Genetic Characterization of Bull Trout from the Walla Walla River Basin

by

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Abstract

A total of 25 collections of bull trout were analyzed from eight different collection sites within the Walla Walla River Basin and two collection sites in the Yakima River Basin. Adult bull trout were analyzed from five sites and juvenile bull trout were analyzed from five sites. Sixteen nuclear microsatellite DNA loci that are included in the standardized suite of loci were used to examine the levels and patterns of genetic variation. The multi-locus genotypes generated for these bull trout were analyzed to determine population structure of the adult and juvenile bull trout collections. Tests of population subdivision, factorial correspondence analysis, and the neighbor-joining tree suggested the five adult collections were significantly different from one another. The five juvenile collections were also all significantly different from one another. The Touchet River adult collection clustered with the juvenile collections, but was significantly different from them in the genotypic tests of differentiation. The collections of juvenile bull trout from the North Fork Touchet, Wolf Fork and Burnt Fork were significantly different from one another with most of the statistical comparisons we employed, while bull trout from the Lewis and Spangler Creek collections could not be differentiated from the other groups and tended to overlap or group with bull trout from the North Fork Touchet or Wolf Fork. Assignment tests were used to determine stock-of-origin percentage of migratory adult bull trout that were collected at Dayton Dam Trap. The highest percentage of migratory bull trout at the Dayton Dam came from Wolf Fork (50.6%) and the N.F. Touchet River (39.0%), while Lewis Creek and Spangler Creeks accounted for 5.2% each, and no adult bull trout assigned to Burnt Fork.

Introduction

The Walla Walla River Basin is located in southeastern Washington and northeastern Oregon, stretching from the Blue Mountains in the east to the Columbia River on the west. Major tributaries in the Walla Walla River Basin include Mill Creek, Touchet River, and the Walla Walla River. The Umatilla-Walla Walla Chapter of the Bull Trout Draft Recovery Plan (chapter 10, USFWS 2002) initially described these three tributaries within the Walla Walla River Basin as core populations. However, core areas were revised to include only the Walla Walla and Touchet River core areas in the latest draft recovery plan (Chapter 10, USFWS 2004). The latest draft plan separates the Walla Walla core area into upper Mill Creek and upper Walla Walla local populations, while the Touchet River core area is comprised of the three discrete populations (North Fork Touchet River, South Fork Touchet, and the Wolf Fork of the Touchet River).

The population designations by the Umatilla-Walla Walla Chapter of the Bull Trout Draft Recovery Plan (USFWS 2004) were not made with genetic data, but with limited local population studies, extensive literature review, and assessment of geographic separation of spawning areas. A microsatellite analysis by Spruell et al. (2003) on 65 populations of bull trout from the Northwestern part of the United States included samples from the Walla Walla River Basin. That analysis concluded that there was little genetic variation within bull trout populations but substantial divergence among populations. They lumped bull trout from the Walla Walla River Basin with other populations in the Snake and mid-Columbia River Basin into a broad group. Current information on the status of bull trout populations is inconsistent given the difference between the bull trout recovery plan and genetic analysis by Spruell et al. (2003).

Recovery and management of bull trout in the Walla Walla River Basin requires better information and planning. Managers need to know if there is evidence of mixing and/or reproductive isolation of bull trout among the major tributaries in the Walla Walla River Basin and nearby basins. Samples of migratory adult bull trout were collected from

traps in the three primary drainages within the Walla Walla Subbasin (Mill Creek, Touchet River, and Walla Walla River) and samples of juvenile bull trout were collected from five locations that had both spawning and juvenile rearing (Lewis Creek, Spangler Creek, North Fork, Wolf Fork, and Burnt Fork) in the Touchet River drainage. These samples along with bull trout data from two collections in the Yakima River basin were analyzed with a microsatellite DNA analysis to address the following management goals:

- Document and describe the genetic composition or stock structure for migratory bull trout of the Walla Walla River Basin by analyzing adult bull trout samples from each of the three major drainages. Specifically, are there significant genetic differences among populations of adult migratory bull trout in the Mill Creek, Walla Walla River, and Touchet River drainages? If so, should bull trout in these areas be managed as separate populations?

- Document and describe the genetic composition or stock structure from juvenile bull trout in spawning locations within the Touchet River drainage. Also, use stock-of-origin assignment tests on migratory adult bull trout collected at Dayton Dam Trap (on the Mainstem Touchet River) to determine stock percentage of each spawning population downstream of their spawning locations to answer the following questions: 1) are there significant genetic differences among juvenile (generally less than 200 mm fork length) bull trout captured during summer from five isolated spawning areas in the Touchet River drainage and could they be reproductively isolated; 2) are these juvenile populations different enough to be managed separately?

- Provide evidence (if possible) that bull trout in the tributaries of the Walla Walla River Basin have undergone a genetic bottleneck or are inbreeding. Calculate effective population size (N_e) for each group or collection if possible.

- Compare the genetic characteristics and stock structure of bull trout in another Columbia River Basin (Yakima River Basin) with the Walla Walla River Basin to determine genetic relatedness among bull trout in these two basins.

Methods

Collections

Washington Department of Fish and Wildlife (WDFW) provided genetic sampling kits used by Oregon Department of Fish and Wildlife staff that operated the Nursery Bridge Trap (near Milton Freewater, Oregon) and US Forest Service staff at the trap in upper Mill Creek (at the City of Walla Walla's municipal water intake Dam) and requested they collect fin clips for this study. Also, at the request of WDFW Fish Management staff, WDFW staff from the Snake River Laboratory enumerated bull trout captured at the trap and collected fish lengths (fork length in mm) and tissues at the Dayton Dam while trapping steelhead for hatchery Broodstock (Bumgarner et al. 2003, 2004). Samples of juvenile bull trout (primarily less than 200 mm) from five known spawning areas in the Touchet River drainage were collected by WDFW Fish Management staff by electrofishing numerous sites over several years in July and August (Mendel et al. 2000, 2001, 2002, 2003a, 2004, 2005, 2006). At each site, bull trout fork length (mm) and unique identification label were recorded for each fish, and a small partial clip of caudal fin was placed in individually labeled vials of 100% ethanol for preservation immediately after collection.

Adult bull trout samples were analyzed from three major drainages in the Walla Walla River Basin and juvenile samples from five isolated spawning areas in the Touchet River. Data were also included from the Yakima River Basin (Naches River and Ahtanum Creek) for comparison.

Laboratory Analyses

Genomic DNA was extracted by digesting a small piece of fin tissue using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

A total of 16 microsatellite loci were assessed in this study. Twelve of the loci were selected by a group of five participating laboratories for standardization with an additional four loci to be used for regional studies; however allele standardization has not occurred among participating labs. Data generated by the different labs is therefore not standardized and cannot be used unless an assessment of allele sizes is conducted. Microsatellite alleles were sized using an internal size standard. GENEMAPPER (Version 3.7) software (Applied Biosystems) was used to collect and analyze the microsatellite data. Allele binning and naming were accomplished using MicrosatelliteBinner-v1h (Young, WDFW, available from the WDFW Molecular Genetics Laboratory). MicrosatelliteBinner creates groups (bins) of alleles with similar mobilities (alleles with the same number of repeat units). The upper and lower bounds of the bins are determined by identifying clusters of alleles separated by gaps (nominally 0.4 base pairs in size) in the distribution of allele sizes. The bins are then named as the mean allele size for the cluster rounded to an integer.

Statistical Analyses

Analysis was performed on all collection groups to detect any individuals with matching genotypes using the EXCEL Microsatellite Toolkit v.3.1 (Park 2001). If matching genotypes between two samples were detected we assumed an individual fish was sampled twice and one of the two individuals was removed from the analysis.

Tests for Hardy-Weinberg proportions between all pairs of loci within each subpopulation were performed using GENEPOP (version 3.4; Raymond and Rousset

1995). Allele frequencies were calculated using CONVERT (version 1.3; Glaubitz 2003).

Observed and expected heterozygosity was computed for each subpopulation using GDA (Lewis and Zaykin 2001). Allelic richness and inbreeding coefficient (F_{IS} from Weir and Cockerham 1984) were computed for each subpopulation with FSTAT (version 2.9.3.2; Goudet 1995). Linkage disequilibrium was compared between each locus for each collection using GENEPOP v 3.4 (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). Statistical significance for the linkage disequilibrium analysis was evaluated using a Bonferroni correction of p-values (Rice 1989). The Bonferroni correction is a procedure that is employed to minimize Type I errors (declaring a significant difference due to chance) by dividing the 0.05 significance level by the total number of tests being conducted. Values that are significant after correction can then be evaluated based on their true significance and not by chance alone.

Within a group, the coefficient of identity was calculated between each pair of samples in all collections using Queller and Goodnight (1989) estimator of relatedness in the program IDENTIX v.1.1 (Belkhir et al. 2002). Using this measure of relatedness, a value of 0.5 is expected for a full-sibling relationship (individuals sharing the same mother and father) between two individuals.

Evaluation of the number of alleles and the expected genetic diversity (H_e or expected heterozygosity at Hardy Weinberg proportions) was conducted using the program BOTTLENECK (Cornuet and Luikart 1996) to determine if any of the populations have undergone a reduction in effective population as the result of a genetic bottleneck. We used the two-phased model and a two-tailed Wilcoxon sign-rank test for this evaluation. The Wilcoxon test is best suited for this data set because it can be used with any number of individuals and as few as four polymorphic loci (Cornuet and Luikart 1996). Expectation is that the number of alleles would be reduced more quickly and the expected Hardy-Weinberg frequencies would be lower than the observed. A significant

p-value indicates a collection that fits the expectation and identifies a population that has undergone a genetic bottleneck.

Pairwise estimates of genotypic differentiation and F_{ST} were computed to examine population structure using GENEPOP 3.4. These estimates use allelic and genotypic frequency data to assess differences between subpopulation pairs. Statistical significance of both estimates was tested (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). Statistical significance for the genotypic differentiation analysis was evaluated using a Bonferroni correction of p-values (Rice 1989).

Genetic distance between pairs of subpopulations was estimated using Cavalli-Sforza and Edwards (1967) chord distance as performed in PHYLIP (version 3.5c, Felsenstein 1993). Bootstrap calculations were performed using SEQBOOT followed by calculations of genetic distance using GENDIST. The NEIGHBOR-JOINING method of Saitou and Nei (1987) was used to generate the dendrograms and CONSENSE to generate a final consensus tree from the 1,000 replicates. The dendrogram generated in PHYLIP was plotted as a radial tree using TREEVIEW (version 1.6.6, Page 1996).

We used GENETIX (version 4.03, Belkhir et al. 2002) to provide a factorial correspondence analysis and a graphical representation of the genetic variation among all individual samples in multi-dimensional space. Genotypic data for an individual sample is transformed into a value and plotted using the value. The multi-dimensional data space represents all the individual values. Each axis (three-dimensional in this case) is derived from the individual values where the first axis (x) is a line, analogous to a least squares regression, which encompasses the maximum amount of variation present among all loci and populations. The second and subsequent axes are derived from a decreasing amount of observed variation.

We used GENECLASS2 (version 2.0.g, Piry et al. 2004) to perform maximum likelihood jackknife assignments of each Touchet bull trout in the juvenile baseline collections. In

the jackknife procedure, each individual fish is removed from the dataset, the allele frequencies of the baseline subpopulations are recalculated, and the fish is assigned to the most likely group. Jackknife assignments were used to evaluate the reliability of the assignments of the temporal collections, and to determine the relationships among subpopulations in the Touchet River Basin. Correct jackknife assignment relies upon a robust baseline as well as true distinctions among groups.

For the analysis of migratory adults from the Dayton Dam Trap, we used GENECLASS2 (version 2.0.g, Piry et al. 2004) to determine possible relationships between the mixed samples and the juvenile baseline groups in the Touchet River Basin by assigning individuals to their most likely stock-of-origin.

Results and Discussion

Collections

DNA was obtained and analyzed from ten collection sites (Figure 1) and a total of 469 individuals (Table 1). Thirty-three of the individuals were dropped from analysis because they failed to amplify DNA (indicated as missing data in Table 1).

Figure 1. Map of the Walla Walla River subbasin and collection sites of bull trout samples.

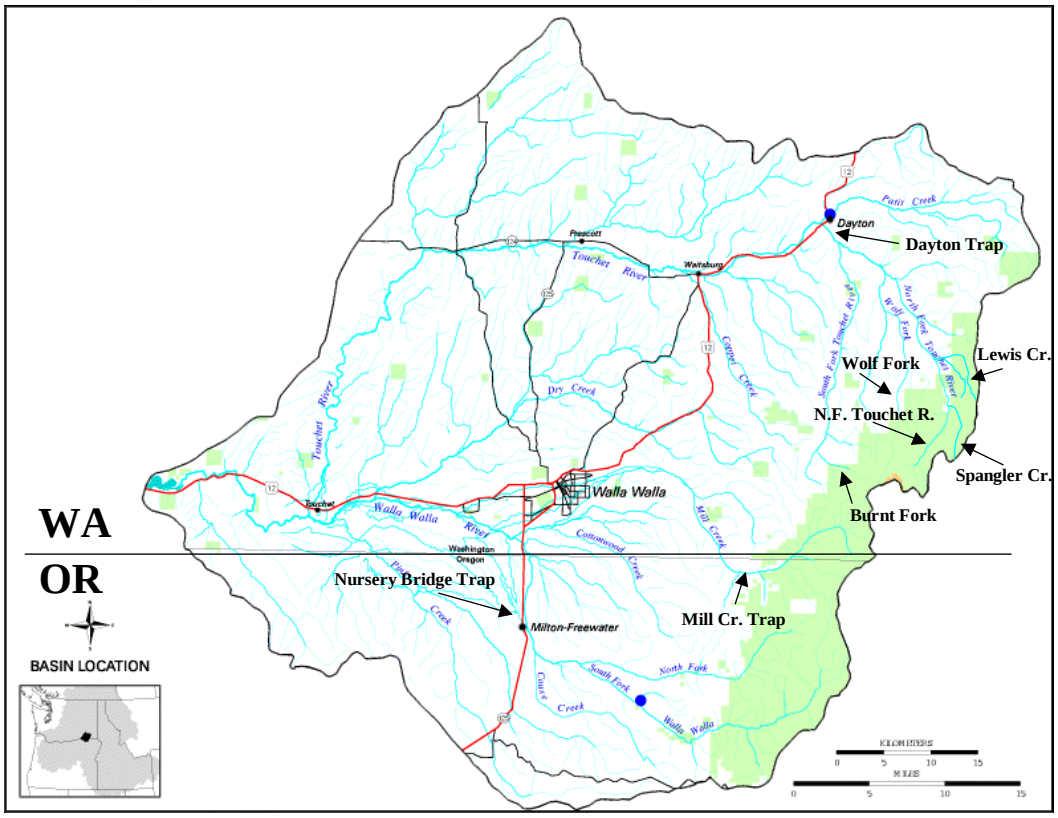


Table 1. Collection code, collection location, identification as an adult or juvenile collection, total number of samples collected, and number analyzed for collections of adult and juvenile bull trout taken from the Walla Walla River and Yakima River Basins.

Walla Walla River Basin – Adult Collections

Collection code	Collection location	Adult/ Juvenile	N = Total	N = Analyzed
99AL	Dayton Dam - Touchet River	adult	16	11
00AN	Dayton Dam - Touchet River	adult	21	11
03LC	Dayton Trap - Touchet River	adult	40	39
03LM [A]	Dayton Dam - Touchet River	adult	23	16
Dayton Dam - Touchet River - Total			100	77[B]

[A] samples from the 03LM collection were taken in 2001 and 2002

[B] 15 samples were dropped because an identical genotype was detected at another sample indicating the same fish had been sampled twice

[B] 8 samples were dropped because of missing data

98AI	Mill Creek, OR	adult	40	40
00AU	Mill Creek, OR	adult	50	42
Mill Creek, OR - Total			90	82[A]

[A] 1 sample was dropped because an identical genotype was detected at another sample indicating the same fish had been sampled twice

[A] 7 samples were dropped because of missing data

98LS	Walla Walla River, OR	adult	7	7
99AM	Walla Walla River, OR	adult	2	2
00AO	Walla Walla River, OR	adult	14	14
Walla Walla River, OR - Total			23	23

Touchet River Basin – Juvenile Collections							
Collection code	Collection location	Adult/ Juvenile	N = Total	N = Analyzed			
00AN	Burnt Fork	juvenile	9	9			
Burnt Fork - Total			9 [A, B]	9			

[A] samples from the 00AN collection were taken in 2000 (N = 8) and 2005 (N = 1)

[B] Samples > 200 mm, but < 300 mm, and one was 340 mm

99EW	Lewis Creek	juvenile	1	1			
02AAA	Lewis Creek	juvenile	2	1			
03LR	Lewis Creek	juvenile	7	4			
05GV	Lewis Creek	juvenile	4	4			
06HS	Lewis Creek	juvenile	3	3			
Lewis Creek - Total			17 [B]	13 [A]			

[A] 4 samples were dropped because of missing data

[B] One sample was > 200 mm (231mm)

03LQ	Spangler Creek .	juvenile	5	5			
06HR	Spangler Creek .	juvenile	14	14			
Spangler Creek - Total			19 [A]	19			

[A] All samples less than 171 mm

03LO	Wolf Fork	juvenile	46 [A]	38			
04DG	Wolf Fork	juvenile	41 [B]	41			
Wolf Fork - Total			87	79 [C]			

[A] Samples were less than 133 mm, plus three samples > 200 mm, but < 300 mm, and three were > 300mm (343, 365, 553 mm)

[B] All less than 133 mm

[C] 1 sample was dropped because an identical genotype was detected at another sample indicating the same fish had been sampled twice

[C] 7 samples were dropped because of missing data

03LP	N.F. Touchet River	juvenile	27 [A]	20			
04DF	N.F. Touchet River	juvenile	45 [B]	45			
N.F. Touchet River - Total			72	65 [C]			

[A] Samples were < 200 mm, plus one sample was > 200, but < 300 mm, and three samples were > 300 mm (310, 350, 430 mm)

[B] All samples < 141 mm

[C] One sample was dropped because an identical genotype was detected at another sample indicating the same fish had been sampled twice

[C] 6 samples were dropped because of missing data

Yakima River Basin – Adult Collections							
Collection code	Collection location	Adult/ Juvenile	N = Total	N = Analyzed			
01AAE	S.F. Ahtanum Creek	adult	6	5			
01AAF	M.F. Ahtanum Creek	adult	16	16			
01AAG	N.F. Ahtanum Creek	adult	8	8			
Ahtanum Creek - Total			30	29[A]			
[A] 1 sample was dropped because of missing data							
03GG	Naches River	adult	22	22			
Naches River - Total			22	22			

Eighteen samples were dropped because a matching multilocus genotype was found in another sample (likely from sampling the same individual fish in two different years). One of the two samples that were matching remained in the subsequent analyses. Bull trout return data collected at Dayton Dam on the Touchet River has identified that a portion of the returns have been captured in previous years (Table 2). Matching genotypes from multiple collection years on the Touchet River was therefore likely, and was evident by the 15 samples identified by the genetic analysis (top of Table 1).

Table 2. Bull trout capture summary from Touchet River trap for all years of operation.

Year	Number of Unique Fish Handled	Total Fish Captures	Number of New PIT Tags Implanted	Total Recapture Events	Recapture Events In-season (on same year)	1st Year Recaptures	2nd Year Recaps	3rd Year Recaps	4th Year Recaps
1993	0	0	0	0					
1994	3	3	0	0					
1995	0	0	0	0					
1999	20	20	0	0					
2000	22	31	0	9	9 [A]				
2001	43	43	25	0	0				
2002	22	22	12	2	0	2			
2003	45	60	41	16	15	1	0		
2004	65	87	55	32	17 [B]	14 [C]	1	0	
2005	49	60	41	18	11 [D]	5	1	1	0
2006	54	84	39	42	23 [E]	14 [F]	4 [G]	0	1

[A] based on in-season fin clips for DNA samples.

[B] This includes 12 individual fish that were recaptured after fall back once (7) or twice (5) in 2004.

[C] This includes 9 individual fish recaptured from initial PIT tagging in 2003, but 5 of these fell back and were recaptured two times in 2004; another 4 fish fell back and were recaptured once in 2004.
[D] This includes 9 individual fish recaptured from initial PIT tagging in 2004, but 2 of these fell back and were recaptured two times in 2005; another 7 fish fell back and were recaptured once in 2005.
[E] This includes 18 individual fish that were recaptured after fall back once (13) or twice (10) in 2006.
[F] This includes 8 individual fish recaptured from initial PIT tagging in 2005, but 1 fish fell back twice and was recaptured a total of three times in 2006; 4 individual fish fell back and recaptured two times in 2006; plus 3 fish were recaptured only once in 2006.
[G] This includes 3 individuals recaptured from initial PIT tagging in 2004, but one fell back in 2006 and was recaptured.

Locus Statistics

Tests of Hardy-Weinberg equilibrium for each locus and population did not reveal any significant deviation after Bonferroni correction (Rice 1989); therefore all loci and populations were included in the analyses. Loci and populations that are not in HW equilibrium suggest there has been non random mating of individuals (inbreeding or assortative mating) in the population (evident by an increase in homozygotes, known as a Walhund effect), the populations are small and subject to genetic drift, or there have been errors in the scoring the locus (null alleles). Any locus or population that is not in equilibrium for multiple collections or loci is therefore dropped from the analyses.

Allele frequencies for all collections analyzed are in Appendix 1 and information for each locus is shown in Table 3. Observed and expected heterozygosity was also calculated for all loci. Three loci (*Sfo-18**, *Sco-102**, and *Sco-215**) had fewer than five alleles scored and observed heterozygosity of less than 0.152. The remaining loci had between 5 – 29 alleles and observed heterozygosity was between 0.603 – 0.835. Heterozygosity is a measure of the molecular variation at a given locus and is utilized in statistical analyses to determine if the variation meets the expected values in Hardy Weinberg proportion to describe the population and locus.

Table 3. Microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci used in the analysis of bull trout from the Walla Walla River and Yakima River Basins. The four loci shown in bold identify the ones used in specific region analyses and are not part of the 12 standardized loci. Also included are the observed (H_o) and expected (H_e) heterozygosity for each locus. Results of the Hardy-Weinberg analysis over all populations did not reveal any significant difference between the observed and expected heterozygosity values for any of the loci. Publication source for each locus is shown if available.

					Heterozygosity		
Multiplex	Locus	Annealing temp ^{o}C	# Alleles/ Locus	Allele Size Range (bp)	H_o	H_e	Source
Sco-A	***Sco-107****	55	14	250 - 314	0.761	0.824	WDFW unpublished
	*Sco-109**	55	29	257 - 402	0.835	0.882	WDFW unpublished
Sco-B	***Sco-106****	55	17	132 - 241	0.800	0.849	WDFW unpublished
	*Sfo-18**	55	1	178	0.000	0.000	Angers and Bernachez 1996
	*Smm-22**	55	25	221 - 333	0.777	0.900	Crane et al. 2004
Sco-C	***Omm-1130****	57	16	263 - 340	0.765	0.876	Rexroad et al. 2001
	*Sco-102**	57	4	166 - 182	0.152	0.171	WDFW unpublished
None	*Sco-212**	57	11	272 - 351	0.605	0.694	DeHaan & Ardren 2005
Sco-E	*Omm-1128**	47	12	267 - 353	0.606	0.749	Rexroad et al. 2001
	*Sco-105**	47	12	139 - 212	0.701	0.788	WDFW unpublished
Sco-I,1	*Sco-200**	56	10	151 - 187	0.660	0.765	DeHaan & Ardren 2005
	*Sco-202**	56	5	152 - 169	0.603	0.635	DeHaan & Ardren 2005
	Sco-218*	56	17	233 - 297	0.676	0.772	DeHaan & Ardren 2005
Sco-I,2	*Sco-220**	56	14	316 - 406	0.673	0.792	DeHaan & Ardren 2005
Sco-J	*Sco-215**	55	2	317 - 321	0.059	0.058	DeHaan & Ardren 2005
	*Sco-216**	55	7	263 - 295	0.652	0.683	DeHaan & Ardren 2005

Population Statistics

The estimates of genetic diversity, including heterozygosity and allelic richness, within these bull trout groups ranged from 0.524 to 0.661 and from 3.1 to 4.4, respectively (Table 4).

Table 4. Collection location and population statistics [heterozygosity (expected (H_e) and observed (H_o), allelic richness (A_o), F_{IS}, and Linkage Disequilibrium)] for adult and juvenile bull trout collections taken from the Walla Walla River and Yakima River Basins. P-values for F_{IS} tests were defined as significant after implementation of Bonferonni correction for multiple tests (Rice 1989) and identifies if the value is significantly different than zero (alpha p-value = 0.0003). Linkage disequilibrium is shown as the number of significant locus comparisons after Bonferroni correction of p-values over the total number of comparisons (0.05/120 = alpha of 0.0004).

Walla Walla River Basin – Adult Collections					
	Heterozygosity				
Collection location	H_o	H_e	A_o	F_{IS} (p-value)	Linkage Disequilbrium
Dayton Dam – Touchet River	0.592	0.601	4.0	0.028 (0.0516)	3 / 104
Mill Creek, OR	0.566	0.580	3.7	0.023 (0.0981)	1 / 91
Walla Walla River, OR	0.572	0.575	3.7	0.006 (0.4216)	0 / 78

Yakima River Basin - Adult Collections					
	Heterozygosity				
Collection location	H_o	H_e	A_o	F_{IS} (p-value)	Linkage Disequilbrium
Ahtanum Creek	0.535	0.569	3.4	0.060 (0.0322)	3 / 91
Naches River	0.661	0.668	4.4	0.010 (0.3856)	1 / 91

Touchet River Basin - Juvenile Collections					
	Heterozygosity				
Collection location	H_o	H_e	A_o	F_{IS} (p-value)	Linkage Disequilbrium
Burnt Fork	0.660	0.535	3.1	-0.255 (1.0000)	0 / 91
Lewis Creek	0.524	0.605	3.7	0.140 (0.0013)	0 / 91
Spangler Creek	0.602	0.594	3.9	-0.013 (0.6838)	1 / 78

Wolf Fork	0.599	0.600	3.9	0.003 (0.4241)	2 / 105
N.F. Touchet River	0.561	0.562	3.6	-0.003 (0.5616)	4 / 105

Overall, genetic diversity was quite similar among all collections and comparable to other analysis of bull trout (Bettles et al. 2005, Hawkins and Von Bargen 2006, Small and Bowman 2007). Genetic diversity (heterozygosity and allelic richness) is a measure of the diversity detected in a population sample and is affected by the number of individuals contributing to that population (e.g. populations with few individuals or populations with related individuals will have low genetic diversity). Observed heterozygosity was not significantly different than expected for samples from any collection site and therefore did not indicate few, or related, parents for the progeny sampled.

Estimates of within population variation, or the inbreeding coefficient (F_{IS}), were also assessed to determine the level of variation within each population to determine if the individuals were potentially inbred (Table 4). F_{IS} values can range from negative 1.0 – 1.0 and p-values for F_{IS} will determine if a value is significantly different from zero. Any significant value is an indicator that there are lower heterozygosity values within that population (because of small sample size or that the population is inbred) than would be expected in Hardy-Weinberg equilibrium. All F_{IS} values shown in Table 4 are not significantly different than zero after Bonferroni correction was applied. The Lewis Creek collection had the highest F_{IS} value (0.140) among all of the collections that were analyzed; however the sample size for Lewis Creek was low indicating these results may reflect the effects of a small effective population size and not inbreeding (i.e. mating between closely related individuals). If a population were inbred then the heterozygosity and allelic richness values would be low because there are fewer individuals mating and therefore fewer possible allele combinations. The values for F_{IS} would be high and contrast with the genetic diversity values. F_{IS} is a measure of the heterozygosity within a population; therefore a higher value indicates fewer heterozygotes implying that more closely related individuals were breeding together.

The low genetic diversity values along with the low F_{IS} values for all collections does not support a conclusion that the bull trout populations are comprised of siblings, but is the result of small population size from each collection site.

Tests for linkage disequilibrium revealed low levels of disequilibrium in these collections of bull trout (Table 4). Linkage disequilibrium can be caused by genetic drift, inclusion of family groups within collections, assortative mating and/or analysis of an admixed collection. Low levels of disequilibrium indicate that there were no associations between alleles at different loci; therefore all collections were included in the subsequent statistical analyses.

Genetic Relatedness within Sample Groups

The analysis of identity (relatedness) among samples revealed between 0.0% and 8.3% of the comparisons to be 0.5 or greater indicating what could be a full-sibling relationship (Table 5). Two collections (Ahtanum and Burnt Fork) had the highest values (6.2 and 8.3%) while all other collections ranged between 0.0 and 2.0%. The collections of adult bull trout from the Touchet River at Dayton Dam Trap had 15 individuals that had matching genotypes suggesting the same fish had been sampled multiple times over several years. Recapture data for the Touchet River at Dayton Dam Trap identified between 0.0% - 51.2% of the captures in a given year to be recaptures (Table 2). The high level of recaptures in some years suggests that populations of bull trout in the Touchet River are small while the identity analysis does not reveal a large percentage of full-sibling relationships. Determining actual sibling relationships in a population versus the effects of inbreeding within a small-related population are difficult. The mean identity values for each population are low, while the variance of the identity values suggests multiple family groups in the populations. This analysis identifies the potential of sibling relationships, but does not discount the possibility that these results occur from a lack of overall genetic diversity in the populations. Inclusion of the sibling groups in populations used for analysis is appropriate when the individuals are

contributing to the overall reproductive success of the population; therefore all samples were included in the analyses.

Table 5. Identity values from IDENTIX. A value of 0.5 or greater identifies a full-sibling relationship between two samples that are compared. The mean and variance of the mean is calculated over all individuals in a population.

Population	# comparisons 0.5 or greater	Total # of comparisons	% comparisons > 0.5	Mean	Variance
Adult - collections					
Touchet River	48	2,926	1.6%	-0.0123475	0.0476016
Mill Creek	32	3,321	1.0%	-0.0141908	0.0353499
Walla Walla River	0	253	0.0%	-0.0448173	0.0315707
Naches River	2	231	0.9%	-0.0488661	0.0324942
Ahtanum Creek	25	406	6.2%	-0.0359277	0.0757943
Juvenile - collections					
Lewis Creek	1	78	1.3%	-0.0732809	0.0571214
Spangler Creek	3	171	1.8%	-0.0543125	0.0462767
Wolf Fork	17	3,081	0.6%	-0.0161517	0.0339461
Burnt Fork	3	36	8.3%	-0.1227602	0.1338116
N.F. Touchet River	42	2,145	2.0%	-0.0150926	0.0433258

Bottleneck Analysis and Effective Population Size

Samples from each collection site were evaluated to determine if there was a difference in the number of alleles and the expected proportion of heterozygotes indicating that the samples from the site had undergone a reduction in effective population size or bottleneck. A Bonferroni correction (Rice 1989) was applied to the p-values for this analysis (0.05/10 = alpha of 0.005). The juvenile collection from Lewis Creek was the only collection to have a significant signal that identified the collection has undergone a genetic bottleneck (Table 6). The small sample size of the multiple collections from Lewis Creek attributed to the significant value. All other collections exhibited a non-

significant value and therefore identifies that these populations have not undergone a recent reduction in population size. Small sample sizes for some of the collections used in this study and a lack of temporal collections limits our ability in calculating effective population size. Effective population size for collections with appropriate samples sizes (e.g. Wolf Fork) will be evaluated at a later date. Other research by Heath et al. (2002) calculated effective population size (ratio of N_e to N) for three populations of steelhead in British Columbia to be between 0.06 – 0.29 (Heath et al. 2002) and Araki et al. (2006) recently published the ratio of population census size to effective population size for steelhead to be between 0.17 – 0.40.

Table 6. Calculation of p-values for assessment that populations of bull trout have undergone a recent reduction in effective population size or bottleneck. Values in black background with bolded white type identify populations with a significant test. Statistical significance (alpha - 0.05/10 = 0.005) was evaluated using a Bonferroni correction of p-values (Rice 1989).

population	p-value		population	p-value
Adult - collections			**Juvenile - collections**	
Touchet River	0.095		Lewis Creek	**0.000**
Mill Creek	0.104		Spangler Creek	0.007
Walla Walla River	0.08		Wolf Fork	0.252
Naches River	0.013		Burnt Fork	0.903
Ahtanum Creek	0.091		N.F. Touchet River	0.055

Genetic Differences Among Groups

Several statistical tests were conducted to examine the interrelationships among these populations of adult and juvenile bull trout. Tests of genetic differentiation among the multiple collections indicated few significant differences between collections from the same location (Tables 7 and 8). Two adult collections from Mill Creek, two juvenile collections from Spangler Creek, two juvenile collections from N.F. Touchet River, and

three adult collections from Ahtanum Creek were significantly different from the other collections at the same location. The two adult collections from Mill Creek may contain samples that had allele frequencies that varied only by a few alleles that resulted in the significant difference between the two collections. The two juvenile collections from Spangler Creek were both small (N = 5 and 14) and could easily have different allele frequencies resulting in a significant difference. The temporal collections of juvenile bull trout from the N.F. Touchet River could be different because the collections may include a genetic admixture of individuals from nearby Touchet River tributaries. If the samples from the N.F. Touchet River included a mixture of juveniles from several of the spawning locations that are genetically different then the genotypic tests of differentiation would reflect a significant difference. The samples from Ahtanum Creek were collected from the three forks of Ahtanum Creek (North Fork, Middle Fork, and South Fork) and are expected to be different; therefore these results are not surprising. The Ahtanum Creek collections were used for comparison to the populations of bull trout in the Walla Walla River Basin; therefore they were analyzed as one group instead of the three separate collections.

Table 7. P-values for genotypic differentiation tests (below diagonal) for each of the four adult collection sites with more than one years samples and genotypic differentiation tests among all five adult collection locations. Pairwise comparisons of genotypic differentiation tests that were significantly different are highlighted in black fill and white type. Pairwise comparisons were defined as significant after implementation of Bonferonni correction for multiple tests (Rice 1989). The alpha p-value for each group comparison is shown after correction. Pairwise F_{ST} values (above diagonal) for comparisons among the five adult collection locations can range between 0.0000 – 1.0000. The F_{ST} value represents the amount of genetic differentiation that exists between the pairwise groups being tested. The larger the F_{ST} value identifies that the populations are more genetically differentiated.

Adult Collections		Corrected alpha p-value – 0.05/10 = 0.0050				
	Touchet River	Mill Creek	Walla Walla River	Ahtanum Creek	Naches River	
Touchet River	---	0.0951	0.0677	0.2106	0.1790	
Mill Creek	**0.0000**	---	0.0652	0.1868	0.2063	
Walla Walla River	**0.0000**	**0.0000**	---	0.1982	0.2038	
Ahtanum Creek	**0.0000**	**0.0000**	**0.0000**	---	0.2159	
Naches River	**0.0000**	**0.0000**	**0.0000**	**0.0000**	---	

Touchet River		Corrected alpha p-value – 0.05/6 = 0.0083				
	99AL	00AN	03LM	03LC		
99AL	---					
00AN	0.5536	---				
03LM	0.6741	0.7640	---			
03LC	0.7954	0.4986	0.4926	---		

Mill Creek		alpha p-value = 0.05, no correction because there is only one test				
	98AI	00AU				
98AI	---					
00AU	**0.0066**	---				

Walla Walla River		Corrected alpha p-value – 0.05/3 = 0.0167				
	98LS	99AM	00AO			
98LS	---					
99AM	0.9958	---				
00AO	0.0515	0.6119	---			

Ahtanum Creek		Corrected alpha p-value – 0.05/3 = 0.0167				
	01AAE	01AAF	01AAG			
01AAE	---					
01AAF	**0.0000**	---				
01AAG	**0.0000**	**0.0000**	---			

Assessment of the pairwise F_{ST} estimates was conducted on the groups of adults from each sampling location (Table 7). Estimates among the adult collections from the Walla Walla River Basin were between 0.0652 – 0.0951. These values indicate that approximately 6 – 9% of the variation in bull trout occurs between the collections that were analyzed. For comparison, the F_{ST} estimate of bull trout within the Yakima River basin (the Naches River and Ahtanum Creek collections) analyzed in this study reveals a pairwise F_{ST} value of 0.2159 (Table 7) while values for bull trout collections in the American and Naches Rives (Yakima River basin) was 0.0108 (Denise Hawkins, unpublished data). Variation in F_{ST} values among collections depends on the overall genetic variation of the populations being analyzed and is therefore a reference to that difference. The F_{ST} values for the bull trout within the Walla Walla River basin reveal that these populations are more genetically differentiated from each other than the American and Naches River populations, while the Naches River and Ahtanum Creek populations are much more differentiated than either of the other comparisons.

Comparison of the F_{ST} values between the Walla Walla Basin adult groups and Yakima River Basin (Naches and Ahtanum Creek) was between 0.1790 and 0.2106. These values reveal greater genetic difference between the two basins than what was detected within the Walla Walla River basin indicating there has been minimal gene flow between these geographic areas. Similar values were detected between the Naches River and Ahtanum Creek also suggesting these two areas are genetically divergent from each other.

Assessment of pairwise F_{ST} estimates was also conducted on the groups of juveniles from each sampling location in the Touchet Basin (Table 8).

Table 8. P-values for genotypic differentiation tests (below diagonal) for each of the four juvenile collections sites with more than one years samples and genotypic differentiation tests among all five collections of juvenile populations. Pairwise comparisons of genotypic differentiation tests that were significantly different are highlighted in black fill and white type. Pairwise comparisons were defined as significant after implementation of Bonferonni correction for multiple tests (Rice 1989). The alpha p-value for each group comparison are shown after correction. Pairwise F_{ST} values (above diagonal) for comparisons among the five juvenile collection locations can range between 0.0000 – 1.0000. The F_{ST} value represents the amount of genetic differentiation that exists between the pairwise groups being tested. The larger the F_{ST} value identifies that the populations are more genetically differentiated.

Juvenile Collections		Corrected alpha p-value – 0.05/10 = 0.0050				
	Burnt Fork	Lewis Creek	Spangler Creek	Wolf Fork	N.F. Touchet River	
Burnt Fork	---	0.1127	0.1073	0.0716	0.1013	
Lewis Creek	**0.0000**	---	0.0407	0.0556	0.0323	
Spangler Creek	**0.0000**	**0.0000**	---	0.0413	0.0488	
Wolf Fork	**0.0000**	**0.0000**	**0.0000**	---	0.0602	
N.F. Touchet River	**0.0000**	**0.0000**	**0.0000**	**0.0000**	---	

Lewis Creek		Corrected alpha p-value – 0.05/9 = 0.0056				
	99EW	02AAA	03LR	05GV	06HS	
99EW	---					
02AAA	not possible	---				
03LR	0.9787	0.9998	---			
05GV	0.6066	0.9891	0.2043	---		
06HS	0.8543	0.9747	0.4956	0.9629	---	

Spangler Creek		alpha p-value = 0.05, no correction because there is only one test				
	03LQ	06HR				
03LQ	---					
06HR	**0.0000**	---				

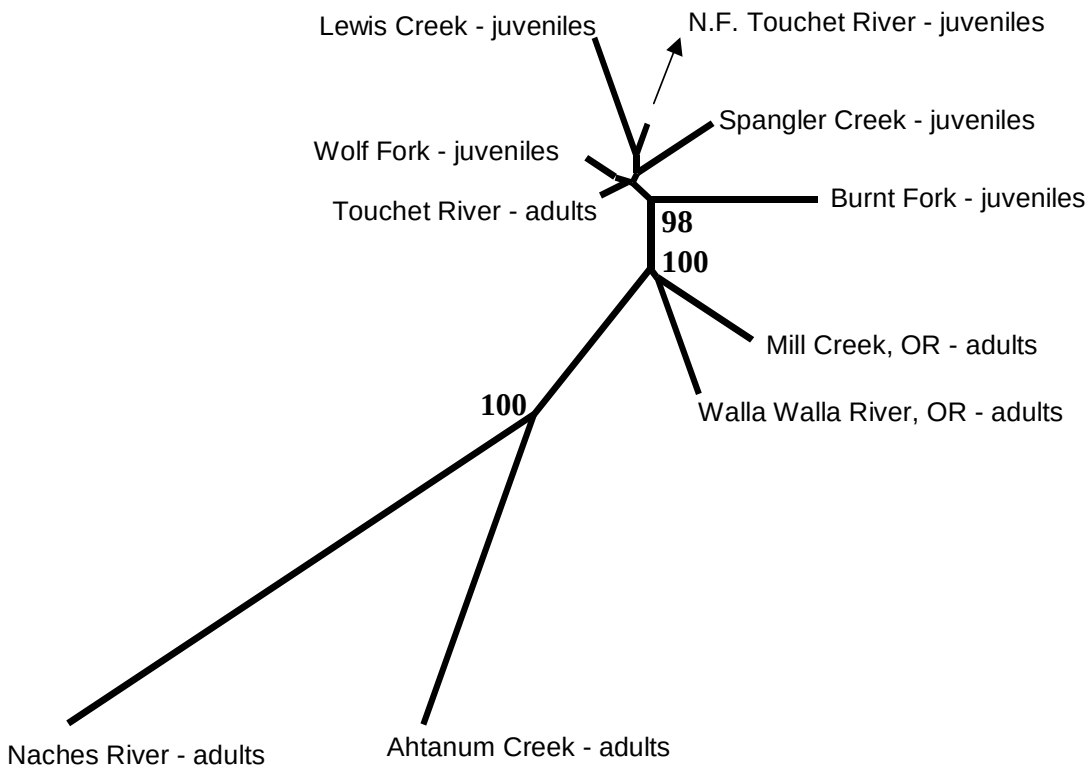
Wolf Fork		alpha p-value = 0.05, no correction because there is only one test				
	03LO	04DG				
03LO	---					
04DG	0.1230	---				

N.F. Touchet River		alpha p-value = 0.05, no correction because there is only one test				
	03LP	04DF				
03LP	---					
04DF	**0.0018**	---				

Estimates ranged between 0.0407 – 0.1127 with the higher estimates occurring between the Burnt Fork collection and the four other collections. This suggests the samples from the Burnt Fork in the S.F. Touchet River are more different from the four separate collections in the N.F. Touchet River. There were only nine samples collected from the Burnt Fork so these results may have been influenced by the small sample size.

The genetic relationship among collection groups was examined by assessing the groups in the neighbor-joining tree (Figure 2). The following groups were associated with over 90% bootstrap support: 1) the two collections from the Yakima River Basin (Naches River and Ahtanum Creek) were grouped together but well apart from both the adult and juvenile collections in the Walla Walla River Basin; 2) the collections of adult bull trout from Mill Creek and Walla Walla River were together, and apart from the adult and juvenile collections from the Touchet River and Yakima River; and 3) the Burnt Fork juveniles were genetically different and separated from all the other groups.

Figure 2. Relationship of adult migratory bull trout from the Walla Walla River and Yakima River Basins and juvenile bull trout from the Touchet River Basin based on the genetic distance matrix using Cavalli-Sforza and Edwards (1967) chord distance. Clusters with bootstrap values over 90% are shown.



These results identify substantial genetic differentiation between bull trout in the Yakima River Basin and the Walla Walla River Basin. Additionally, the two adult collections of bull trout within the Walla Walla River and Mill Creek are differentiated from the Touchet River collections. All of the collections from the Touchet River juveniles group together, with the exception of the Burnt Fork collection, which is the only tributary of the S.F. Touchet River with spawning bull trout. A couple of possible reasons exist to explain the difference of the Burnt Fork juveniles to other Touchet River juvenile collections: 1) there were only nine samples from the S.F. Touchet River drainage collected during two years (2000 – N = 8; 2005 – N = 1, plus several years of sampling effort with no bull trout collected) and therefore the allele frequencies of those samples may not be representative of the entire population; 2) isolation of bull trout in the S.F. Touchet River has resulted in the divergence of those into a genetically different population than bull trout in the N.F. Touchet River. The results of the bottleneck analysis did not have a positive signal that the Burnt Fork group had undergone a genetic bottleneck and other statistical tests (F_{IS} and Linkage Disequilibrium) also did not provide any cues to suggest the collection was inbred or comprised of only a few family groups.

The factorial correspondence analysis on the adult individuals from the three collection areas identifies no overlap in the distribution of individuals from each of the defined areas based on the polygons (Figure 3). This separation between the groups identifies that the groups are genetically distinct from one another. All of the variation between the adult individuals was distributed on two axes (1 and 2). Analysis of the juvenile collections (Figure 4) was conducted on two collection sites in the N.F. Touchet River drainage (N.F. Touchet River and Wolf Fork) with large sample sizes and one collection from the S.F. Touchet River (Burnt Fork). There was almost complete separation among the collection groups with exception of three individuals from the Wolf Fork that fell within the N.F. Touchet River polygon and one individual from the Burnt Fork that fell within the Wolf Fork polygon. This separation among these three groups identifies that the individuals from each of these collection groups are genetically unique and the variation is distributed along two axes (1 and 2). The results (Figure 4) are more

complicated if Spangler and Lewis Creeks are added to the analysis: 1) Spangler Creek samples show a slight amount of overlap with Lewis Creek, 2) Lewis Creek samples have a substantial overlap with the North Fork Touchet samples, and 3) samples from these two groups add a large component of the variation in the third (z) axis.

Figure 3. Factorial correspondance Analysis conducted with GENETIX showing the distribution of individual migratory adult bull trout from three primary watersheds of the Walla Walla River Basin. All the variation is distributed on axes 1 and 2.

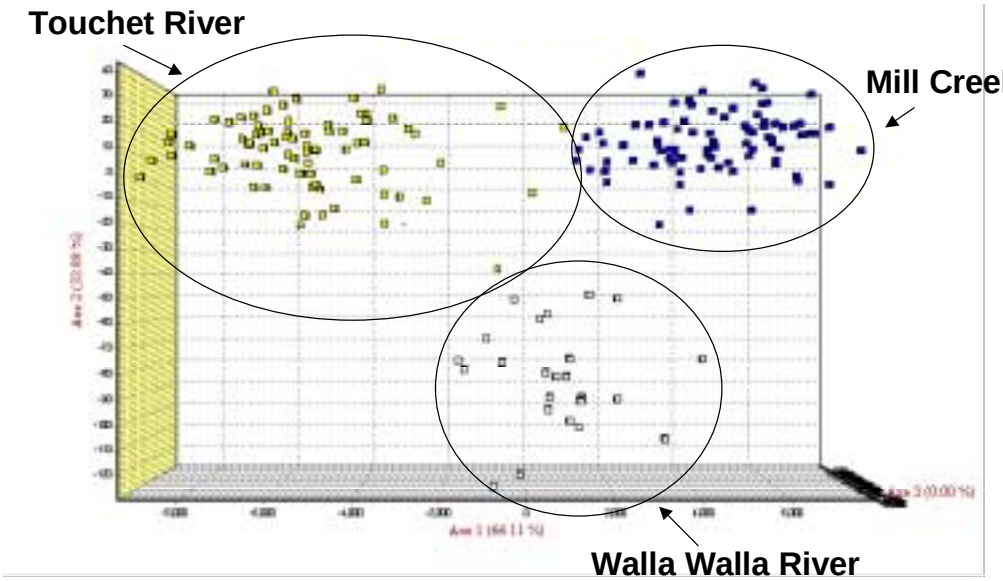
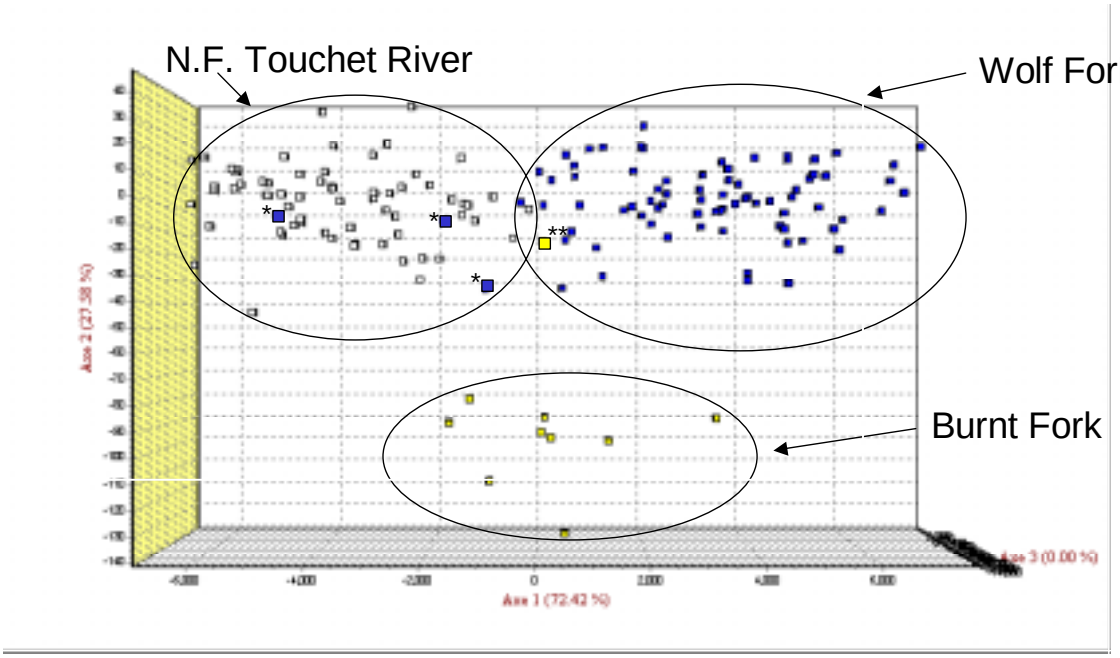


Figure 4. Factorial correspondance Analysis conducted with GENETIX showing the distribution of individual juvenile bull trout from three primary watersheds in the Touchet River drainage. All the variation is distributed on axes 1 and 2.



*Three samples from the Wolf Fork that fall within the N.F. Touchet River polygon
**One individual from Burnt Fork that falls within the Wolf Fork polygon.

Results by Spruell et al. (2003) lumped bull trout from the Columbia River together including samples from Mill Creek, Touchet River, and the Walla Walla River based on microsatellite analysis of only four loci. The results presented here are based on data from a total of 16 loci and therefore provide more information on the genetic differentiation of bull trout populations in the Walla Walla River Basin. An analysis by Homel et al. (unpublished) at Utah State University on collections of bull trout in the Walla Walla River basin did not reveal any genetic differentiation of samples collected from three spawning locations (F_{ST} values were between 0.0003 – 0.0093) or between resident or migratory bull trout. Our limited results of bull trout samples within the Walla Walla River mainstem also do not provide any evidence of genetic differentiation in

spawning collections of bull trout in the Oregon portion of the Walla Walla Basin; however we did not analyze resident or migratory samples. Comparison of the eight loci in common revealed a larger total number of alleles in their analysis and a lower level of overall heterozygosity.

Assignment Tests

Assignment tests were used to assess the reproductive contributions of the bull trout from four spawning areas of the N.F. Touchet River and one location (Burnt Fork) from the S.F. Touchet River drainage. Jackknife assignment tests for juvenile bull trout from five isolated spawning areas provided information regarding the genetic uniqueness of fish from these individual locations and the genetic relationship among them (Table 9).

Table 9. Results of the jackknife analysis for five collection areas where juvenile bull trout spawn in the Touchet River Basin and stock-of-origin assignments for a mixture sample of adult bull trout collected at Dayton Dam Trap on the mainstem Touchet River. Shading indicates correct assignment back to stock-of-origin in the jackknife analysis.

Baseline Juvenile Collections – Counts						
	Burnt Fork	Lewis Creek	Spangler Creek	Wolf Fork	N.F. Touchet R.	Total N
Burnt Fork	7	0	0	2	0	9
Lewis Creek	0	9	2	0	2	13
Spangler Creek	0	0	17	0	2	19
Wolf Fork	1	2	3	71	2	79
N.F. Touchet R.	0	1	1	2	61	65
Touchet River Adults	0	4	4	39	30	77

Baseline Juvenile Collections – Percentages						

	Burnt Fork	Lewis Creek	Spangler Creek	Wolf Fork	N.F. Touchet R.	
Burnt Fork	77.8%	0.0%	0.0%	22.2%	0.0%	
Lewis Creek	0.0%	69.2%	15.4%	0.0%	15.4%	
Spangler Creek	0.0%	0.0%	89.5%	0.0%	10.5%	
Wolf Fork	1.3%	2.5%	3.8%	89.9%	2.5%	
N.F. Touchet R.	0.0%	1.5%	1.5%	3.1%	93.8%	
Touchet River Adults	0.0%	5.2%	5.2%	50.6%	39.0%	

The N.F. Touchet River juveniles had the highest percentage (93.8%) of individuals assign back to the correct stock-of-origin while the Lewis Creek samples had the lowest percentage (69.2%). Mis-assignments of Burnt Fork juveniles were all to the Wolf Fork while mis-assignment of juveniles from the other locations were split among multiple areas. The Wolf Fork juvenile collections had larger sample sizes than from the other locations and therefore would more accurately characterize the genetic variation and result in higher self-assignment. The overall assignment values of 69% or greater identifies that each fish group from a separate spawning location is genetically unique, since a majority of samples assign back to the correct stock-of-origin.

In addition to examining the relationships among the juveniles at isolated spawning areas, assignment tests were performed using the adult bull trout collected at Dayton Trap as unknowns. The results of this analysis reveal the percentage of migratory bull trout adults collected at Dayton Dam (Table 9) from the five baseline sources. The highest percentage of individuals in the analyzed sample was assigned to the Wolf Fork (50.6%) while no samples were identified to be from the Burnt Fork.
Based on enumeration of redds in each of these spawning areas, the Wolf Fork has substantially more spawning bull trout than all other spawning areas in the Touchet

Basin; with the N.F. Touchet River second in abundance (Mendel et al. 2006). Few redds have been documented in each of the other spawning areas.

We were able to compare genetic assignments of six radio tagged adult bull trout captured at the Dayton Dam Trap with their subsequent migrations to spawning areas in 2001 and 2002. Genetic assignments matched telemetry results for four of six individuals (Table 10). Although genetic tests did not assign any of the radio tagged adults to the Burnt Fork, WDFW fish management staff successfully tracked one of these bull trout into the Burnt Fork in 2001 (Mendel et al. 2003b). The individual bull trout that was tagged at Dayton Dam and subsequently migrated to the Burnt Fork was assigned to the N.F. Touchet with the genetic analysis, therefore identifying the stock-of-origin where it likely had been produced. Presence of this fish in the Burnt Fork is either by the fish straying from its original stock-of-origin into the Burnt Fork or the power of the genetic assignment was low and assigned the wrong stock-of-origin.

Table 10. Comparison of stock-of-origin assignments by genetic analysis and radio telemetry results of adult bull trout tracked into tributaries or reaches of the Touchet River drainage. Telemetry results reflect bull trout that were tagged at Dayton Dam Trap and then tracked to spawning areas. Assignments in bold disagreed between the results of the two methods.

Sample	Genetic Assignment	Telemetry Results
03LM 04	Wolf Fork	Wolf Fork
03LM 17	**North Fork**	**Burnt Fork**
03LM 19	North Fork	North Fork
03LM 20	**North Fork**	**Wolf Fork**
03LM 21	Wolf Fork	Wolf Fork
03LM 23	Wolf Fork	Wolf Fork

Conclusions

Evaluation of the genetic analysis to the specific management questions is addressed in the following:

1. Are there significant genetic differences among populations of adult migratory bull trout in the Mill Creek, Walla Walla River, and Touchet River drainages? If so, should bull trout in these areas be managed as separate populations?

- Assessment of migratory adult bull trout from the Walla Walla River Basin consistently identified genetic differences among groups. Results of the tests for genotypic differentiation revealed the individual collections of adults from the Walla Walla, Touchet, and Mill Creek were all significantly different, and the F_{ST} values indicated differences among the collections per location. The neighbor-joining tree supports the genotypic tests, factorial correspondence analysis, and F_{ST} tests by separating the three collection groups with high bootstrap support. All the results from this analysis identify that these three populations of adult bull trout in the Walla Walla River Basin are genetically distinct and should be managed as separate populations.

2. Are there significant genetic differences among juvenile (generally less than 200 mm fork length) bull trout captured during summer from five isolated spawning areas in the Touchet River drainage; and are these juvenile populations different enough to be managed separately?

- Analysis of the combined collections of juveniles revealed that all five populations were highly significantly different from one another with the genotypic differentiation tests. The F_{ST} values indicate the difference between the Burnt Fork samples to the other four collections is between 0.0716 – 0.1127, while the difference among the other four collections within the N.F. Touchet River are lower (between 0.0323 – 0.0602). The difference between the Burnt Fork group

and relationship of the other four groups to each other is not surprising given that the Burnt Fork is geographically isolated as a tributary to the S.F. Touchet River, while the other collections are part of the N.F. Touchet River. The neighbor-joining tree does not separate the juvenile collections in the N.F. Touchet River with any statistical significance or support; however the Burnt Fork group is separated from those groups with 98% bootstrap support. The factorial correspondence analysis of juvenile bull trout collections; however does show strong separation between the N.F. Touchet mainstem, Wolf Fork, and Burnt Fork even though the neighbor-joining tree does not indicate separation with any statistical support. The jackknife analysis of Burnt Fork and Lewis Creek had the lowest assignment power with less than 78% of the juveniles assigning back to the correct stock-of-origin while the remaining three collections assigned over 89% of the juveniles to the correct stock-of-origin. The Burnt Fork (N = 9) and Lewis Creek (N = 13) had the smallest sample sizes and that could contribute to the lower assignment power. Results of the assignment tests for the migratory adults collected at Dayton Dam revealed over 89% of the individual samples were from the Wolf Fork and the N.F. Touchet River. Considering the escapement to each of the five locations (Mendel et al. 2006) this result may is not surprising. The overall results of the genetic analyses determines the five groups can be genetically differentiated, however the small sample sizes for the Lewis Cr., Spangler Cr., and Burnt Fork limits the confidence level of differentiation for these sites. The combined results of multiple statistical tests in this report supports that the N.F. Touchet River mainstem, Wolf Fork, and Burnt Fork (even though the collection had a small sample size) are differentiated and should be managed as separate groups.

3. Provide evidence (if possible) that bull trout in the tributaries of the Walla Walla River Basin have undergone a genetic bottleneck or are inbreeding. Calculate effective population size (N_e) for each group or collection, if possible.

- Analysis to determine relatedness revealed values that suggest the possibility of full sibling pairs in the collection groups. The analysis of linkage disequilibrium and the inbreeding coefficient (F_{IS}), however were low and did not support a conclusion that there were sibling groups in the collections. A more detailed assessment of the individual samples would be required to test for sibling relationships. The rationale for eliminating samples based on sibling relationship within sample groups would have to be considered, however no samples were removed from this analysis for that reason. If samples are randomly collected and determined to be from family groups, but they are contributing to the reproductive output of the population then the genetic identity of those samples should be included in population level analyses because they represent the population. The analysis to determine if the collections have undergone a bottleneck indicates the populations have not undergone any recent reductions in population size and suggests that the populations of bull trout have been small for some time. Evaluation of the effective population size was not conducted due to the small sample sizes for some collections and the lack of temporal samples; however evaluation on the collections with larger samples sizes (e.g. N. Fork Touchet, Wolf Fork, and Mill Creek) should be conducted at a later date.

4. Compare the genetic characteristics and stock structure of bull trout in another Columbia River Basin (Yakima River Basin) with the Walla Walla River Basin to determine genetic relatedness among bull trout in these two basins.

- Adult bull trout that were analyzed from the Yakima River basin and compared to adult bull trout in the Walla Walla River Basin were much more different based on the results of all the statistical tests. The level of genetic variation and differentiation between bull trout in the Walla Walla River Basin and the Yakima River Basin identifies that the separation and isolation of these groups has been longer than the separation of bull trout within the Walla Walla River Basin.

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Appendix 1. Allele frequencies of adult and juvenile bull trout collections in the Walla Walla River and Yakima River Basins at 16 microsatellite loci.

Sco-107

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	79	80	22	27	21	6	12	19	78	66
250	---	---	0.046	---	---	---	---	---	---	---
254	---	---	0.068	---	---	---	---	---	---	---
258	0.015	0.013	0.091	---	---	---	0.083	---	---	0.068
262	---	---	---	---	0.024	---	---	---	---	---
270	0.062	---	---	---	0.238	---	0.083	0.105	0.051	0.061
274	0.039	---	---	---	0.024	---	---	0.158	0.013	---
278	0.069	---	0.023	0.407	0.286	---	---	0.026	0.026	0.030
282	0.208	0.213	0.500	0.222	0.167	0.083	0.167	0.132	0.141	0.379
286	0.269	0.275	0.136	---	0.048	0.250	0.292	0.158	0.212	0.167
290	0.123	0.069	---	---	0.143	---	---	0.053	0.141	0.061
294	0.162	0.363	0.091	0.370	0.024	0.500	0.250	0.290	0.359	0.159
298	0.054	0.069	0.046	---	---	0.167	0.125	0.079	0.045	0.076
302	---	---	---	---	---	---	---	---	0.013	---
314	---	---	---	---	0.048	---	---	---	---	---

Sco-109

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	87	58	21	28	21	9	13	19	78	66
257	0.014	---	---	---	---	---	---	---	---	0.023
261	0.027	---	---	---	---	---	---	---	0.051	---
265	0.069	---	0.048	---	---	---	0.154	---	0.128	0.015
269	0.103	---	0.214	---	---	0.056	0.077	0.184	0.141	0.076
273	---	---	---	---	---	---	---	0.026	---	---
290	---	0.026	---	---	---	---	---	---	---	0.008
297	0.322	0.293	0.357	---	0.024	0.500	0.039	0.395	0.295	0.349
302	0.082	0.043	---	---	0.214	0.056	0.154	0.079	0.064	0.114
306	0.034	0.078	---	---	---	0.222	0.039	---	0.013	0.091
310	0.014	0.345	---	---	0.024	---	---	---	0.058	---
314	---	0.086	0.048	---	0.429	---	---	---	---	---
315	---	---	---	---	---	---	---	---	0.006	---
318	0.075	---	---	---	0.143	---	---	0.026	0.051	0.076
322	---	---	---	0.054	0.024	---	---	---	---	---
326	---	---	---	---	0.095	---	---	---	---	---
331	---	---	---	0.036	0.024	---	---	---	---	---
335	---	---	---	0.054	---	---	---	---	---	---
351	0.041	---	0.024	0.464	0.024	---	0.115	---	0.045	0.068
355	---	0.043	---	---	---	---	0.039	0.026	---	---
359	0.014	---	---	---	---	---	---	---	---	0.023

363	0.041	---	---	0.107	---	---	0.154	0.158	0.006	0.038
367	---	0.017	---	---	---	---	---	---	---	---
375	---	0.060	---	---	---	---	0.154	---	---	---
382	0.137	0.009	0.191	0.018	---	0.056	0.039	0.053	0.128	0.046
386	0.027	---	0.119	0.125	---	0.111	0.039	---	0.006	0.076
390	---	---	---	---	---	---	---	0.053	---	---
394	---	---	---	0.036	---	---	---	---	0.006	---
398	---	---	---	0.089	---	---	---	---	---	---
402	---	---	---	0.018	---	---	---	---	---	---

Sco-106

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	91	82	23	29	22	9	13	19	79	66
132	---	0.085	0.130	---	---	---	---	---	---	---
136	0.040	---	---	---	---	---	0.192	0.079	0.025	---
169	0.204	---	---	---	---	0.056	0.346	0.105	0.171	0.394
173	0.007	---	0.065	---	---	---	---	---	---	---
178	0.145	0.445	0.109	0.138	0.068	0.111	---	0.211	0.114	0.083
181	0.040	0.073	0.087	---	0.136	0.444	0.154	0.079	0.032	0.106
185	0.059	0.085	---	0.466	---	0.222	---	0.211	0.146	0.182
193	---	---	---	---	0.023	---	---	---	---	---
197	0.099	---	---	---	0.159	---	0.039	---	0.082	0.068
201	---	0.024	---	0.345	0.091	---	---	---	---	---
205	---	0.024	0.044	---	---	---	---	---	0.006	---
209	0.388	0.256	0.391	---	---	0.167	0.269	0.184	0.380	0.167
213	0.020	0.006	0.174	---	---	---	---	0.132	0.044	---
229	---	---	---	---	0.091	---	---	---	---	---
233	---	---	---	---	0.091	---	---	---	---	---
237	---	---	---	0.052	0.318	---	---	---	---	---
241	---	---	---	---	0.023	---	---	---	---	---

Sfo-18

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	89	79	23	29	22	9	13	19	80	66
178	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Smm-22

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	65	77	23	27	22	7	12	19	77	65
221	---	---	---	---	0.341	---	---	---	---	---
225	---	---	---	---	0.023	---	---	---	---	---
229	---	---	---	---	0.114	---	---	---	---	---
232	---	---	0.022	0.333	---	---	---	---	---	---
236	0.241	0.013	0.044	---	0.114	0.071	---	0.290	0.338	0.139

240	0.045	---	---	---	0.091	---	---	---	0.052	0.015
244	0.071	0.162	0.022	---	---	0.143	0.042	0.026	0.013	---
248	---	0.007	0.087	---	---	---	0.083	---	---	---
252	0.214	0.188	0.239	---	---	0.500	0.333	0.211	0.110	0.377
256	0.018	0.097	0.174	0.056	---	---	0.083	0.026	0.013	0.054
260	0.018	0.039	0.087	---	---	0.286	---	---	0.052	---
264	0.107	0.033	0.022	0.037	0.091	---	---	---	0.208	0.015
268	0.152	0.039	0.044	---	---	---	0.125	0.237	0.065	0.208
272	0.089	0.162	0.044	0.056	0.046	---	---	0.132	0.078	0.131
276	---	0.110	0.022	0.093	---	---	---	0.053	0.013	0.015
280	0.009	0.013	0.022	0.148	0.136	---	---	---	0.007	---
284	---	---	---	0.093	0.023	---	---	---	0.007	---
288	0.036	---	---	---	---	---	0.208	0.026	0.020	0.046
292	---	0.039	0.065	---	0.023	---	---	---	---	---
296	---	0.007	0.044	---	---	---	---	---	---	---
300	---	---	0.022	---	---	---	---	---	0.007	---
305	---	0.065	---	---	---	---	---	---	---	---
308	---	0.026	0.044	---	---	---	0.125	---	0.020	---
325	---	---	---	0.167	---	---	---	---	---	---
333	---	---	---	0.019	---	---	---	---	---	---

Omm-1130

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	56	81	18	27	22	8	9	19	74	64
263	---	---	---	---	---	---	---	0.053	---	---
267	---	---	---	0.037	0.023	---	---	---	---	---
271	---	0.025	---	0.130	0.318	---	---	---	---	---
275	0.245	0.068	---	0.093	0.250	0.625	0.111	0.053	0.223	0.156
279	0.102	0.191	0.361	0.037	0.023	0.063	0.389	0.132	0.108	0.273
283	0.010	---	---	---	0.023	---	0.167	---	---	0.016
287	0.061	0.235	0.250	---	0.364	---	0.111	0.184	0.088	0.234
291	0.122	0.179	0.056	---	---	0.125	---	0.079	0.169	---
295	0.133	0.130	---	0.148	---	---	---	0.026	0.074	0.016
299	0.010	0.006	---	---	---	---	---	---	0.014	---
311	---	---	---	0.426	---	---	---	---	---	---
323	---	---	---	0.093	---	---	---	---	---	---
328	---	---	0.278	---	---	---	---	0.079	---	---
332	0.153	0.012	0.056	0.037	---	---	0.056	0.158	0.203	0.039
336	0.163	0.148	---	---	---	0.188	0.167	0.237	0.122	0.266
340	---	0.006	---	---	---	---	---	---	---	---

Sco-102

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	67	82	23	27	22	9	13	19	80	66
166	---	---	---	0.167	0.250	---	---	---	---	---

170	0.947	1.000	1.000	0.685	0.568	0.667	1.000	1.000	0.875	0.947
174	0.053	---	---	0.037	0.159	0.333	---	---	0.125	0.053
182	---	---	---	0.111	0.023	---	---	---	---	---

Sco-212

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	90	83	23	29	22	9	12	19	80	66
272	0.100	0.084	0.065	---	0.296	0.222	0.042	0.026	0.144	0.136
280	---	0.012	---	---	---	---	---	---	---	---
284	---	0.199	---	---	---	---	---	---	---	---
288	0.240	0.084	0.370	0.172	0.068	0.056	0.333	0.316	0.131	0.333
292	---	---	---	---	0.318	---	---	---	---	---
302	---	0.102	---	---	---	---	---	---	---	---
324	0.580	0.494	0.522	0.052	0.159	0.722	0.292	0.500	0.656	0.500
328	0.067	---	0.044	---	0.023	---	0.333	0.158	0.050	0.030
332	0.013	0.024	---	---	0.046	---	---	---	0.019	---
336	---	---	---	0.707	0.091	---	---	---	---	---
351	---	---	---	0.069	---	---	---	---	---	---

Omm-1128

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	89	78	23	29	22	7	12	19	79	66
267	---	---	---	---	---	---	---	---	0.006	---
271	0.007	---	---	---	---	---	---	0.053	0.063	---
275	0.196	---	0.022	0.017	---	0.286	0.250	0.079	0.177	0.288
279	0.487	0.378	0.283	0.207	---	0.500	0.417	0.500	0.411	0.621
283	0.007	0.019	0.109	0.362	---	---	---	0.079	0.006	0.015
287	---	---	---	0.035	---	---	---	---	---	---
291	---	---	---	---	0.159	---	---	---	---	---
337	---	0.026	---	0.017	---	---	---	---	---	---
341	0.095	0.295	0.348	0.328	0.046	0.214	0.083	---	0.165	---
345	0.196	0.263	0.065	0.035	0.409	---	0.250	0.263	0.171	0.046
349	0.014	0.019	0.044	---	0.386	---	---	0.026	---	0.030
353	---	---	0.130	---	---	---	---	---	---	---

Sco-105

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	88	79	23	29	22	7	13	19	79	66
139	---	---	---	---	0.114	---	---	---	---	---
156	---	---	---	0.328	0.432	---	---	0.026	---	0.008
160	0.048	---	---	---	---	---	0.039	0.105	0.019	0.099
164	0.206	0.399	0.261	0.672	---	0.643	0.192	0.237	0.234	0.258
168	0.233	0.184	0.239	---	---	0.286	0.231	0.263	0.354	0.159
172	0.151	0.222	0.304	---	---	---	0.231	0.105	0.139	0.046

176	---	0.006	---	---	---	---	---	---	---	---
188	---	---	---	---	0.318	---	---	---	---	---
200	---	---	0.022	---	0.091	---	---	---	---	---
204	---	---	0.044	---	---	---	---	---	---	---
208	0.363	0.184	0.130	---	0.046	0.071	0.308	0.263	0.253	0.432
212	---	0.006	---	---	---	---	---	---	---	---

Sco-200

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	89	80	23	29	22	9	13	19	80	66
151	---	---	---	---	0.114	---	---	---	---	---
155	---	---	---	0.017	0.364	---	---	---	---	---
159	---	---	---	---	0.341	---	---	---	---	---
163	0.047	0.594	0.239	0.103	0.023	---	0.077	0.395	0.075	0.046
167	---	---	0.065	0.586	---	---	---	---	---	---
171	0.014	---	0.065	---	---	---	---	---	---	---
175	0.446	0.206	0.283	0.293	0.159	0.611	0.346	0.316	0.388	0.508
179	0.291	0.125	0.174	---	---	0.278	0.385	0.132	0.200	0.341
183	0.203	0.075	0.174	---	---	0.111	0.192	0.158	0.331	0.106
187	---	---	---	---	---	---	---	---	0.006	---

Sco-202

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	91	81	23	29	22	9	13	19	80	66
152	---	---	---	0.259	0.227	---	---	---	---	---
156	0.559	0.210	0.391	---	0.546	0.333	0.731	0.553	0.650	0.765
160	0.171	0.265	0.261	0.397	0.068	0.611	0.115	0.053	0.138	0.099
164	0.263	0.525	0.348	0.345	0.159	0.056	0.154	0.395	0.206	0.136
169	0.007	---	---	---	---	---	---	---	0.006	---

Sco-218

	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	84	73	21	29	22	7	12	19	79	65
233	0.015	---	---	---	---	---	---	---	0.006	0.008
237	---	---	---	---	---	---	---	---	0.006	---
241	0.544	0.171	0.405	---	---	0.571	0.625	0.395	0.582	0.523
245	0.094	0.007	0.095	---	---	---	---	0.053	---	0.031
249	0.130	0.048	---	---	0.023	0.286	0.167	0.053	0.101	0.208
253	0.015	0.007	---	---	---	0.071	---	0.026	0.038	0.085
257	0.073	---	---	0.379	---	---	---	0.184	0.146	0.069
261	0.007	0.007	---	---	0.091	---	---	---	0.013	0.008
265	0.094	0.473	0.214	0.328	0.068	0.071	0.208	0.263	0.108	0.069
269	0.029	0.110	0.214	0.103	0.068	---	---	0.026	---	---
273	---	---	---	0.172	0.068	---	---	---	---	---

278	---	---	---	---	0.250	---	---	---	---	---
282	---	---	---	---	0.023	---	---	---	---	---
285	---	0.116	0.024	---	0.227	---	---	---	---	---
289	---	0.027	0.048	0.017	0.136	---	---	---	---	---
294	---	---	---	---	0.046	---	---	---	---	---
297	---	0.034	---	---	---	---	---	---	---	---

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	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	90	77	22	29	22	7	12	19	78	66
316	---	---	---	---	0.068	---	---	---	---	---
320	---	---	---	---	0.091	---	---	---	---	---
324	---	---	---	0.259	0.182	---	---	---	---	---
328	---	---	---	---	0.091	---	---	---	---	---
332	0.193	0.156	---	---	0.091	0.071	---	0.132	0.115	0.167
336	0.280	0.169	0.227	---	0.023	0.214	0.375	0.395	0.147	0.553
340	0.187	0.007	---	0.190	---	0.357	0.292	0.395	0.231	0.136
344	0.267	0.494	0.773	0.328	---	0.357	0.083	0.079	0.449	0.106
348	---	0.007	---	0.224	---	---	---	---	0.006	---
352	0.073	0.007	---	---	---	---	0.250	---	0.051	0.038
356	---	0.162	---	---	0.091	---	---	---	---	---
379	---	---	---	---	0.023	---	---	---	---	---
402	---	---	---	---	0.273	---	---	---	---	---
406	---	---	---	---	0.068	---	---	---	---	---

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	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	80	83	23	29	21	8	12	19	80	66
317	0.985	0.958	1.000	1.000	1.000	1.000	0.833	1.000	0.981	0.939
321	0.015	0.042	---	---	---	---	0.167	---	0.019	0.061

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	Touchet R. Adults	Mill Cr. Adults	Walla Walla R. Adults	Ahtanum Cr. Adults	Naches R. Adults	Burnt Fork Juveniles	Lewis Cr. Juveniles	Spangler Cr. Juveniles	Wolf Fork Juveniles	N.F. Touchet R. Juveniles
Size/N =	91	81	21	29	22	9	13	19	80	66
263	---	---	---	---	0.227	---	---	---	---	---
267	0.322	0.309	0.357	0.414	0.091	0.056	0.039	0.079	0.256	0.121
271	0.329	0.642	0.619	0.500	0.114	0.722	0.577	0.684	0.431	0.409
275	0.290	0.006	---	---	---	0.167	0.385	0.237	0.238	0.424
279	0.046	---	---	---	0.227	---	---	---	0.006	---
291	0.013	0.043	0.024	0.086	0.205	0.056	---	---	0.069	0.046
295	---	---	---	---	0.136	---	---	---	---	---