adult BCT were captured in the trap at the inlet to the broodstock pond. Females made up less than half the run. In total, 31 female BCT were spawned yielding 25,328 fertilized eggs. The fecundity increased from 735 per female in 2010 to 873 per female in 2011. Capture dates ranged from May 4, 2011 through June 15, 2011. Table 3 shows egg survival statistics. At the time this report was prepared, these fry have been ponded in small raceways at the Grace State Fish Hatchery.

Table 3. Egg production for the 36 females spawned in 2011. Asterisk indicates culled eggs due to disease or relatedness concerns.

Female	# green eggs	# eyed eggs	% eye-up	Comment
1	1060	892	84.15%	
2	831	809	97.35%	
3	864	844	97.69%	
4	956	478	50.00%	
5	746	706	94.64%	
6	1208	1194	98.84%	
7	472	372	78.81%	
8	400	368	92.00%	
9	1136	1077	94.81%	
10	900	887	98.56%	
11	424	410	96.70%	
12	630	616	97.78%	
13	1344	1326	98.66%	
14	801	787	98.25%	
15	1131	1100	97.26%	
16	1097	1082	98.63%	
17	713	658	92.29%	
18	842	828	98.34%	
19	840	778	92.62%	
20	1001	975	97.40%	
21	1110	1043*	93.96%	culled ELISA
22	795	82	10.31%	bad male
23	472	356	75.42%	
24	1680	942	56.07%	
25	564	541*	95.92%	culled related
26	1024	677	66.11%	
27	990	778*	78.59%	culled ELISA
28	979	966	98.67%	
29	318	307	96.54%	
30	0	0		no egg development
31	0	0		no egg development
32	0	0		not mature
Totals	25,328	21,879	86.38%	
Total minus culls		19,517		

Fish produced from the spawning effort in 2010 were released as age-1 fish in 2011. The first release was at the Kackley Springs complex (Figure 4). Stocking began in April and continued through the 18th of July. Figure 5 shows the locations where BCT were released. Table 4 shows the dates and number of BCT released at each site.

Broodstock Disease Testing

Female Bonneville cutthroat trout spawners at Grace Hatchery were tested for *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD) in both 2010 and 2011. It is well documented that cutthroat in general are moderately susceptible to clinical BKD, and that viable *R. salmoninarum* organisms can be passed via the egg from one generation to the next. The enzyme-linked immunosorbent assay (ELISA) was used to measure the presence of bacterial antigen in kidney samples collected when each individual was spawned. Advantages of ELISA are that the test is relatively sensitive and it is quantitative (i.e. results are expressed in a numerical value that we believe correlates with the level of infection within an individual fish and with the risk that bacteria may be transmitted with the eggs). The arbitrary designations of "negative" (ELISA value <0.100), "low" (0.100 – 0.249), and "high" ($\geq$0.250) have been developed from Chinook salmon management and may not be directly applicable to cutthroat trout. With that in mind, most of the Bonneville cutthroat results for both years were in the negative to low ranges. Those numbers reflect the normal "background" level of detections expected from any wild/feral cutthroat population, and that risk of transmitting bacteria in the eggs of these fish was acceptably low. A few individuals (5 fish in 2010 and 1 fish in 2011) had high ELISA values, with a maximum value of 0.440. Such values are only marginally high and fall in a very grey area for risk interpretation. Taking a very conservative position in order to protect the Bonneville cutthroat program and other production programs at Grace Hatchery, the IDFG culled the eggs from those individuals (Example shown in Table 3). Full disease reports on female broodstock are found in Appendex A.

Figure 4. Photograph showing the first release of Bonneville cutthroat trout from the broodstock program. The release was on April 20, 2011 in the Kackley Springs Complex.

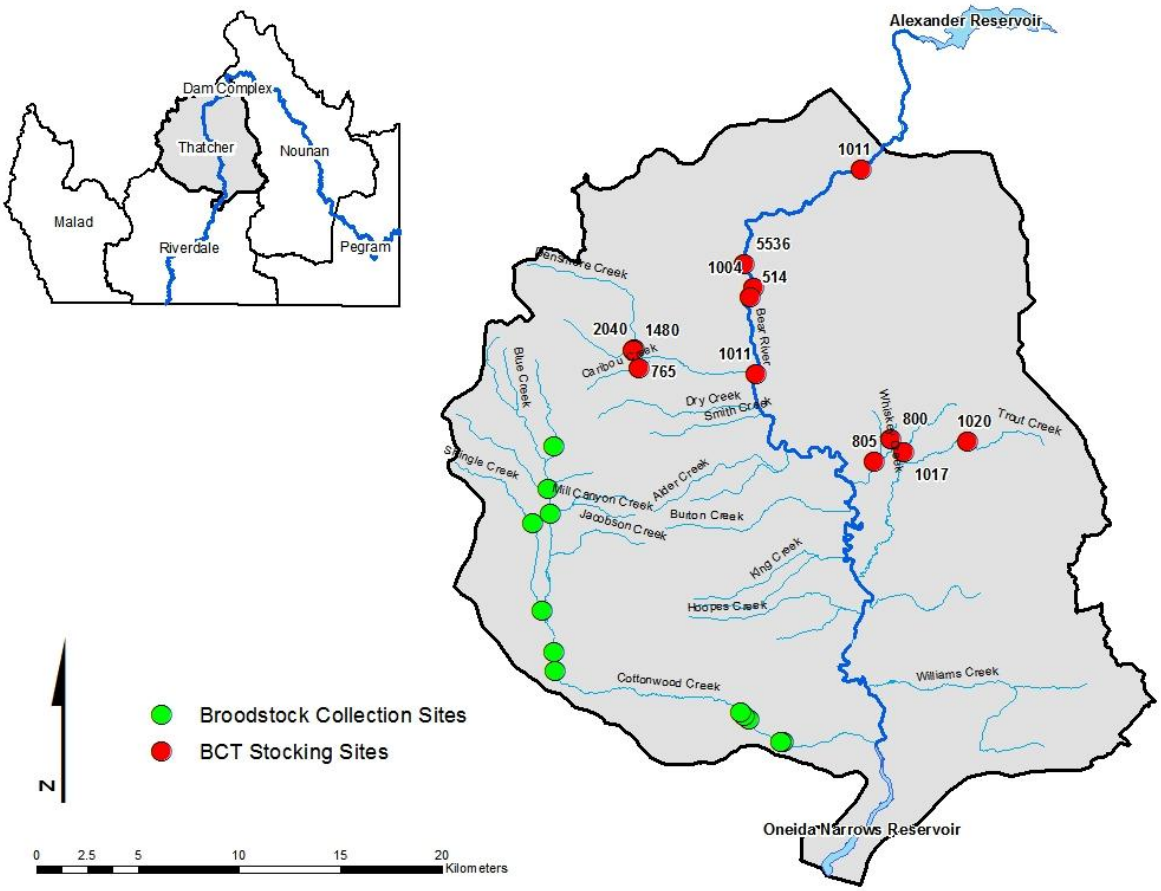

Figure 5. Map showing the 2011 stocking locations in the Thatcher Management Area. The number above each location identifies the number of Bonneville cutthroat trout released.

Table 4. 2011 stocking records for BCT in the Thatcher Management Area.

Location	Date	Number
Kackley Springs	20-Apr-11	1,004
Kackley Springs	2-Jun-11	514
Trout Creek	2-Jun-11	1,017
Trout Creek	14-Jun-11	1,020
Densmore Creek	14-Jun-11	2,040
Cottonwood Creek	14-Jun-11	1,480
Caribou Creek	14-Jun-11	765
Bear River (Cheese Plant)	18-Jul-11	1,011
Bear River (Black Canyon)	18-Jul-11	5,536
Bear River (Grace Dam)	18-Jul-11	1,011
Whiskey Creek	2-Jun-11	1,605
	Totals	17,003

Budget Summary

During the three year broodstock development period, IDFG received a total distribution of $337,256 from PacifiCorp to fund the program. As of January 2012, IDFG expenditures for broodstock development total $322,031. At the time this report was prepared there was an estimated unspent balance of $15,225. Figure 6 shows the major spending categories.

Modifications to the Grace Hatchery were necessary to implement the program. A primary objective at the onset of the program was to isolate water used for raising BCT from all other rearing operations at the hatchery. Introducing wild stock to the Grace Hatchery introduces disease. To minimize the risk, only disinfected eggs are brought on station. Water isolation further reduces risks of spreading or transferring disease. A second objective related to isolating water sources was to bury spring inflows. The middle and west spring sources were buried. About $47,000 was spent on isolating water sources which is reported as capital expenses (Figure 6). Other facility maintenance costs include, lining small vats used to raise BCT, construction of steel isolation incubation tables, replacing some windows and doors, and construction of a shed to store spawning equipment. Construction of the inlet channel at the broodstock pond was also a capital costs.

Operating expenditures make up the largest portion of the budget. Operating costs include, broodstock collections, PIT tags and PIT tag readers, genetic sample collection and laboratory analysis, fish feed, disease testing, vehicle and travel expenses, spawning equipment, and stocking costs.

Personnel expenses account for about 27% of expenditures. Personnel costs include hatcheries, genetics lab, and biologists that collected and tagged broodstock.

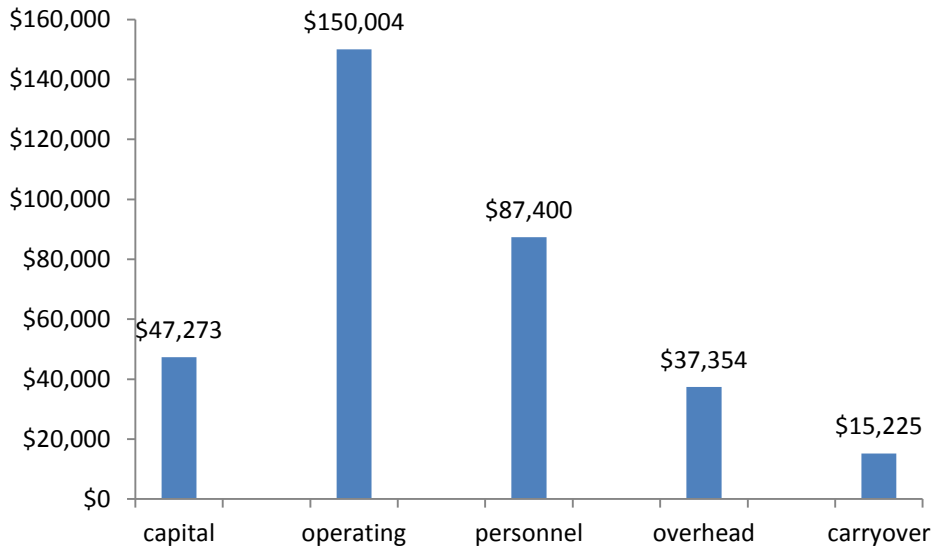

Figure 6. Expenditure breakdown for broodstock development. The carryover category represents the unspent portion of the total distribution of broodstock funds at the time this report was prepared.

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Appendix A

Disease Results from 2010 and 2011 Spawn Years

GRACE HATCHERY
BONNEVILLE CUTTHROAT TROUT

ReportDate: 1/4/2012

Idaho Department of Fish and Game

Eagle Fish Health Laboratory

1/1/10 TO 12/31/10

Accession	Sample Date	BroodYr	Stock	Species	IHN	IPN	WAVHS	BKD	FNR	ERM	CWD	HAS	VHD	CSH	ExamType	Diagnoses
10-157	5/12/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+							INSPECTION	BKD;URO=A,UHSV=A,ELISA=4+(3 LOCUS,1 HIGH)
Comments:																
10-158	5/18/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+					-		INSPECTION	BKD;IHNU=A,IPNU=A,UHSV=A,ELISA=A(3 LOCUS,3 HIGH),PTD-MYXOB=A,PCR-RS=A
Comments:	FEMALES #4 AND NO PITAS TESTED NEGATIVE FOR RENIBACTERIUM DNA BY PCR. IT IS NOT UNUSUAL FOR WILD FISH OTHER THAN CHINOOK OR SOCKEYE SALMON TO HAVE MODERATE TO HIGH ELISA OPTICAL DENSITY VALUES YET TEST NEGATIVE BY PCR. THERE IS STILL NO GOOD EXPLANATION FOR THIS PHENOMENON. PERHAPS THE FISH HAVE BEEN EXPOSED BUT CLEARED THE LIVE BACTERIA FROM THEIR BODIES, LEAVING ONLY THE CELL WALL PROTEINS BUT NO USABLE DNA.															
10-171	5/21/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+							INSPECTION	RS;IHNU=A,IPNU=A,UHSV=1,ELISA=4(3 LOCUS)
Comments:																
10-173	5/24/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+							INSPECTION	RS; IHNU=1, IPNU=A, UHSV=A, ELISA=A (3 LOCUS),
Comments:																
10-178	5/28/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+							INSPECTION	RS;IHNU=A,IPNU=A,UHSV=1,ELISA=A (3 LOCUS)
Comments:																
10-196	6/4/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+							INSPECTION	BKD;IHNU=A,IPNU=A,UHSV=A,ELISA=4+(3 LOCUS,1 HIGH)
Comments:	SAMPLES FROM SPAWNED FEMALES AND 1 TANK MORT (THE ONLY ELISA HIGH).															
10-211	6/10/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-		+							INSPECTION	RS;IHNU=A,IPNU=A,ELISA=A (3 LOCUS)
Comments:																

Accession	Sample Date	Brood Yr	Stock	Species	IHN	IPN	MAVHS	BKD	FUR	BRM	CWD	PAS	VHS	CSH	ExamType	Diagnoses
10-227	6/1/2010	CAPTIVE	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT									-		INSPECTION	NO PATHOGENS DETECTED;PTID=MIX08-83

Comments: HEADS WERE COLLECTED OVER THE COURSE OF THE SPAWNING SEASON FROM BROOD FISH THAT WERE SACRIFICED AND SAMPLED FOR OTHER DISEASE TESTS(SEE ACCESSIONS #-10-1, #-10-11, #-10-13, #-10-15, #-10-4, AND #-2-10).

GRACE HATCHERY
BONNEVILLE CUTTHROAT TROUT

Report Date: 1/3/2012

Idaho Department of Fish and Game

Eagle Fish Health Laboratory

1/1/11 TO 12/31/11

Accession	Sample Date	Brood Yr	Stock	Species	IHN	IPN	WAVHS	BKD	PKR	ERM	CMO	WAS	VHS	CSH	Exam Type	Diagnoses
11-172	5/4/2011	CAPTIVE	B BAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-		+					-		INSPECTION	RS;URO +/5 , ELISA 1/2 (1 LOCI),PTD-MYXOB +/2
Comments:																
11-181	5/12/2011	CAPTIVE	B BAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-		+					-		INSPECTION	RS;URO +/5 ,ELISA 1/5 (1 LOCI),PTD-MYXOB +/5
Comments:																
11-187	5/18/2011	CAPTIVE	B BAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+					-		INSPECTION	RS;URO +/5 ,UHSV +/5 ,ELISA +/5 (4 LOCI),PTD-MYXOB +/5
Comments:																
11-190	5/25/2011	CAPTIVE	B BAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-		+					-		INSPECTION	RS;URO +/5,ELISA 2/5(2 LOCI),PTD-MYXOB +/5
Comments:																
11-208	6/1/2011	CAPTIVE	B BAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-		+					-		INSPECTION	RS;URO +/5 ,ELISA 3/5 (3 LOCI),PTD-MYXOB +/5
Comments:	ERRORING ON THE SIDE OF CAUTION,FEMALE #21 WAS CULLED DUE TO ELISA VALUE = +/2.5.															
11-217	6/8/2011	CAPTIVE	B BAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-	-	+					-		INSPECTION	BKD;URO +/5 ,UHSV +/5 ,ELISA +/5 (5 LOCI, 1 HIGH),PTD-MYXOB +/5
Comments:	RECOMMEND CULLING EGGS FROM FEMALE #21 IF SUFFICIENT EGGS FROM NEGATIVE OR LOW FEMALES REMAIN ON HAND TO MEET PROJECT GOALS.															
11-223	6/15/2011	CAPTIVE	B BAR RIVER	BONNEVILLE CUTTHROAT TROUT	-	-		-					-		INSPECTION	NO PATHOGENS DETECTED;URO +/5 ,ELISA +/5 ,PTD-MYXOB +/5
Comments:																

Accession	Sample Date	BroodYr	Stock	Species	IHN	IPN	WAVNS	BKD	FUR	ERM	CWD	MAS	VHS	CSH	ExamType	Diagnoses
11261	7/19/2011	2011	BEAR RIVER	BONNEVILLE CUTTHROAT TROUT					-	-	-	-			DIAGNOSTIC	NO PATHOGENS DETECTED;BACTERIA

Comments: NEWLY-FEEDING FRY WERE FOUND TO HAVE RUPTURED THE VENTRAL AEROMINAL SUTURE AND THE STOMACHS WERE PROTRUDING THROUGH THE RUPTURES. TO ASCERTAIN THE PROBLEM WAS A FEED/FEEDING DIGESTABILITY ISSUE, BUT TOOK BACTERIOLOGY SAMPLES TO MAKE SURE THERE WAS NOT AN INFECTIOUS AGENT. CHANGES IN FEEDING RATE, FEED SIZE, AND FEED MANUFACTURER HAVE NOT PROVIDED A COMPLETE SOLUTION TO DATE.

Microsatellite PCR Optimization and Relatedness Estimation of SY2010 Hatchery Stock Bonneville Cutthroat Trout in the Bear River Drainage, Thatcher Management Unit, Idaho

Final Report

By

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ABSTRACT

As part of the larger goal to conserve Bonneville cutthroat (*Onchorhynchus clarkii utah*), the Idaho Department of Fish and Game established a population of candidate broodstock donors collected from Cottonwood Creek, within the Thatcher Management Unit (MU) in the Bear River Drainage. The goals of this project were to develop a microsatellite DNA multiplex with enough power to compare genetic diversity among wild and captive Bear River cutthroat trout (BRCT) populations and to determine relatedness among the 2010 spawners. A multiplex of 12 microsatellite loci was developed and used to describe genetic diversity for the captive spawners and wild populations within the Thatcher MU. Two maximum likelihood methods and one relatedness estimator were evaluated using captive broodstock crosses of known relationships to determine the most effective for use with future spawners. The 2010 captive spawners showed genetic diversity similar to that of Cottonwood Creek, the main tributary in the Thatcher MU and all relatedness estimators were successful in identifying related individuals.

INTRODUCTION

All cutthroat trout in the Bonneville basin are taxonomically considered Bonneville cutthroat trout *Onchorhynchus clarkii utah*. For conservation and management purposes the subspecies is grouped into four geographic regions: the Bear River drainage (north slope Uinta Mountains, Smith's Fork, Thomas Fork, Cub, Logan, Little Bear and others), northern Bonneville drainages (Ogden, Weber, Jordan, Provo and Spanish Fork rivers), western Bonneville drainages (Deep Creek mountains, Wheeler Peak, Snake Valley) and southern Bonneville drainages (Sevier, Beaver and Virgin rivers). This report focuses on Bonneville cutthroat trout populations within the Bear River drainage in Idaho and samples are referred to as Bear River cutthroat trout (BRCT) in this report.

Bonneville cutthroat trout are considered a Game fish by the State of Idaho and a Sensitive Species by the USDA Forest Service and U.S. Bureau of Land Management. Several non-governmental organizations as well as other state's wildlife agencies have petitioned for Bonneville cutthroat trout to be designated as Threatened or Endangered. These petitions, summarized in Teuscher and Capurso (2007), were largely prompted by studies indicating that the subspecies only occupies an estimated 63% of its' historically available habitat and fish densities in the remaining available habitat are low. Currently, Idaho Department of Fish and Game (IDFG) objectives for ensuring the long-term viability and persistence of BRCT in Idaho include preserving the genetic integrity and diversity of existing populations and providing opportunities for genetic exchange. One management option for meeting these objectives includes the development of a broodstock for supplementation and reintroduction efforts. This action, in addition to current efforts to improve and re-connect habitat, should lead to increased fish densities in the basin. In 2007, IDFG began capturing wild sub-adult BRCT in the Thatcher MU to initiate broodstock development and some of these fish were spawned in the spring of 2010. This report describes efforts to develop genetic methods to assess the relatedness among fish collected in the wild and guide future broodstock management decisions. There were three major objectives of this work:

1.) Optimize a robust and powerful suite of microsatellite loci for BRCT.
2.) Screen the optimized suite of loci on a subset of samples with known relationships to assess the accuracy of different statistical methods for estimating relatedness.
3.) Assess relatedness among the 2010 spawners.

Results of this work should assist in the development of a conservation broodstock/supplementation program for the Thatcher Unit and provide a framework from which managers could initiate supplementation programs in other MUs in the Bear River drainage in Idaho.

METHODS

Objective 1: Microsatellite Optimization

A total of 19 microsatellite loci were chosen for optimization on the 3730 DNA Analyzer (Applied Biosystems) (Table 1). Six loci (*Fgt3*, *Ocl1*, *Ogo4*, *Omm1036*, *Ots107*, and *Ssa85)* were used in a previous study by IDFG on Yellowstone cutthroat trout (YCT) (Cegelski et al. 2006) and the 13 remaining loci were chosen based on reported allelic diversity, total number of alleles per locus (N_A) or expected heterozygosity (H_e) observed in previous *O. clarkii* studies . From the Cegelski et al. (2006) study, the population with the highest average number of alleles (A=9.83) and highest average expected heterozygosity (H_e =0.77) was Tincup Creek-2003 (N = 24). Due to its variability, this population was chosen to optimize all 19 loci and standardize projects. All DNA used in this study was extracted using a fish tissue protocol for Nextecc extraction kits (Nextecc, Leverkusen, Germany). Samples underwent multiplex PCR in 10 ul reactions using Qiagen® Multiplex Master Mix at 1X concentration and 1 ul of DNA. The thermal profile for all PCR panels started with 95^0C for 2 min, 40 cycles of 95^0C for 30 s, 60^0C for 30 s, 72^0C for 30 s, and a final extension at 72^0C for 30 min The PCR products were run on the 3730 DNA Analyzer using GS500 ROX size standard (Applied Biosystems) with GeneMapper v4.0 software. All 19 loci were assessed for peak height (amplification strength), peak morphology, spikes and blips, allelic variability, and estimated allelic size range using electropherograms produced from the 3730 analyzer. The outcome of these five factors determined whether or not any changes were needed in the PCR master mix or thermal profile for a locus. Conditions were manipulated until a locus exhibited optimal results relative to previous performance within this study or performance based on use in a past study. The final primer concentrations and annealing temperatures varied depending on the primer and panel (Table 2).

Following initial locus optimization and multiplex construction, an additional three sets of samples were screened for further optimization and testing. These included samples from Glenwood Hatchery (N=22, “southern” Bonneville cutthroat), Bear Lake (N=27, BRCT), and rainbow trout samples from several resident hatchery stocks (N=93, RBT). These sample sets were evaluated for allelic size range and diversity, and conformation to Hardy-Weinberg Equilibrium and Linkage Disequilibrium expectations using GENEPOP on the Web (Raymond and Rousset 1995). Rainbow trout samples were screened to determine whether any loci might be useful in testing for hybridization. Finally, we screened BRCT samples collected from tributaries in the Bear River Drainage (N = 152; 2003, 2005, and 2009) to provide a baseline of the genetic diversity within the drainage. These baseline samples came

from some of the same streams in which the broodstock was collected, allowing for comparison of genetic diversity and representativeness of the wild collection to the brood collection.

Objective 2: Parentage and Relatedness Estimation

Samples:

In the spring of 2010, IDFG managers at Grace Fish Hatchery spawned 36 females with two males each, for a total of 72 sub-families. All of these adults had been previously sampled for genetics and PIT tagged. However, discrepancies identified during sample inventory indicated that some PIT tags may have been recorded inaccurately or not linked to the correct sample. For quality control, re-clips from culled spawned females were genotyped. It was the intention to resample males as well, but they were inadvertently returned to the holding pond after spawning, prior to sampling. In August of 2010, 93 offspring from 24 crosses were genetically sampled, representing 49 pairs of full-siblings and 88 pairs of half-siblings.

Parentage:

Adult and juvenile samples were genotyped with the 13 optimized loci. Parentage was first evaluated using conditional formatting in Microsoft Excel as a quick way to identify any genotyping or inheritance problems. CERVUS 3.0 (Kalinowkski et al 2007) was then used to help assign parentage to see if we could resolve some of the pit tag discrepancies. The simulation in CERVUS was run in using a 95% confidence level and a 0.01 locus genotyping error rate. To account for any additional errors in adult sampling and missing genotypes we selected 0.90 and 0.80 as the proportions of mothers and fathers sampled, respectively.

Relatedness Estimators:

There are two main types of relatedness estimators: pairwise methods that provide a relatedness coefficient for every pair of samples within the dataset (i.e. fraction of alleles identical by descent), and likelihood methods that classify pairs into a specific relatedness category (full-sib, half-sub, or unrelated). In this study, we chose to test two maximum likelihood methods that categorize pairs of fish into full-sib, half-sib or un-related pairs and one pairwise method using the Queller and Goodnight

relatedness coefficient. We tested how well these estimators could detect the 49 known full-sib pairs and 88 known half-sib pairs using two different software programs (COLONY and KINGROUP).

COLONY is user-friendly and has already proven to be valuable in other population relatedness projects involving salmonids (Palm et al. 2008). COLONY utilizes a maximum likelihood method which places crosses into two possible dyads, Full-sib or Half-sib (Wang 2004, Wang and Santure 2009). Parameters used in COLONY analysis included: male and female polygamy, no inbreeding, dioecious population, diploid population, medium length of analysis run, use of Full-likelihood analysis method, no updates to allele frequencies, and no prior information on sibship size. Results were given as a probability value for each pair, and the two output files were the Full-Sib Dyad and Half-Sib Dyad. These result files list the pairs of individuals that had the maximum likelihood ($M\text{-}L_W$) of being either full- or half-sibs according to Wang (2004). Any crosses not found in either dyad were considered to be unrelated. If crosses showed up in more than one dyad the one having the probability ≥ 0.5 was used as the relationship designation. The full-sib family group result file in COLONY was also used to easily identify related groups of fish, which allows one to evaluate how many of collected fish are related.

The program KINGROUP (Konovalov et al. 2004) performs pedigree relationship reconstruction and kin group assignments also using a maximum likelihood method described by Goodnight and Queller, 1999. Two null hypotheses, Half-sibs and un-related, were tested separately against the primary hypothesis of Full-sibship. KINGROUP also produces pairwise relatedness coefficients (r-values). Distribution of r-values for full-sibs, half-sibs and un-related pairs can be graphed and the level of overlap between groups can be used to determine miss-classification rates. This is done by taking the mean r-value of each group (full vs. half vs. un-related) and then using the mid-point between the two groups as the cut-offs.

For each of the estimates, Type I and II errors rates were calculated by the using the number of known crosses, both full- and half-sib, and un-related fish incorrectly assigned to another group. Type I errors are crosses that are identified as being more related than they actually are (example: an un-related pair is mistaken to be a full-sib pair). Type II errors are crosses that are identified as being less related than they actually are (example: a full-sib pair is mistaken to be un-related).

Objective 3: Genetic Diversity and Relatedness of broodstock spawned in 2010

Allelic diversity of 2010 spawners was calculated using Microsatellite Toolkit in Microsoft Excel and compared to BCT samples from Glenwood Hatchery (N=22), and BRCT samples from Bear Lake

(N=27), Cottonwood Cr (N=74), Hoopes Cr (N=25), First Cr (N=11), Maples Cr (N=11), and Mill Cr (N=20), along with one population of YCT (Tincup Cr, N=24). The expected heterozygosity, observed heterozygosity, and allelic richness for the wild populations were calculated and compared to that of the 2010 spawners. The inbreeding coefficients (F_{IS}) were calculated in FSTAT (Goudet 1995) and compared to the same populations. The 2010 spawners, Cottonwood Cr, and Hoopes Cr were evaluated for the presence or absence of any rare (less than 5% in a population) or private alleles (unique to a population).

There were 105 genotypes from the 2010 spawners run in COLONY and KINGROUP. The parameters used in COLONY were the same as those listed in Objective 2, with the same results used to evaluate estimated relatedness (full- and half-sib dyads). The same hypotheses were tested in KINGROUP as those listed in Objective 2 for the 2010 spawners as well. Related crosses identified in each analysis were recorded. An estimate of effective population size (N_e) was calculated for the 2010 spawners using LDNE (Waples and Do 2008). Effective population size is an estimate of the number of parents contributing genetically to a population.

RESULTS

Objective 1: Microsatellite Optimization

Of the 19 loci initially tested, 6 were dropped from multiplex optimization. Four were dropped due to exhibiting large amounts of stutter (*Ocl4, Ocl8pig, Fgt3, and Ogo1c*), one was dropped because it failed to amplify (*Ogo3*), and one was dropped because it was monomorphic in BRCT (*Och35*). It was however, diagnostic between rainbow trout and BRCT and could be incorporated into future multiplex panels if needed. No other loci exhibited diagnostic alleles, however allele frequencies did allow for accurate population assignment (unpublished data, S. Dauwalter). Tests of Hardy-Weinberg equilibrium and linkage disequilibrium revealed no patterns among the remaining 13 loci that suggested they should be removed prior to further analyses (Table 3).

Objective 2: Parentage and Relatedness Estimation

Parentage:

Parentage confirmation using Excel supported all known assignments in 81 of the 93 offspring samples. In these 12 samples offspring failed to match parental genotypes at more than six of the 13 loci. CERVUS results indicated that of the 12 miss-matches, four were apparently due to male parents that were inadvertently un-sampled, six were due to single parent miss-assignment, and 2 were most likely due to an error in tube labeling at the time of juvenile sampling. There was no indication that assignment failures were due to lack of power in the microsatellite locus set. This was supported by the simulations in CERVUS that indicated either no mother or father could be assigned, or the only other possibilities had greater miss-matched loci (Appendix 1).

While most offspring were successfully assigned parentage in CERVUS, locus miss-matches existed between known adults and juveniles. There were twenty cases of loci not matching at just one locus, and one instance of 2 loci not matching. *H114* and *Och29* made up the majority of the mismatches with 13 and 6, respectively (*Och18* and *Ots107* contributed one mismatch each). Four pairs of full-sibs made up eight of the 13 mismatches at *H114* and two pairs of full-sibs accounted for six of the mismatches at *Och29*. In all instances, parents and offspring were re-genotyped to check for errors, however genotypes matched the originals. Neither one of these loci were found to be out of Hardy-Weinberg equilibrium or in linkage disequilibrium, but there appeared to be some presence of a null allele or some other problem with inheritance. Results for Hardy-Weinberg equilibrium and linkage disequilibrium supported the use of all 13 loci in parentage and relatedness estimates, but the performance of *H11*4 in parentage assignment was questionable. The majority of the single-loci miss-matches were due to *H114* (13 of the 21) and therefore this locus was dropped from the multiplex. In the few cases where offspring genotypes did not match parental genotypes for *Och9, Och18, and Ots107*, offspring were coded as having "failed". This left 12 loci for the next phase of analyses.

Relatedness:

COLONY accounted for 47 of the 49 full-sib crosses in either the Full-sib or Half-sib Dyad results file and 68 of the 88 half-sibs were accounted for in the dyads (Table 4). Using the dyads as cutoffs for calculating error rates resulted in a Type II error rate for full-sib crosses of 4.1% (would be mistaken to be unrelated and would go undetected) and 22.7% for half-sib crosses (would be mistaken to be unrelated and would go undetected). The Type I error rate for unrelated crosses was 2.0% (considered

to be related). COLONY estimated 61 full-sib family groups out the known 49 full-sib pairs entered. The extra families were due to known full-sibs being placed in family groups alone. There was only one case of a miss-assignment where a half-sib was placed with a full-sib family.

The same number of possible full-sib, half-sib, and un-related crosses were entered into KINGROUP for the BY2010 analysis as in the COLONY analysis. The Full vs Half hypothesis resulted in 43 crosses considered full-sibs, 39 of those were known to be full-sib crosses (20.4% Type II error rate), three were known half-sibs crosses, and one was un-related. This hypothesis did a very good job at designating between full- and half-sib crosses, but the high Type II error rate was not ideal. The Type I error rate for un-related crosses was 0.1%. The second hypothesis tested (Full vs Unrelated) resulted in 252 crosses considered significant and therefore full-sibs. Most of the known full-sib crosses were identified (48 of 49), as well as 52 of 88 half-sib crosses, and 152 unrelated crosses. This hypothesis gave a Type II error of 2.0% for full-sib crosses and 40.9% for half-sibs, and under-estimated the number of un-related crosses with a Type I error rate of 3.7% for unrelated crosses (Table 5). Full vs Un-related would answer more relatedness questions than Full vs Half due to the lower Type II error rate for full-sib crosses, and by including more half-sib crosses into the full-sib crosses (Table 5). Therefore, only Full vs Un-related results will be compared to the M-L_W results.

The distribution of R_{QG}-values for BY2010 samples produced by KINGROUP (Figure 1) displayed three separate distributions for the three different relationships defined by the study; however these distributions showed some overlap. This overlap in distributions resulted in Type II errors for full and half crosses of 16.3% and 22.7%, respectively when unadjusted cut-offs were used (Table 6). In an effort to reduce Type II error rate to 2.0% the cutoff value for full-sibship was adjusted to ≥ 0.2, but this increased the Type I error rate of un-related crosses to 11.9%. To achieve a 0.0% Type II error rate the cutoff would need to be reduced to ≥ 0.0, this would increase the Type I error rate to 56.2%.

Objective 3: Relatedness of broodstock spawned in 2010

Expected and observed heterozygosity for the 2010 spawners was compared to nine other sample groups (Figure 3), however the Cottonwood Creek and Hoopes Creek samples were of the most interest because of their location within Thatcher MU. The 2010 spawners exhibited levels of expected and observed heterozygosity similar to those observed in the Cottonwood Creek sample group and higher than levels observed in the Hoopes Creek sample group. The 2010 spawners also exhibited higher gene diversity and allelic richness than both the Cottonwood Creek and Hoopes Creek sample

groups, although 95% confidence bounds overlapped among all three sample groups (Figures 3 and 4). Inbreeding coefficients for 2010 spawners and Cottonwood Creek were close to 0 (F_{IS} = 0.05 and 0.07, respectively). Hoopes Creek had a slightly lower estimate with F_{IS} = -0.08. Possible values for F_{IS} are between -1 and 1, so values for the Thatcher MU populations are in the mid-range and indicate little evidence of inbreeding or admixture. The previous Cottonwood Creek collection contained only one allele at one locus that was not represented in the 2010 spawners at a low frequency (Allele 253, Freq =0.06). Hoopes Creek did not contain any private alleles at a high enough frequency (>5%) that would indicate anything other than an effect of sampling error being responsible for the missing allele in other populations. Our initial results suggest that the fish spawned in 2010 are representative of wild fish found in the Thatcher MU and are suitable for supplementation purposes. However, sample sizes from Cottonwood Creek and Hoopes Creek are pretty low and we recommend that additional sampling occurs within the Thatcher Mu to improve the robustness of these genetic diversity comparisons.

The effective population size (N_e) was calculated in LDNe (Waples and Do 2008), and N_e= 62. Therefore, it is estimated that 62 individuals contributed to the 105 individuals making up the 2010 spawners. Relatedness estimates supported this calculation. Maximum likelihood methods in COLONY estimated that 4.65% of the crosses were related; KINGROUP estimated 6.33% were related (Table 4 and 5). Of these related crosses, COLONY indicated four were crosses spawned in 2010, KINGROUP indicated five (Table 7). Three of the crosses were the same between the two analyses. COLONY reported 87 full-sib family groups with an average of 1.23 individuals per family. Unadjusted pairwise relatedness estimation in KINGROUP (R_{QG}) resulted in only one cross being identified as related; the adjusted cut-off resulted in eight crosses (Table 7). However, the adjusted cut-off is not reliable considering the high Type I error rate reported previously. The one cross identified with the unadjusted cut-off was identified in both M-L methods. The average pairwise relatedness of the 2010 spawners as a whole using R_{QG} was 0.0, which is within the un-related range (Figure 2).

DISCUSSION

This study was able to successfully optimize 12 microsatellite loci which provided enough genetic diversity for parentage assignment and relatedness estimation to occur with a high degree of credibility and reliability. The multiplex proved its efficacy in distinguishing full-sib crosses in both M-L analyses. However, in COLONY both full- and half-dyads had to be used to account for most of the full-

sib crosses and in KINGROUP the hypothesis test Full vs Un-related had to be used. The two M-L methods used in COLONY and KINGROUP had low Type II error rates, with M-L_{QG} having the lowest with 2.0% compared to 4.1% of M-L_W, meaning the method used in COLONY slightly underestimates the full-sib pairs in the population. M-L_{QG} had the higher Type II error rate for half-sibs, but more half-sibs were identified as related in the M-L_W analysis than with COLONY. M-L_{QG} had the higher Type I error rate for unrelated crosses which means it identified more un-related crosses as related than M-L_W. The estimated loss in heterozygosity due to Type II error rates for full- and half-sib crosses is 2.5% for M-L_W and 3.5% M-L_{QG}, despite M-L_{QG} having a lower Type II error rate for full-sibs. The ability of M-L_W to pick out more half-sib crosses ultimately lowers the potential inbreeding.

Pairwise relatedness estimates of R_{QG} had high Type II error rates 16.3% for full-sibs and 22.7% for half-sibs when using the un-adjusted cutoff values for relationship designation. One of the benefits of using the R_{QG} method is that adjustments can be made to the cutoff values depending on the goals of the program. When applying the lowered cutoff value to a data set of unknown individuals more crosses would be identified as being full-sibs. This would ultimately increase the number of fish needed to be incorporated in the broodstock as many fish would be excluded from spawning together, but would ultimately lead to identifying more full- and half-sib crosses. The benefit of lowering the cutoff is obviously increasing the chance of identifying all full-sib crosses, as well as an increased chance of identifying half-sib crosses.

Overall, the use M-L procedures in either COLONY or KINGROUP were reliable in accounting for full-sib crosses, while minimizing Type I and Type II errors. Both COLONY and KINGROUP are user-friendly and input files are easily built. COLONY provides family group assignment, and more half-sib cross identification resulting in a lower inbreeding coefficient. KINGROUP runs faster and identifies more full-sib crosses. It is recommended that both programs be used to compile a consensus list of related fish representing both full- and half-sib crosses.

Based on preliminary test of genetic diversity using SY2010 and Cottonwood Cr, the BCT broodstock appears to be representative of the wild populations in Thatcher MU. Only one rare allele was found in Cottonwood Cr that was not accounted for in the 2010 spawn group. This allele was found at a low frequency, so it is possible it may be found in fish already held at Grace, or could be incorporated from fish collected from the wild in the future. As this study progresses it will be important to continue to monitor the genetic diversity within Thatcher MU.

The future direction of the BRCT broodstock at Grace Hatchery could progress in several different trajectories and could create a conservation broodstock program or supplementation program.

A broodstock program would require fish to be maintained for subsequent generations on an IDFG hatchery. Supplementation would require the spawning of adults, with the rearing and release of offspring, without maintaining a resident population at a broodstock. While there is a difference in fish management for these two programs they both require relatedness estimation of spawning candidates. Regardless of the direction of the program, the analyses evaluated in this report can be used separately or in combination for either task. Maximum likelihood estimates in COLONY and KINGROUP can be used separately, or in conjunction, to limit the number of individuals taken from the same family group before being incorporated into a broodstock or supplementation program, and random mating can occur. If a population requires more a larger more in depth broodstock matrix, R_{QG}-values produced KINGROUP can be used. This would be of interest if managers wanted to use a spawning matrix to reduce inbreeding among pairs. This could be also be used in the supplementation program after maximum likelihood tests, if managers were concerned every related fish wasn't being identified.

RECOMMENDATIONS

Overall, the sampling and collecting of candidate fish appears to have been successful in providing a diverse broodstock. However it is encouraged that future candidates for incorporation into the broodstock undergo relatedness testing and family size is equalized before spawning events occur. Limiting the number of individuals with co-ancestry may be the best way to maintain genetic diversity (Cabellero and Toro 2000, Lacy 1995). The results concerning relatedness among the spawned crosses was relatively low considering both M-L analyses identified many related pairs within the spawned individuals. When COLONY and KINGROUP results are combined, six spawned crosses were identified and the offspring for two of these crosses had already been placed in production. So while the random spawning method worked with relatively little negative impacts this year, the same method may not prove to be as effective in future spawn years. These fish may have come from the same location in the same year, increasing the likelihood they were related and then spawned. Implementing relatedness estimators could identify related individuals prior to spawning. A meeting in January with hatchery managers and genetic lab personnel outlined a potential plan for the future of the BRCT hatchery program. It was decided a supplementation program would work best at this point in time, using the following guidelines.

There are five main genetic guidelines under this management plan:

1. **Genotyping wild fish pre-spawn**: Genotyping wild fish while they are being held at Kent Clegg's pond

2. **Analyze spawners for relatedness**: Relatedness analyses will determine if any fish in the wild collections are related (full- or half-siblings).

3. **Equalize family size pre-spawn**: Family size will be equalized to increase N_e. One fish from each family group would be kept in the spawning group; all others would be released back into the wild population. A minimum number of females and males will be kept according to managers requirements for egg production. Spawning will be kept at 1:1 between females and males.

4. **Culling spawners**: All spawners would be culled to prevent being used again in future spawning events. Spawners used in 2010 should also be culled, without being used in future spawning events.

5. **Track Thatcher MU Genetic Diversity:** Compare wild fish sampled in step 1 to other sampling events within Thatcher MU. Since offspring will be used to stock various reaches of Cottonwood Cr and other tributaries in Thatcher MU, family size will not be equalized within the offspring. It will be important to track the genetic diversity in locations where fish are stocked and in places where small populations currently exist.

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Table 1. Description of the 19 loci used in optimization on the 3730 DNA Analyzer. Annealing temperatures (^{o}C), average expected heterozygosity (H_e), and the number of alleles (N_A) are values reported in literature for *O. clarkii.* NR denotes values not reported in the literature.

ocus	Repeat Motif	Fluorescent Label	Anneal ^{o}C	H_e	N_A	Citation
Fgt3	di	6-Fam	50	NR	NR	Sakamoto et al. 1994, Cegelski and Campbell 2006
Ocl1	di	6-Fam	60	0.84	9	Condrey and Bentzen 1998, Cegelski and Campbell 2006
Ogo4	di	NED	59	0.89	16	Olsen et al. 1998, Cegelski and Campbell 2006
Omm1036	tetra	VIC	59	NR	NR	Rexroad et al. 2002, Cegelski and Campbell 2006
Ots107	tetra	PET	50	NR	NR	Nelson and Beacham 1999, Cegelski and Campbell 2006
Ssa85	di	VIC	56	0.81	12	Wenburg and Bentzen 2001, Cegelski and Campbell 2006
H114	tetra	6-FAM	55	0.57	4	Pritchard et al. 2007
J103	tetra	VIC	55	0.86	13	Pritchard et al. 2007
Ocl8	di	NED	60	0.89	13	Condrey and Bentzen 1998
Ocl4	di	6-FAM	55	0.85	9	Condrey and Bentzen 1998
Ogo3	di	6-FAM	59	0.90	16	Olsen et al. 1998
Ogo1c	tetra-di	6-FAM	60	0.95	24	Olsen et al. 1998
Och18	tetra	PET	60	NR	40	Robinson et al. 2009
Och20	tetra	6-FAM	58	NR	24	Robinson et al. 2009
Och24	tetra	6-FAM	64	NR	18	Robinson et al. 2009
Och27	tetra	NED	58	NR	26	Robinson et al. 2009
Och29	tetra	VIC	58	NR	24	Robinson et al. 2009
Och30	tetra	NED	64	NR	13	Robinson et al. 2009
Och35	tetra	VIC	64	NR	19	Robinson et al. 2009

Table 2. The 13 microsatellite loci used in Bear River Relatedness Multiplex PCR. The PCR is split into 5 PCR reactions and then combined into three (A, B, or C) 3730 panels. The concentration, [p], is for both forward and reverse primers for each locus, the annealing temperature (^{0}C), number of cycles in the PCR reaction, allelic size range according to species, and the number of observed alleles (A^0) according to species is given.

Locus	[p] (uM)	Annealing ^{0}C	# of Cycles	PCR panel	3730 panel	BCT Range (b.p.)	BCT A^0	RBT Range (b.p.)	RBT A^0
Ogo4	0.30	60	35	1	A	117-125	5	115-133	8
Omm1036	0.40	60	35	1	A	180-260	20	204-280	13
Ocl1	0.30	60	35	1	A	142-196	10	188-220	10
Ssa85	0.30	60	35	1	A	95-153	10	95-177	17
Ots107	0.60	52	35	5	A	154-234	24	170-222	11
J103	0.50	56	40	2	A	277-405	25	-	0
H114	0.40	56	40	2	A	210-418	31	206-286	19
Och27	0.60	56	40	2	A	222-470	49	226-490	21
Och20	0.50	64	35	3	B	240-356	25	228-320	18
Och29	0.50	64	35	3	B	245-305	13	-	0
Och30	0.35	64	35	3	B	100-196	23	100-152	12
Och18	0.50	56	35	4	C	158-258	26	170-210	12
Och24	0.20	56	35	4	C	163-227	13	195-259	13

Table 3. Results for Exact Tests of Hardy –Weinberg equilibrium for each locus/population in GENEPOP (Raymond and Rousset 1995). P-values ≤ 0.001 (grayed out) denote populations not in equilibrium at a locus (TC03 = Tincup Creek, BL03 = Bear Lake, GWH03 = Glenwood Hatchery, CTWD = Cottonwood Creek).

Locus	TC03 N=24	BL03 N=27	GWH03 N=22	CTWD N=57
Ogo4	0.811	1.000	-	0.686
Omm1036	0.109	0.467	0.376	0.112
Ocl1	0.377	1.000	0.926	0.224
Ssa85	0.736	0.926	1.000	0.095
Ots107	0.733	0.732	0.347	0.296
J103	0.956	0.494	0.000	0.035
H114	0.970	0.042	0.002	0.037
Och27	0.053	0.809	0.100	0.000
Och20	0.823	0.381	0.738	0.255
Och29	0.865	0.480	0.437	0.000
Och30	1.000	0.585	0.331	0.196
Och18	0.843	0.800	0.213	0.513
Och24	0.883	0.230	0.017	0.017

Table 4. Number of crosses in each dyad produced by COLONY (Wang 2004, 2009) for BY2010 and SY2010. Estimated error rates calculated by membership in dyad. * = information not given as result file in COLONY.

	Dyad			Error	
BY2010	**Full**	**Half**	**Un**	**Type I**	**Type II**
Full (n=49)	33	14	2		4.1%
Half (n=88)	2	66	20	2.3%	22.7%
Un	0	83	4058	2.0%	
SY2010					
All	49	215	5407*	-	-
Spawned	1	3	68*	-	-

Table 5. Number of crosses identified as Full-, Half-sib, or Un-related for BY2010 and SY2010 according to hypothesis tested by M-L_{QG} in KINGROUP (Konovalov et al 2004). Type I and II error rates calculated using number of known full-, half-sib, and unrelated crosses with 0.5 > p > 0.5.

	Ho 1			Ho 2		
BY2010	**Full vs Un**	**Type I**	**Type II**	**Full vs Half**	**Type I**	**Type II**
Full (n=49)	48	-	2.0%	39	-	20.4%
Half (n=88)	52	59.1%	40.9%	1	1.1%	-
Un	152	3.7%		3	0.1%	
SY2010						
All	359	-	-	51	-	-
Spawned	5	-	-	2	-	-

Table 6. Mean Relatedness values using Queller and Goodnight's method in KINGROUP (Konovalov et al 2004) with cutoff ranges and Type I and II error rates for each relationship group in BY2010.

BY2010	Mean	Cutoffs	Type I error	Type II error
Full	0.525	> 0.3765	-	16.3%
Half	0.228	0.3765 - 0.115	21.5%	22.7%
Un	0.002	< 0.115	9.1%	-

Table 7. Spawned crosses in 2010 from BCT Broodstock identified as full-sibs in COLONY and KINGROUP. Crosses in COLONY identified using both Dyads, M-L_{QG} crosses in KINGROUP identified using the hypothesis Full vs Un-related, R_{QG} crosses identified with original cutoff and adjusted cutoffs.

COLONY	**KINGROUP**	**KINGROUP**	**KINGROUP**
All Dyads	M-L: Full vs Un	$R_{QG\ (\geq 0.3765)}$	$R_{QG\ (\geq 0.2)}$
F2 x M2A	F2 x M2A	-	F2 x M2A
-	-	-	F5 x M5A
F8 x M8B	-	-	-
-	F13 x M13B	-	F13 x M13B
F11 x M11A	F11 x M11A	-	F11 x M11A
-	-	-	F20 x M20B
F24 x M24B	F24 x M24B	F24 x M24B	F24 x M24B
-	-	-	F28 x M28A
-	F32 xM32A	-	F32 x M32A

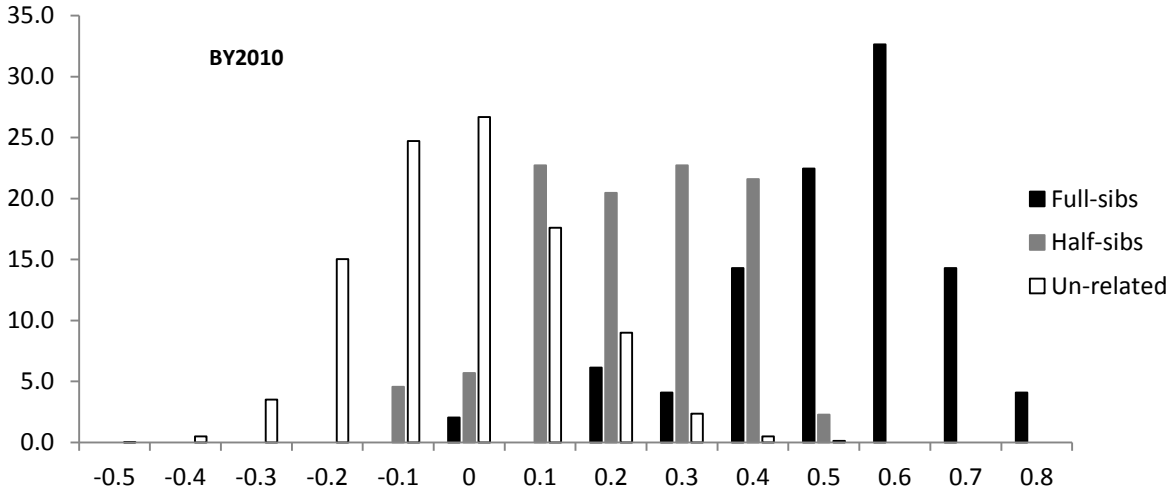


Figure 1. Distribution of R_{QG}-values for BY2010.

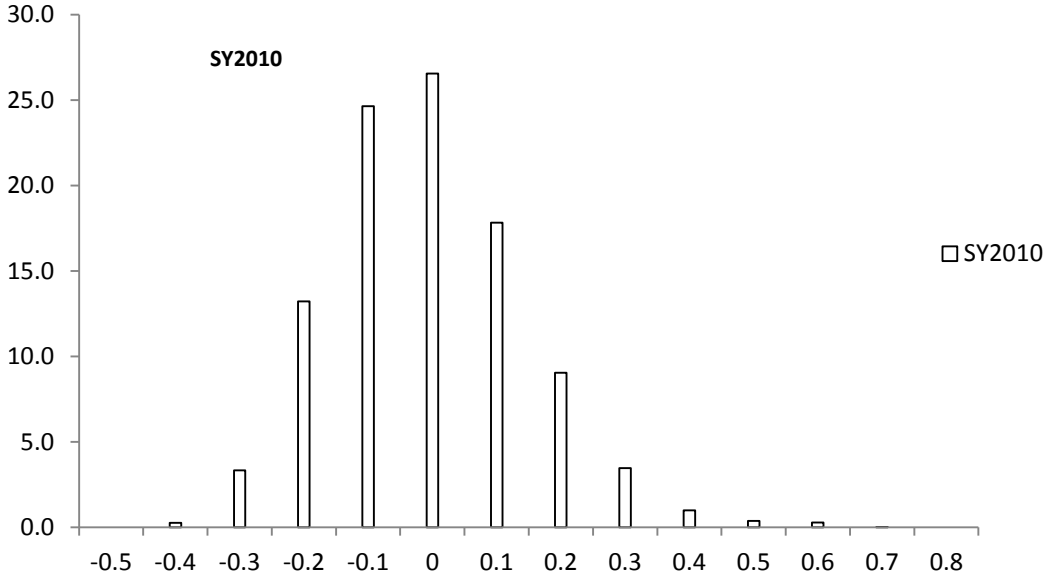


Figure 2. Distribution of R_{QG}-values for BY2010.

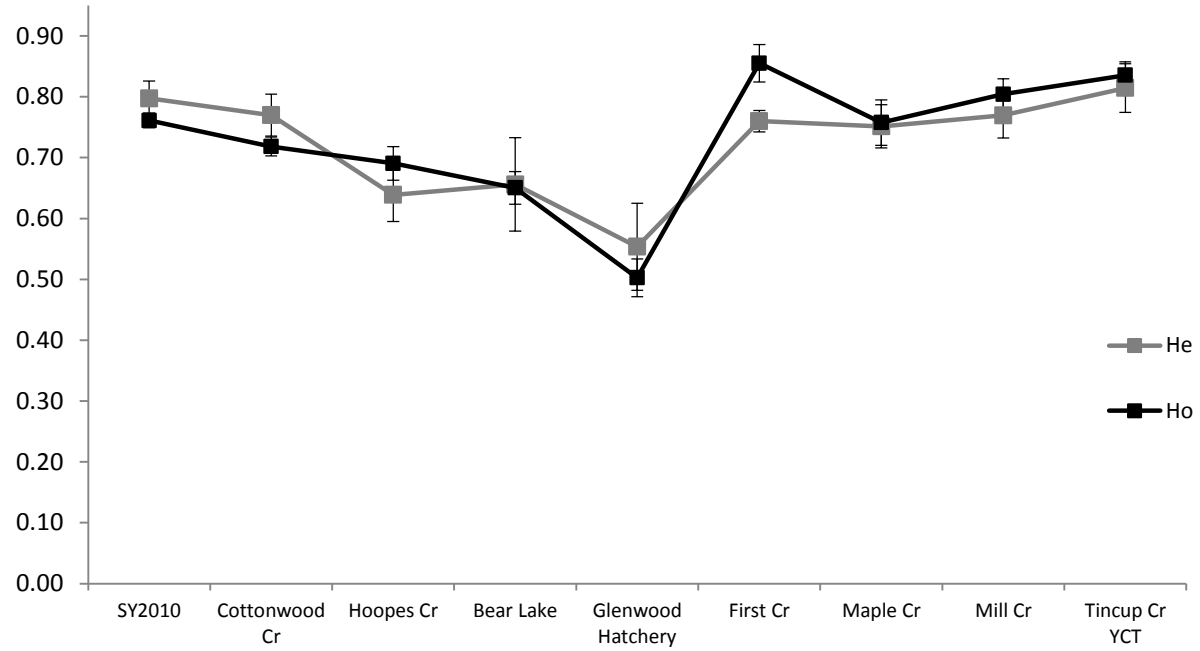


Figure 3. Expected and observed heterozygosity with SD across nine BCT populations and one YCT population.

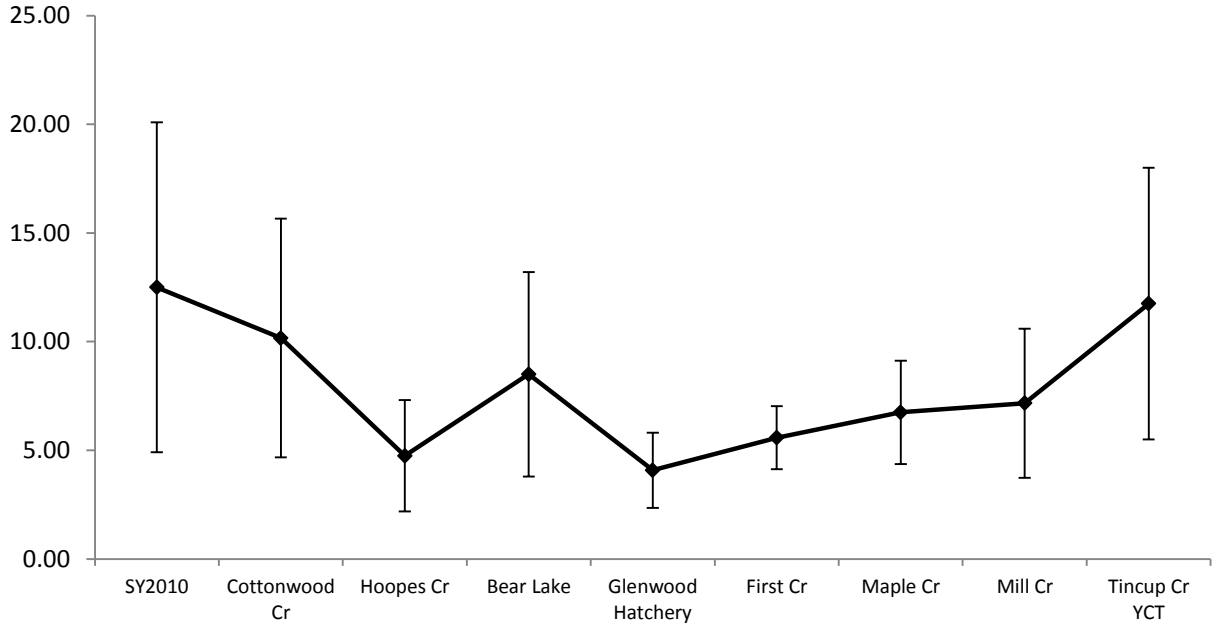


Figure 4. Average number of alleles per locus for each population across nine BCT populations and one YCT population.

Appendix 1. Parentage and Relatedness Summary Results for 2010 Spawners. Color codes denote individuals belonging to the same Full–sib family group.

2010 Cross						Relatedness Estimator			
Female	**Male**	**Offspring Location**	**Pit-tag Discrepancy**	**Parentage Discrepancy (# of offspring)**	**# Offspring in analyses**	**M-L_W**	**M-L_{QG}**	**R_{QG}**	**R_{QGAdj}**
F1	M1A	Isolation	M1A		2				
	M1B	Isolation			2				
F2	M2A	Production				Related	Related		Related
	M2B	Production							
F3	M3A	Isolation	F3		2				
	M3B	Isolation			2				
F4	M4A	Isolation			2				
	M4B	Isolation			2				
F5	M5A	Production							Related
	M5B	Production							
F6	M6A	Isolation		No father assigned-2	2				
	M6B	Isolation			2				
F7	M7A	Isolation			2				
	M7B	Isolation			2				
F8	M8A	Isolation	M8A		2				
	M8B	Isolation			2	Related			
F9	M9A	Production							
	M9B	Production							
F10	M10A	Production							
	M10B	Production							
F11	M11A	Isolation			2	Related	Related		Related
	M11B	Isolation			2				
F12	M12A	Isolation			2				
	M12B	Isolation			2				
F13	M13A	Production							
	M13B	Production					Related		Related
F14	M14A	Isolation			2				
	M14B	Isolation			2				
F15	M15A	Production							
	M15B	Production							
F16	M16A	Production							
	M16B	Production							
F17	M17A	Isolation			2				
	M17B	Isolation			2				
F18	M18A	Isolation			2				
	M18B	Isolation		No father assigned-2	2				
F19	M19A	Production							
	M19B	Production							
F20	M20A	Isolation			2				
	M20B	Isolation			2				Related
F21	M21A	Isolation			2				
	M21B	Isolation			2				
F22	M22A	Isolation			2				
	M22B	Isolation			2				
F23	M23A	Isolation			2				
	M23B	Isolation		M31A father assigned-2	2				

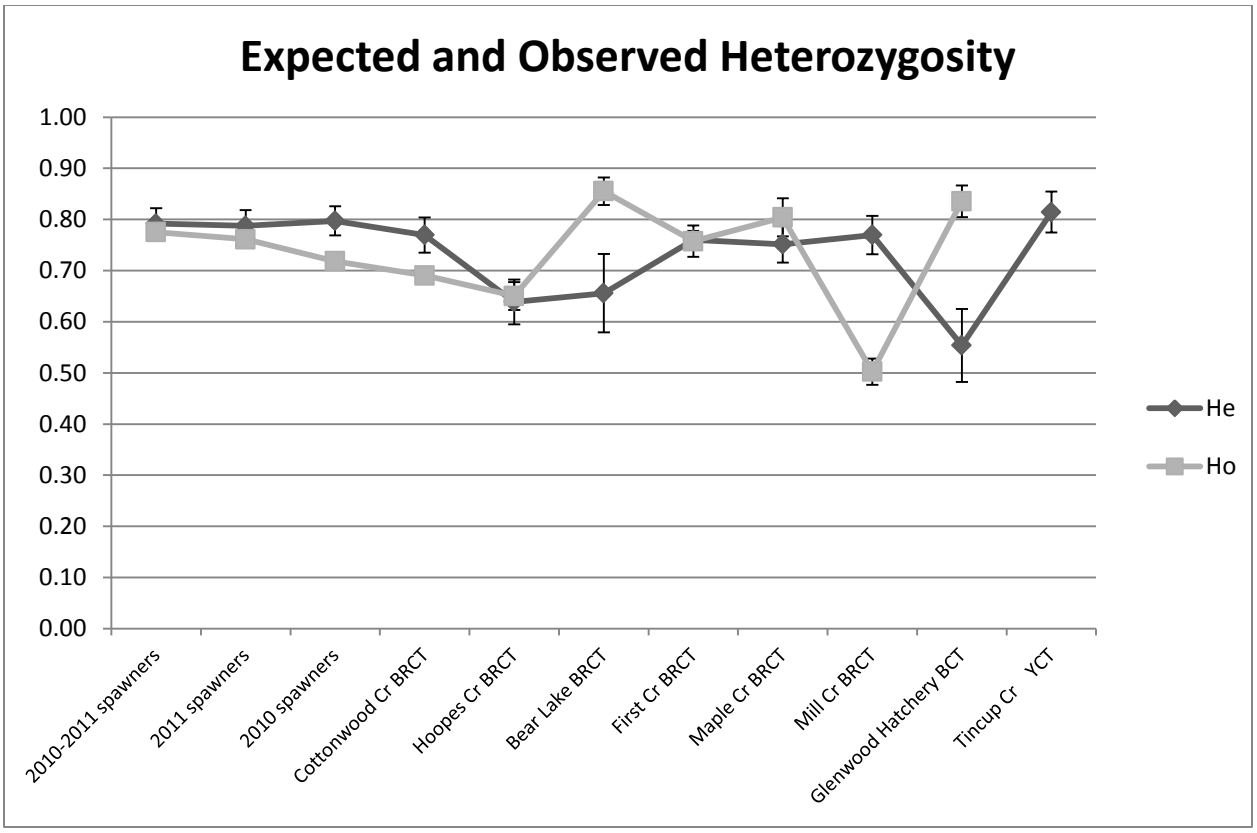
2010 Cross						Relatedness Estimator			
Female	**Male**	**Offspring Location**	**Pit-tag Discrepancy**	**Parentage Discrepancy (# of offspring)**	**# Offspring in analyses**	**M-L_W**	**M-L_{QG}**	**R_{QG}**	**R_{QGAdj}**
F24	M24A	Isolation			2				
	M24B	Isolation		F25xM25B family assignment-1	1	Related	Related	Related	Related
F25	M25A	Isolation		M25B father assigned-1	1				
	M25B	Isolation			4				
F26	M26A	Isolation			2				
	M26B	Isolation			2				
F27	M27A	Isolation			2				
	M27B	Isolation			2				
F28	M28A	Production							Related
	M28B	Production							
F29	M29A	Production							
	M29B	Production							
F30	M30A	Isolation			2				
	M30B	Isolation			2				
F31	M31A	Production	M31A						
	M31B	Production							
F32	M32A	Isolation			2		Related		Related
	M32B	Isolation			2				
F33	M33A	Isolation			2				
	M33B	Isolation			2				
F34	M34A	Production							
	M34A	Production							
F35	M35A	Isolation			2				
	M35B	Isolation			1				
F36	M36A	Isolation	F36		2				
	M36B	Isolation							

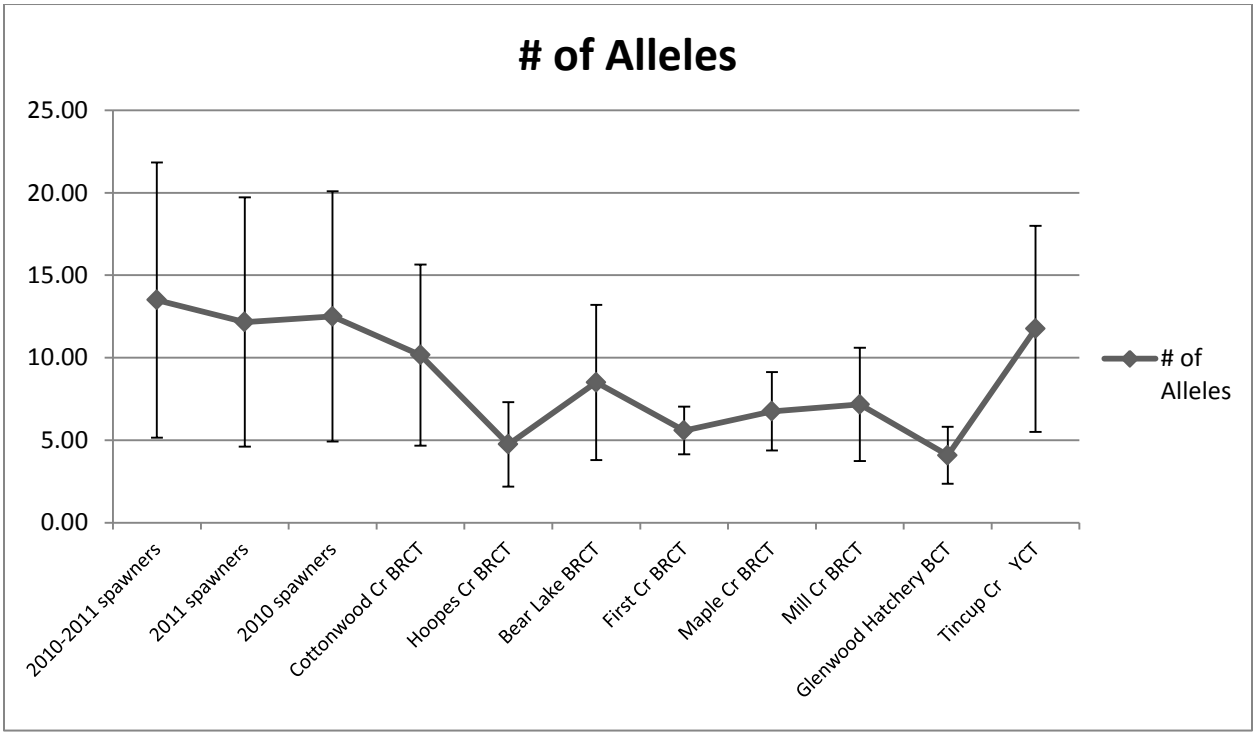
2011 BCT Genetic Spawn Summary:

The 2011 Bear River Cutthroat spawning resulted in one related cross (F25 xF25A). Relatedness analyses indicated this individual was most likely a Half-sib cross, so the removal of the eggs from this cross from production was supported. In 2010 there were six related crosses identified. The decrease in the number of related crosses from 2010 to 2011 was most likely due to chance as fish were used at random. In 2010, 29.5% of individuals were identified as full-sibs to at least one other individual; in 2011 this was 16.8%. Despite less individuals being spawned in 2011 the effective population estimate (N_e) did not change much from the previous year. This is due to the fact that the broodstock was more related in 2010 than 2011. The future genetic screening of pre-spawn adults prior to ponding will ensure that the broodstock is more genetically diverse and less related overall.

Estimates of observed and expected heterozygosity and the number of alleles did not differ significantly between 2010 and 2011. When data for 2010 and 2011 was combined (leaving out repeat individuals), there was a slight increase in heterozygosity and allele count estimates (diversity). This supports the practice of using individuals for one spawn year for this broodstock, avoiding the re-use of males, in order to ensure that diversity is maximized. As the project continues genetic monitoring will continue to be an important component, either post- spawning as the current system is set up, or pre-spawn with wild collections. We advocate that fish are genetically screened prior to ponding to ensure that inbreeding is minimized and genetic diversity is maximized.

	2010	2011
N	105	83
N_{males}	69	27
$N_{males\ re\text{-}used\ from\ previous\ yrs}$	0	25
$N_{females}$	36	31
$N_{females\ re\text{-}used\ from\ previous\ yrs}$	0	0
N_e (95%CI)	72 (50-101)	70 (49-100)
# of unique genetic families	87	74
Avg # individuals in family	1.2	1.1
Range of family size	1-9	1-6


Expected and Observed Heterozygosity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
2010-2011 spawners
2011 spawners
2010 spawners
Cottonwood Cr BRCT
Hoopes Cr BRCT
Bear Lake BRCT
First Cr BRCT
Maple Cr BRCT
Mill Cr BRCT
Glenwood Hatchery BCT
Tincup Cr YCT
He
Ho


# of Alleles
25.00
20.00
15.00
10.00
5.00
0.00
2010-2011 spawners
2011 spawners
2010 spawners
Cottonwood Cr BRCT
Hoopes Cr BRCT
Bear Lake BRCT
First Cr BRCT
Maple Cr BRCT
Mill Cr BRCT
Glenwood Hatchery BCT
Tincup Cr YCT
# of Alleles