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TECHNICAL REPORT 11-02

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2010 Annual Report Genetic Assessment of Columbia River Stocks

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February 16, 2011

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GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS

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Prepared for:
U.S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife
P.O. Box 3621
Portland, OR 97283-3621
Project Number: 2008-907-00
Contract Number: 41224
Reporting Period: 3/1/10-3/31/11
Submitted: February 16, 2011

ABSTRACT
This project combines four inter-related studies from the Fish & Wildlife Program Accords that address these current and future objectives: 1) discover and evaluate SNP markers in salmon and steelhead; 2) expand and create genetic baselines for multiple species (Chinook, steelhead, sockeye, and coho); 3) implement Genetic Stock Identification (GSI) programs for mainstem Chinook fisheries and 4) GSI of fish passing Bonneville Dam (steelhead and Chinook). In the second year of this project, SNP discovery goals (Objective 1) were achieved with successful development of 22 new assays for Chinook salmon and 24 new assays for steelhead. These newly discovered SNP markers will be combined with existing SNP markers to generate genetic baselines and for two applications of genetic stock identification (GSI). For genetic baseline expansion (Objective 2), we successfully genotyped 96 SNP markers in 32 new Chinook salmon collections and 192 SNP markers in 61 steelhead collections from the Columbia River Basin. Results from population genetics analyses suggest SNPs are a class of markers that perform well for distinguishing populations, and these baselines will be useful for estimating stock composition in GSI applications. Results also indicated that some loci may be candidate markers and valuable for analyses based on selective divergence. The second year of the project included two broad applications of GSI, stock composition of Chinook salmon fisheries (Objective 3), and stock composition of Chinook salmon and steelhead passing Bonneville Dam (Objective 4). Results of Objective 3 indicate that spring-run Chinook salmon harvested in the 2010 commercial, sport, and test fisheries were primarily composed of three adipose-clipped stocks (in descending order of stock composition): Rapid River Hatchery/Clearwater R., Upper Columbia R. (i.e., Carson stock), and Mid-Columbia R. These three Chinook salmon stocks were also the most strongly represented at Bonneville Dam. A fourth spring-run stock, Williamette R., was found primarily in harvests spanning an earlier part of the season and locations closer to the mouth of the Columbia R. For fall Chinook fisheries, the sport and commercial fisheries below Bonneville Dam contained large proportions of Lower Columbia fall stocks (20-55% composition), as well as the following stocks (in descending order): upper Columbia R. summer/fall, Snake R. fall, and Deschutes R. fall. The entire Zone 6 tribal Chinook fishery was heavily comprised of Upper Columbia R. summer/fall stock (30-70% depending on region), but Region 1 (closest region to Bonneville Dam) of Zone 6 contained far greater proportion of Lower Columbia R. fall stock (~54%) than Region 2 (< 6%), whereas Snake R. and Deschutes R. fall stocks were similar in both regions (9-20% and ~3%, respectively). Validation of the GSI results using coded wire tag information indicated that the percentage of individuals correctly assigned to a particular reporting group was on average 80% and ranged from greater than 90% (Willamette R. spring-run, Upper Columbia R. summer/fall, and lower Columbia R. fall stocks) to a low of 57% (Snake R. fall-run stock). For Objective 4, we calculated estimates of the run-timing distributions and abundance (units in 1000's of fish) of the "major" stocks of Chinook salmon sampled as they migrated past Bonneville Dam in 2010, and these estimates are listed in order of median day of peak run-timing as follows: Upper Columbia R. (May 1st, 102k), Rapid R./Clearwater R. (May 1st, 127k), middle Columbia R. spring-run (May 6th, 29k), Upper Salmon R. (June 6th, 23k), and South Fork Salmon R. (June 13th,16k), Snake R. fall (Sep 5th, 113k), lower Columbia R. fall (Sep. 7th, 59k), Upper Columbia R. summer/fall (Sep. 8th, 376k), and Deschutes R.

fall (Sep. 11th, 28k). Future work will include the newly developed SNP baseline of steelhead populations to analyze recent years 2009-2010 of steelhead samples in a similar way.

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Acknowledgements

The authors of this report would like to thank the following individuals and organizations for providing time and expertise toward this project: The Bonneville Power Administration for providing funding for this research project and to its staff for their assistance. Tissue samples were collected and provided by William Ardren, Bill Arnsberg, Bobby Begay, Steve Boe, Chris Brun, Peter Cleary, Roger Dick Jr., Roger Dick Sr., Dani Evenson, Chris Kern, James Kiona, Paul Kuneki, Jacinda Mainord, Alfred McConnville, Jim Nagler, Winfred Perez, Terra Schultz, Jason Vogel, Devin Volenec, John Whiteaker, Marc Whitman, Bill Young, and Joe Zendt. Collection of samples from the commercial, sport, and test fishery were coordinated with Joe Hymer. We are grateful for extensive contributions in the genetics laboratory from Stephanie Harmon, Vanessa Jacobson, Amanda Matala, Lori Maxwell, Megan Moore, and Jeff Stephenson.

Introduction

This project combines four inter-related studies from the Fish & Wildlife Program Accords that address these current and future objectives: 1) discover and evaluate SNP markers in salmon and steelhead; 2) expand and create genetic baselines for multiple species (Chinook, steelhead, sockeye, and coho); 3) implement Genetic Stock Identification (GSI) programs for mainstem Chinook fisheries and 4) GSI of fish passing Bonneville Dam (steelhead and Chinook). These four projects are highly related since SNP markers are needed to complete species specific baselines, and these baselines are requisite to complete GSI. The results of these four objectives address needs for distinguishing specific stocks, determining genetic diversity, stock specific run timing, and estimating stock composition to provide information for fisheries management and harvest.

Objective 1) SNP Discovery

One of the highest priorities in the full-scale implementation of SNPs for salmon genetics is the discovery and development of a sufficient number of these markers to characterize population variability. These polymorphisms represent the most abundant variation in the genome of most organisms, and are spread throughout the entire genome at high density (Morin et al. 2004). Thus SNPs can be discovered through sequencing known regions of DNA and converted to high throughput assays (e.g., Campbell and Narum 2008a). Further, mutation rates, mutation models and error rates for SNPs are generally well understood, providing a foundation for estimating genetic divergence between populations. SNP markers also offer the potential of a more cost-effective and less error-prone alternative to existing genetic tools that may be used independently or in tandem with existing microsatellite markers to improve accuracy and precision of stock assignments. The combined power of these two marker types is expected to improve stock composition accuracy (Narum et al. 2008a) and allow researchers to meet rigorous stock composition and assessment needs for timely management of fisheries.

Objective 2) Baseline Expansion

Currently, genetic baselines of microsatellite markers are in place for Chinook salmon across the coastwide range (Seeb et al. 2007) and steelhead in the interior Columbia River Basin. Despite large, representative sample sizes from many populations and very high microsatellite allelic diversity, the resolution of specific stocks and populations in these baselines is limited in some cases. For example, fall Chinook salmon in the Columbia River are closely related and remain difficult to distinguish even with a powerful set of 13 microsatellite markers. Several other closely related populations in the Chinook salmon baseline are similarly difficult to distinguish and thus have been pooled into a single reporting unit for GSI applications. In some cases (i.e., mainstem Columbia R. Chinook fisheries), a finer level of stock discrimination is necessary for management of fisheries. Additional SNP loci will increase stock assignment reliability where greater resolution is required. Given the difficulty and expense of inter-laboratory standardization, additional microsatellite markers may not the most efficient choice. In this regard, SNP markers are the preferred option for additional loci since they offer many beneficial characteristics that make them amenable to adding loci to existing baselines.

Objectives 3 &4) Genetic Stock Identification

Genetic Stock Identification (GSI) methods have proven to be effective in determining the proportion of stock origin in several mixed stock applications (Shaklee et al. 1999, Beacham et al. 2006, Narum et al. 2008b). This proposal includes two GSI projects that will utilize genetic baselines: 1) GSI to Evaluate Catch; and 2) GSI of fish passing Bonneville Dam.

This study will include GSI analysis of Chinook salmon collected from commercial, recreational, and tribal fisheries in the Columbia River. (Subsequent years of the study will include steelhead and coho fisheries as possible.) Implementation of GSI technology could make monitoring individual production units in mixed stock areas possible. Tissues will be sampled annually from fisheries with existing programs in place with Washington Department of Fish and Wildlife (WDFW) and Oregon Department of Fish and Wildlife (ODFW). We plan to genotype representative samples from fisheries of primary interest. The GSI estimates may help refine CWT based estimates of stock composition used in fishery management.

The second application of GSI analysis in this proposal includes sampling unknown origin salmon and steelhead at Bonneville Dam for genetic analysis. Samples will be collected over the entire length of the run on a weekly basis, and genetic baselines will be utilized to determine the stock composition of these runs. Few studies have been able to determine the extent of overlap among life history types of salmon and steelhead, but GSI of each life history type will allow us to determine the stock composition of the different runs through Bonneville Dam with greater accuracy than current methods. Population genetic methods and statistical assignment models have advanced dramatically in recent years, and estimating stock composition is now possible using either Bayesian or Maximum Likelihood methods (Anderson et al. 2008). Therefore, we plan to estimate stock composition of multiple species passing Bonneville Dam and provide this information on a timely basis to fisheries managers.

Report Structure

This report is divided into four sections, one for each of the objectives of the study. The first section reports on SNP discovery efforts and the second section on genotyping SNP markers in Chinook salmon and steelhead to create genetic baselines. The third section contains GSI estimates of stock composition of Chinook salmon sampled in mainstem fisheries compared to stock composition of adults migrating over Bonneville Dam in 2010. The fourth section includes analysis of run-timing distributions and estimated abundance of adult Chinook salmon stocks migrating over Bonneville Dam in 2010.

Section 1: SNP Discovery

Introduction

Conservation genetics projects such as genetic stock identification (GSI), reproductive success, and selection studies require the use of an informative suite of genetic markers. Although many types of markers have been used historically, single nucleotide polymorphisms (SNPs) are the most common type of variation in the genome, genotyping assays can be designed for a number of high throughput platforms, and the resulting genetic information can be easily exchanged between collaborating laboratories (Melton, 2003). This section describes the sequencing and identification of SNPs in two species of Pacific salmonids, steelhead (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*), and the development of genotyping assays. Target goals for this objective were to produce 10 informative assays for *O. mykiss* and 15 for *O. tshawytscha*.

Methods

DNA sequences from *O. mykiss* and *O. tshawytscha* were collected from online database entries in TIGR and NCBI for primer design. Primers were designed using either the embedded primer design tool on NCBI's database or the primer3 program (http://frodo.wi.mit.edu/primer3/) to amplify a PCR product of 400 to 800 base pairs in length (Table 1). All primer pairs (N = 80) were tested for amplification of a PCR product suitable for sequencing using 2uL of extract DNA from 2 individuals per species. PCR conditions for testing were 1x Qiagen multiplex master mix, 250nM primers, and 1-2uL of extract DNA in a 10uL reaction. PCR were done in 96-well PCR plates and thermal cycling conditions were [95ºC for 15 min; (95ºC for 30 sec, 60ºC for 30 sec, 72ºC for 45 sec, repeated 40x); 4ºC hold]. Amplified products separated on a 1% agarose gel and stained with Sybr-green. Products were visualized using a UV light source and images were captured on a CCD camera. Primer pairs that produced suitable products were used to amplify products from an ascertainment panel of 32 individuals per species. Individuals chosen for ascertainment panel were from across each species' range but with the greatest representation within the Columbia River drainage (Table 2).

For amplification of the ascertainment samples, primers were multiplexed such that each reaction produced four products. PCR and cycling conditions remained the same as in the test. PCR products were cleaned by treatment with Exonuclease 1 and Shrimp alkaline phosphatase (New England Biolabs) to remove unincorporated dNTPs and primers. Sequencing reactions were performed in one direction using the Big Dye v.1.1 sequencing kit (Applied Biosystems) and MJ research and Applied Biosystems thermal cyclers using 2uL of each product. The reactions were cleaned up by ethanol-EDTA-NaAcetate precipitation prior to separation by capillary electrophoresis using a 3730 instrument (Applied Biosystems). Chromatogram data was analyzed by using the Sequencing Analysis program v.5.4 (Applied Biosystems) to assign base calls followed by Sequencher v.4.7 (Gene Codes) to align and edit the data. Observed SNPs and small indels were recorded and minor allele frequencies calculated for each.

Suitable SNP sites were used to design Taqman™ assays by submitting to Applied Biosystems via their FILE BUILDER program. Once received, the assays were used for genotyping using the Fluidigm EP-1 96.96 system using the manufacturer's suggested protocol, but modified slightly by including a sample pre-amplification step and increasing the PCR cycles to 50. The assays were validated by comparison of genotyping data to sequencing data in the ascertainment samples. Assays were further evaluated by genotyping one or more populations within the Columbia River drainage to examine allele frequencies, deviations from Hardy-Weinberg expectations, and linkage disequilibrium using the program GENEPOP (Raymond and Rousset 1995).

Results

For *O. mykiss*, 52 loci were amplified and sequenced, producing 48 sequence alignments totaling 12,008 unique consensus bases with an average contig length of approximately 364 base pairs. 264 total SNP sites were identified and the number of SNPs per locus ranged from 1 to 22. Based on minor allele frequency and distribution in the ascertainment panel, 30 SNP sites were chosen for assay design. The 30 assays were used to genotype the ascertainment samples as well as 188 additional samples representing 7 regions (lower, middle, and upper Columbia R., S.E. Alaska, Snake R., McCloud hatchery strain, and a mixture of steelhead taken from Bonneville Dam 2004). Twenty-four of the assays were validated by comparison of genotyping data to sequencing data and 20 of the validated assays showed a high occurrence of the minor allele in Columbia River populations. The 24 validated assays are listed in Table 3. Assays were further tested by genotyping individuals from 6 hatcheries in the Snake River (N = 16 individuals per hatchery) and testing for deviations from Hardy-Weinberg expectations. The genotypes showed no significant linkage with one another and no significant deviations from Hardy-Weinberg expectations were observed.

In *O. tshawytscha*, 67 loci were amplified and sequenced producing 42 sequence alignments totaling 14,217 unique consensus bases with an average contig length of approximately 365 base pairs. 118 total SNP sites were identified in the screen and 28 were chosen for development into genotyping assays based on minor allele frequency and distribution within the ascertainment panel. Twenty-two of the assays were validated by concordance to sequence data (Table 3). Assays were further evaluated by genotyping individuals from three populations in the Columbia River (N = 32 individuals per population) and testing for deviations from Hardy-Weinberg expectations (HWE). Sixteen of the assays showed a high occurrence of the minor allele in the tested populations and none showed significant deviations from HWE.

Discussion

Initial analysis of the validated assays showed that for each species the target number of informative assays was achieved. However, further testing will be required to determine which assays are the most effective for differentiation of Columbia River stocks and allow us to compare them to assays developed by others. Ultimately, these assays will be

used to generate genotypes from a larger set of collections and rank the most informative markers with descriptive statistics such as F_{ST} and locus ranking programs (e.g., BELS; Bromaghin 2008). Some of the assays that showed very low minor allele frequencies in the populations tested to this point may show much higher frequencies in other populations and prove to be the most informative. Similarly, some of the least informative assays for the Columbia River may work well in other parts of the species' range. All functional assays will be made available by publication in peer reviewed articles. These newly discovered SNP markers were combined with existing SNP markers to generate genetic baselines and for two applications of genetic stock identification (GSI). These markers may also be useful for other applications such as pedigree studies for estimating reproductive success, and evaluating adaptive divergence of populations to specific environments.

References

Bromaghin, J.F. 2008. BELS: backward elimination locus selection for studies of mixture composition or individual assignment. Molecular Ecology Resources 8:568-571.

Melton, L. 2003. Pharmacogenetics and Genotyping: On the trail of SNPs. Nature 422;917-923.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86;248–249.

Table 1: List of targets and primer sequences used for PCR amplification and sequencing.

	Target name:	Abbreviation:	Acc#:	Forward	Reverse
1	peptidylarginine deiminase	pad	FM999001	CTGGGACTCAGCTGCACATA	TGGATTACCTCCAGTGGCTC
2	succinate-CoA ligase	suCoA	FJ607869	AGAGAAGATTGCCGCCCTAC	AGCCAGTGAGAACAGACATTTT
3	nucleoside diphosphate kinase	ndk	FJ607868	AGGAGACACCAACCCATCTG	AAATGACAGGTCAAGGGCAG
4	thymidine kinase	thyK	FJ607866	GGTGCAGGAAGTGTTACGGT	TGATGAGGCAAATCAGACCA
5	C type lectin receptor B	clrB	FJ607865	ACTGGAGATGGGTGGACAAC	GGTGACTGATTGGCAGCTTT
6	branched-chain alpha-keto acid lipoamide acyltransferase	bcAKala	NM_001124203	AGACCACCGGATCATCGAC	TGCAATGGTGCTTTCCATAG
7	carbonic anhydrase 1	carban1	NM_001124220	CAGGATCACGGCAGCTTAAT	GGTTGATTCTTCCTTGGCTG
8	dehydrodolichyl diphosphate synthase	dds	FM212438	TCTGGAATCTGTGTGATGCC	TAGCCCCATAGGGGTAAAAT
9	NILT4 leukocyte receptor precursor	NILT4	FM180058	TGGTTACTATGCCACCGTCA	GGGGCTGATTAGCATGTTGT
10	signal sequence receptor delta	ssrd	FJ591155	ATCCAGCCCCTTTTCTCTGT	CATTGGGATCGATTGAGATGT
11	programmed cell death 6	pcd6	FJ591154	AACTGTGCCAAAGCCAAATC	CAGTTTAAGGCATCATGCACA
12	GHS-R gene for growth hormone secretagogue receptor	GHS-R	AB479381	AAAGGCACAGAATTGGAGACA	TCGGAGGAATGGGACATAAG
13	brain creatine kinase b	bckB	FJ548753	TTCTCTGAGGTGGAGCTGGT	CAATGGGGCAAAGAGACTTTTA
14	glucocorticoid receptor	gluR	EU084718	CACCGGCTCTTTTCTTTCTG	TGGATGACAGCTTTGCACAT
15	gadd45 beta ii	gadd45b2	EU084728	GTTACAGGACGTGGGCAACT	CACGTCGGATAGCATAGGGT
16	Tumor protein p53	p53	NM_001124692	TTCGGTTCCATGTCATTTCA	CAAGCATCAACAGTTCACCG
17	metallothionein B gene	metB	M22487	TTTTATCGATGATCAACGTGGT	TTTAACGTGCCACCAAGTCA
18	metallothionein A gene	metA	BV725496	GGATCCTTGTGAATGCTCCA	GCTGGTATCACAAGTCTTGCC
19	defender against cell death 1	dacd1	FJ849061	ATGTGTTGGAGCCTTCATCC	TCAAGTGGTTTGCAACAATCA
20	Vesicle-trafficking protein SEC22b-B	SEC22b	BT074367	AGAGGAGATAGGGGAGGGGT	TGTTATTAACGGCAAAGCCC
21	Ictacalcin	icta	BT074357	AAACACTGACCAGGCAAAGG	ACCAAAGAGCTTCCTCTCCA
22	SUMO-1-conjugating enzyme UBC9	SUMO1	BT074321	CGAGCACAAGCCAAAAAGTT	TTGAATGGGTTCATTGAGGC
23	insulin-like growth factor II	igf2	NM_001124697	GGACAACTACGTCAGCCACA	CCGCTAAGGATCCACCTAAA
24	thyrotropin beta-subunit	tshb	NM_001124543	GATAACGCTCTCCCTTCCCT	TCCCATTCATGATATTGGTTCA
25	Ntl T-box protein	ntl	GQ241688	CTCCTCAACCACCATCCTGT	ACATAAGGATTGGCCCCTTT
26	Pax7 gene	pax7	FJ713022	CCCATGGTAATGTGGGTAGC	TGATAACGTCTGCTTGCTGG
27	archain 1	arcn1	FM993912	CACCTGCAAGAAGGAATGGT	TTTCTTTGAATGACCTCCCG
28	adenine nucleotide translocator 2	ant2	NM_001160491	TGGTACCATTGACTGCTGGA	CATACCCACTGGAGGACAGG
29	perforin	prf1	NM_001134847	GAGATTTGAGGTGTGGGACG	GATCTGGGGTTTTGACATGG
30	atrial natriuretic peptide	anp	NM_001124211	ATTTCAGCCTGTTTTGGTGG	AGTCTTGCACATCACCAACG

31	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit	dad1	NM_001160576	ATATCATGTGTTGGCGCCTT	TCGGATTGCAACAATCATACA
32	Peptidyl-prolyl cis-trans isomerase E	ppie	NM_001160524	TGCAGAATTGTTAAAAGGGCA	TGGGATCTTTTATTTCAGCTCAT
33	Type-4 ice-structuring protein precursor (afp4)	afp4	FN396363	GCTGATGGACCAGACCAAGT	TTGCATGTCAACAACATTCG
34	apolipoprotein A-II	apoa2	NM_001161448	TGGGTCTGTACGGACACTATG	ATACATGCACAATGTTGAGTGAA
35	Reticulon-3	rtn3	NM_001160516	CAGTCCTACACAGCGTCGAA	AGTGCCCCAGAAACATCAAC
36	Ribonuclease P/MRP protein subunit POP5	pop5	NM_001160634	CAGTACTGAGCTGCACCCTG	CACATGTCCAATGTTTTATTTGC
37	Mitogen-activated protein-binding protein-interacting protein	mapip	NM_001160633	AGACTGTGGGCTTTGGAATG	GCGTGACAGGAACAGACAAC
38	Transcription initiation factor TFIID subunit 12	taf12	NM_001160631	TTGTTTCACCCACGTCTTCA	CAGTCTGGCGATACAAGCAA
39	Crystallin J1A	crj1a	NM_001160629	TGAAGGACAACTTATCCAAGCA	GCAGCTTTGACAGAGCAACA
40	CA050 protein	ca050	NM_001160628	GCACAAACCTTTAGGCAAGC	ACAATCATTTTGCAGGTCCC
41	Eukaryotic translation initiation factor 5	if5	NM_001160627	CCAAAGAGATCCATGCGAAG	TGCAGTCAAGTAAATCCCCA
42	Vesicle-associated membrane protein 5	vamp5	NM_001160626	TGTCAGAGCAAAGGACATGG	TCCTATGGTAAGGCTGAAGCA
43	ADAM 10	ada10	NM_001160624	CGATCCCTCCCACATTTACA	AAAGCTTGCTGGATTTTTGG
44	Isopentenyl-diphosphate delta-isomerase 1	idi1	NM_001160619	CTAGGGGCCCTGGTAAAAAG	AAACGCAACAAAAATTTGGC
45	Signal recognition particle 9 kDa protein	srp09	NM_001160617	GGCTTATGGTGTCCAAGGAA	GGAGGCATGTTTCTTCCACT
46	methionine sulfoxide reductase	msra	NM_001160616	CCGGAGTCTCCTGTCCAATA	GTCAACACACCGTTTCATCA
47	SYS1 homolog	sys1	NM_001160611	CTGGCTCATTCCCTGTGAAC	GGAAAGGACATAACCGAAAGC
48	RNA-binding protein 4B	rbm4b	NM_001160608	GTAATGCATGACCCCTCGTC	AAACAAGCACGTTACCCACC
49	Prefoldin subunit 4	pfd4	NM_001160606	AGGAACAACTGCAGCAGGAA	CCATTGCTGAGAAAAATCATGT
50	Normal mucosa of esophagus-specific gene 1	nmes1	NM_001160604	GAGGGAGTGGACCCATCTAA	TGTTCTGTTGGTGGCATTGT
51	Poly synthetase 3	parp3	NM_001160600	TGCAGACACCTCCAGATGAG	GCATTTGTGGAGTGTTTATTGAA
52	Zinc finger protein 706	zn706	NM_001160599	GGGGGAATATTTGTGTGACG	TACAGGATTTGGCCTCGTTT
53	NipSnap2	nips2	NM_001160597	GACCTCGCCTCTGAAGTAACC	CACGATCCTTAATAAAGCACTGGA
54	S-acyl fatty acid synthase thioesterase	sast	NM_001160595	GCATGGAAAGACATATCATCTGG	TTTGATTTGAGGACTAGTCAGGC
55	Anaphase-promoting complex subunit 7	apc7	NM_001160593	AGCTGTTCTCACCATCCCAG	CCATTTTTCAAAACGTGTGTGT
56	Gastrula zinc finger protein XLCGF57.1	zg57	NM_001160590	GTGGCGACTGTGGGAAAAG	CACAGGCTTTACAAAAGTAAATGAA
57	26 proteasome complex subunit DSS1	dss1	NM_001160587	AAGAATTCCCAGCCGAGG	CCGTGAACTGAACCACCTTT
58	Phosphatidylinositol N-acetylglucosaminyltransferase subunit H	pigh	NM_001160586	GCATACCGACGCTTCAAACT	TCGAGCTTATCAAGTAGGGACA
59	NTF2-related export protein 2	nxt2	NM_001160583	ATGTGACCCCCAGAACTGAA	TTCACCAACAAACAAACGGA
60	Inositol monophosphatase	impa1	NM_001160581	ATGGTGGACCATTGGATCTC	CATACATGACTGGAGACACATATACA
61	Molybdenum cofactor synthesis protein cinnamon	cin	NM_001160580	CAGAAGACTTGAGAGGAGGGC	TCAGTCACGGAGCAGTTGAT
62	Guanine nucleotide-binding protein alpha-11	gna11	NM_001160579	TTGTTACACGGTTGGCTCTG	TGCTGATGAAAGTGAGGCAC
63	DNA-directed RNA polymerase II subunit J	rpo2j	NM_001160575	TGGTGCATAATGTTGGGAAA	AAGCATTTAAGCGGAATGGA

64	Checkpoint protein HUS1	hus1	NM_001160574	AATTGGCCATCCCTCCTACT	CATGAAGGCTGAGCGTGTAG
65	Hepatitis B virus X-interacting protein	xip	NM_001160573	GATTCGGGGAATATTCTAGTGAGA	TTCTACATGGATTTGAAAAATCAGTC
66	S-methyl-5-thioadenosine phosphorylase	mtap	NM_001160570	ACTATCATCCCACAGCCACC	ATGCCCTAAGCACATTCCAC
67	Betaine aldehyde dehydrogenase	badh	NM_001160550	TGAACAACGGGAAGTCAAGG	ACTGCTGCTTGAAATGTGAGG
68	Survival of motor neuron-related-splicing factor 30	spf30	NM_001160549	CCCCTGTAATGGATCAGCAC	TACGTCGCCAGCAGTGATAC
69	Inosine triphosphate pyrophosphatase	itpa	NM_001160547	CTCTGACCAATCGCTCAACA	CTTGGGGACTCCACACACTC
70	Vacuolar ATP synthase subunit F	vatf	NM_001160545	AGGGGGACTTTGGAGACTGT	TCACATGCATGCTCTGACAA
71	Thioredoxin Reducatse Interacting Protein	txnip	*TC145083*	TTTGAGAACACCTGCTCACG	TCAGAGTAGGCAGGCAGGTT
72	Thioredoxin Reductase	trdnx	CA057296	TTTATTGAACGCACCCACAC	ACGTGGTTTTCCTCAAGGTG
73	Glutathione Peroxidase	gshpx	AF281338	ACGAGCTCCATTCGCAGTAT	TCCTTAATATCTGCCTCAATGTCA
74	Glutathione S-transferase A	gsta	BT073173.1	CCCAAACTGGGAGCGTACTA	CACCAGCTATGTGGGTCTGT
75	calreticulin	crt	AY372389.1	GGGAAGAGGAAACTCCCAAG	TGCTTGACACTGAAGGGATG
76	DNA Damage Inducible Factor 4	redd-1	DQ400410	TAGCAGGGGGTCAAGTATGG	TGATTTGCATTTGAAGCCAG
77	Target of rapamycin	tTOR	EU179853.1	TGCATCAGGACCTCTTCTCA	AAGGACCAGGGTCTTGTGG
78	Asparagine Synthetase	asns	BT059666	TCCAGAACCAAAGAGGCGTA	GAATGTGGTGAAGGGGAGGT
79	Leptin	lpl	AB354909.1	CTCCACTATGAGGGGTCTGC	GGAGATGGTGACAGTGGGAT
80	liver X receptor	lxr	FJ470291.1	AGGACCATGAACTGGTGGAG	CTGCCAAACCACACAGAAGA

Table 2: Ascertainment panel for each species screened for SNPs.

Sample	*O. mykiss* Population	Region	*O. tshawytscha* Population	Region
1	Touchet R.	Washington	Carson Cr.	Washington
2	Touchet R.	Washington	Cowlitz Hat. (Spr)	Washington
3	Sopachnaya R.	Kamchatka, Russia	Cowlitz Hat. (Spr)	Washington
4	Sopachnaya R.	Kamchatka, Russia	Rapid R.	Idaho
5	Omak Cr.	Washington	Hanford Reach	Washington
6	Omak Cr.	Washington	Hanford Reach	Washington
7	Satus R.	Washington	King Salmon R.	Alaska
8	Satus R.	Washington	Bistraya R.	Russia
9	Yakima R.	Washington	Tahini R.	Alaska
10	Yakima R.	Washington	McQueston R.	Canada
11	Omak Cr.	Washington	Togiak R.	Alaska
12	Omak Cr.	Washington	North Fork Hat.	Washington
13	Sashin Cr.	Alaska	S.F. Hoh R.	Washington
14	Sashin Cr.	Alaska	Salmon R.	Alaska
15	Sashin Cr.	Alaska	S.F. Umpqua	Oregon
16	Shitike	Oregon	Sacramento R. (Fa)	California
17	Shitike	Oregon	Sacramento R. (Fa)	California
18	Shitike	Oregon	Eel R.	California
19	Spring Cr.	Kamloops Strain	Eel R.	California
20	Spring Cr.	Kamloops Strain	Nanaimo R.	Canada
21	Upper Summit Cr.	Washington	Nanaimo R.	Canada
22	Lower Summit Cr.	Washington	Spring Cr. Hat.	Lower Col. R
23	Goldendale Hat.	N. California	Spring Cr. Hat.	Lower Col. R
24	Goldendale Hat.	N. California	Nestucca R.	Oregon
25	Upper Malad R.	Idaho, Redband	Soos R.	Washington
26	Upper Malad R.	Idaho, Redband	Soos R.	Washington
27	Pahsimeroi	Idaho	Lyons Ferry Hat.	Washington
28	Pahsimeroi	Idaho	Lyons Ferry Hat.	Washington
29	Dworshak	Idaho	Methow R. summer	Washington
30	Dworshak	Idaho	Methow R. summer	Washington
31	Arlee Str.	Hatchery Str.	Methow R. summer	Washington
32	Donaldson Cr.	Hatchery Str.	Methow R. summer	Washington

Table 3: Validated Taqman™ assays designed from screen. Assay names contain a species identifier, a locus identifier, and the SNP position on the contig (i.e. Omy_ada10-71). All forward and reverse primers are unlabeled and probes contain a 5' fluorophore and a 3' quencher and minor groove binder.

Assay name	Primers	Probes
Omy_ada10-71	TCTTTGAGCGACAAAGTCCTTGT	VIC-CTTCCTGCGTCCAATT
	ACCCACACATGAACGCAAAAG	6FAM-CTTCCTGCATCCAATT
Omy_anp-17	GGTAATGCCACATGCGGTAAATT	VIC-CTCTCATTGGTATAGTAACC
	GGCGAAATCTGAAAATGTGCTGTTA	6FAM-CTCATTGGTATATTAACC
Omy_ca050-64	GTCATACAGAACTGTTTTGTTGTGTCAA	VIC-CAGTTTGAAGAATATACTC
	ACCTTGAATTGGTTCCTAATGCTATTGT	6FAM-CAGTTTGAAGACTATACTC
Omy_cin-172	CGCATGGGACAGGTGTGT	VIC-CGCTCACCGTGGTTAC
	GAGAAAGCCTGTAGAACCATGTCT	6FAM-CGCTCACCATGGTTAC
Omy_hus1-52	CTTGCCGGAGGGTAGCT	VIC-CCCATCCCTCCTCCTGG
	CCACAACTTCTCAAATGAATGGAATGT	6FAM-CCCATCCCTTCTCCTGG
Omy_impa1-55	CGCTGAGAGGATTGTCAA	VIC-CGAGATGATGCGTCTACA
	ATTTTTCTTTGTTCAGTCTTCTGTCTC	6FAM-CGAGATGATGCATCTACA
Omy_lpl-220	TGACAATCACTGAGCAACTGAACTC	VIC-AGTTACTCAGTGACAGTCA
	GTCCAGTCTTGCTTCAACTCATTCT	6FAM-AGTTACTCAGTCACAGTCA
Omy_msra-42	CAATAGGGAATGACCACCAACCT	VIC-TTCCTAGTCAGAATCAG
	CCCTGAATATGCTTCAGTACTGCAT	6FAM-TCCTAGTCAAAATCAG
Omy_nips-299	GACAGGATAGGAACGGTTTCTCAAT	VIC-CTGGATTTCACATGTAATAC
	ATCAGAAGTTTAATTCAATATGTACACGATCCT	6FAM-CTGGATTTCACGTAATAC
Omy_ntl-27	GGTGTGTTACTGTAGTTGTGTCCTT	VIC-CAGACAAGAGTACCCCAAGAC
	TGTGTAGCTAGTGATCCTGATTGTCT	6FAM-CAGACAAGAGTACTCCAAGAC
Omy_nxt2-273	CTTTAGAAAAGCCAAGGTATATTTTAACATACTTCT	VIC-ATCGACATTTACTGTGCCTT
	CTGCTGCCCTCTAATGGTAAGATAG	6FAM-ATCGACATTTACTATGCCTT
Omy_pop5-87	GCTCTTGCTACTTGCAGTGTATCTC	VIC-CCCAGTAACAGAACTAC
	GGGCTCTATTCTGTCATGAAATCCA	6FAM-CCCAGTAACATAACTAC
Omy_ppie-232	CTGTTTTAGATTAGAATGTTTTTGGTCAGGT	VIC-AAATAGCGGAGAAAAT
	CTGAACATAGGCTTTCATTTCAGACAT	6FAM-AAAATAGCAGAGAAAAT
Omy_rbm4b-203	CTGAAATTTGATGAATGGAAGCTGCA	VIC-CACGTTATTATGAAAAGGATGT
	CGTATTCAAGTCGATATACAGTCACGAT	6FAM-ACGTTATTATGAAAAAGGATGT
Omy_redd1-410	GTACTCCCACTAACATACAGTAGACTCA	VIC-AAAATATCCTGCAAGGAAT
	GGCACCATTGTGTTTTAGGATGTAG	6FAM-AATATCCTGCAAGAAAT
Omy_rpo2j-39	TTTGACCAGGATTCTATAAAATGACTCTGTTT	VIC-CAAGCATGGCAGTAAT
	GTACTTTGTATACAGAGGCCATGCT	6FAM-CAAGCATTGCAGTAAT
Omy_sast-264	GAAGTAGGGTTTGTTGACCATGTGA	VIC-CTAGCCAATGCGTCTAA
	TGGATTCCATTTTAGGCTGTAATACATCTT	6FAM-ATCTAGCCAATGTGTCTAA
Omy_srp09-37	TAGTTGTATTAACTCTTCTTTGAGTCTAGA	VIC-TTGTGCTATTGACGCCACAG
	TCATTCCAGCTCCGTTCTCTTC	6FAM-TTGTGCTATTGACACCACAG
Omy_sys1-188	CTTAAATGGTGCTGGTTGCTGTATT	VIC-AAACATGTACGACCTGTC
	AGTGATATCTTAGTGGGTCGAGGAAA	6FAM-TGTAAACATGTACTACCTGTC
Omy_taf12-329	CACATAATCCCCCTTCATCTAACATCA	VIC-CTAACTGCAAAAGGTGTACAT
	TGCCATGAAAGTACCCTGAAAACTA	6FAM-CTAACTGCAAAAGATGTACAT
Omy_txnip-343	CCTTCAAACTAACGCATCATAGACATG	VIC-CCAACTGAAGAGATCTG
	GGTCACTTGGCTAATCCCCTTAT	6FAM-CAACTGAAGGGATCTG
Omy_vamp5-303	CTGCTTCCCAATTCAGTATCGTCTT	VIC-TGGCCGTAGTAGTTGGTCA
	AGGCTGAAGCATTTCTGAGTATGAA	6FAM-TGGCCGTAGTTGGTCA
Omy_vatf-406	TTGCTTCATTTTGTCATAACCTTGGG	VIC-TTGCAGATGACTATCCACA
	TGCATGCTCTGACAAATGTTACACT	6FAM-TGCAGATGACTGTCCACA
Omy_zg57-91	CACTCATACACTCACTCACAAAGGA	VIC-CACAGACTGCACAGCC
	AGCAGATAAGCCTTGTGAGTGAATC	6FAM-CCACAGACTTCACAGCC

Assay name	Primers	Probes
Ots_bcAKal-476	TTGGAAAACAAACCTGTTGTATTTCTGAAA	VIC-ATGTCTTTGCACCTTTTT
	GATGAGGGAATGTGTGGG	6FAM-ATGTCTTTGCACCTCTTT
Ots_cin-330	CGCTCTCTCGATACAACGTCAAG	VIC-CAGTTGATTTGCGTCACAC
	TGTGGTTAGACTATCAGTCACGGA	6FAM-CAGTTGATTTGTGTCACAC
Ots_gna11-169	CCATGGCACTTATTGCAAGAGTAG	VIC-TCCCAATCTGGCCCTC
	GGGTGAATGTGCCAATTGTAAACTG	6FAM-TCCCAATCTAGCCCTC
Ots_hus1-52	GTAACACACCTCGTTCAGCTAATCT	VIC-TCTACTGCTCATTAGCACCT
	ACCCTTAGCTAGCACACCTGAT	6FAM-CTGCTCATTGGCACCT
Ots_itpa-79	TCCAACCTCATCTGCCAAAGG	VIC-ACACTCCATCCTCTGTCC
	GGTACAAAAACGACTTATTTAAGACAAATGGA	6FAM-ACTCCATCCACTGTCC
Ots_lpl-242	CCATGCTGAACAACTGTTACTGACA	VIC-AACTGAACTATGATCAACTC
	GTCCAGTCTTGCTTCAACTCATTCT	6FAM-CTGAACTATGCTCAACTC
Ots_metA-199	CAGTTGTAAGAAAGCAAGTAAGTGGTT	VIC-CTATTGGCCTAATAACAC
	CCACCTAATGAGGCAATTGAGGTTAAT	6FAM-AAGACTATTGGCCTAACAC
Ots_msra-224	GAAATAGTTTGGTAATCAAGCACCACAT	VIC-ATGTGCATTCATTTATG
	GCTCGCACCATATTCAATCAGATGTA	6FAM-ATGTGCATTAATTTATG
Ots_mtap-229	TTTGAAACCCCCGTTTTCTGTTTTT	VIC-TCCTTCACATGAAACTA
	GGTCTCAGTTCATTCAAAACCTCACT	6FAM-CCTTCACAAGAAACTA
Ots_ndk-167	TCCAATGGTATTACACCTTTGTCTTTATTAAACA	VIC-AAAGGGTGAAGAGCAAT
	CTTCTCGTTGACAGTCCGCTTAA	6FAM-AAGGGTGAAAAGCAAT
Ots_nips-133	GCTTGACACTTCTGCTTTCACTTC	VIC-CCTGTGTCGGCATCAA
	CAAGTCTGGTTAGAGCCCTACTATCT	6FAM-TCCTGTGTCTGCATCAA
Ots_ntl-255	TGCAGTTACAAGCCTAAGACAATCT	VIC-TTGTAGAGGAAGAATATTC
	CAACTAAAGTAACACACCAGCAACTG	6FAM-TTGTAGAGGAAGTATATTC
Ots_parp3-286	AGTCAGTGTTGGTGTAGTGAAGAGA	VIC-AGTTACAAGTGGTGTTTCA
	CATTTGTGGAGTGTTTATTGAACAGTAACA	6FAM-ACAAGTGGCGTTTCA
Ots_pigh-105	GTTTGGAATGTTTCTCTGATTGTGTTAACAA	VIC-TGACCTGAAAATATATATTTTT
	GCATTACTAAAAACTGGTGTGTGGAA	6FAM-ACCTGAAAATATATTTTTTT
Ots_pop5-96	CTCTTGCTACTTGCAGTGTATCTCA	VIC-TTCTGTTACTGGACTGATG
	AGTTTGAGGGCTCTATTCTGTCATG	6FAM-CTGTTACTGGGCTGATG
Ots_ppie-245	TGTTTTTGGTCATGTATTTTCTCTGCTATTTTT	VIC-ATGTCTGAAATGAAAGCC
	GGACTGGAGCTGCTGAACATA	6FAM-AATGTCTGAAATTAAAGCC
Ots_redd1-187	TTCTGGGTTGCCATACTCTTTCAAT	VIC-ATTCTGACAGCTGTTTTG
	AGTTGAGACCTTCAGTTCTTAGGGTAT	6FAM-CTGACAGCCGTTTTG
Ots_sys1-112	CTGCTATAACGCAGTAGTTGGATCT	VIC-TGTGCAATGTTCCAACTT
	CAGCACCATTTCAGTTAATTTCCAAGT	6FAM-TGTGCAATGTTCTAACTT
Ots_tshB-226	GCCACTTTGCTTGCAATGGA	VIC-ATCCTGGTATTTCTGTCTGC
	AGTCAATGGACAGGAAAGACATGAG	6FAM-ATCCTGGTATTTATGTCTGC
Ots_txnip-321	CCTTCAAACTAACACATCATAGACATGCTT	VIC-TCTGGCGGATTTACA
	TTATCAAACTGAAGGCGGATTTACTGA	6FAM-CTGGCGGGTTTACA
Ots_vatf-251	CTTTTCGGGTTATTCATGCTGTTGT	VIC-AGACCACAAGATACAGTACC
	GCAAGCATTTGAAAAACAGACTGGAT	6FAM-AGACCACAAGATAGTACC
Ots_xip-130	GGCTGTTTTGTAAATCTGTTTGTTCACT	VIC-CTGCAACTTCAATGTGAC
	TTGACTTTTAAATATGACTCTTGGCCAAAC	6FAM-CTGCAACTTCAGTGTGAC

Section 2: Genetic Baseline Expansion

Introduction

Reproductively distinct aggregations of Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) have evolved among naturally reproducing populations through the cumulative effects of selection and genetic drift (Waples 1991; Nielsen et al. 2009). Philopatry (homing to spawn) among these salmonid species is well documented (Hasler and Scholz 1983; McIssac and Quinn 1988; Quinn *et al.* 1991), and is a significant force shaping populations and defining units of productivity. Although it is easier to resolve population distinctions on a large geographic scale where gene flow and reproductive restrictions are better defined by distance, the distribution of suitable spawning habitat and the homing behavior of locally adapted population segments may produce fine scale genetic structure between adjacent stream sections or watersheds (Beacham *et al.* 2006). While homing miscues (straying) are thought to be necessary to buffer loss of genetic diversity in salmon (Milner and Bailey 1989), particularly in small populations, the rate of straying among wild fish is generally low (Quinn 1993; Heard *et al.* 1995), and genetic structure between populations may persist despite moderate gene flow (Neville *et al.* 2007).

Objective two of the BPA project 2008-907-00 (Genetic Assessment of Columbia River Stocks) involves the collection and distribution of single nucleotide polymorphism (SNP) genotypic data and the use of that data to both expand on existing microsatellite (µSAT) genetic data baselines, and to characterize Chinook and steelhead population structure throughout the Columbia River Basin (CRB). Here we describe our efforts to create and build onto existing baselines founded by multi-agency consortiums called GAPS (Genetic Analysis of Pacific Salmonids; Seeb et al. 2007) for Chinook salmon, and SPAN (Stephen Phelps Allele Nomenclature; Stephenson et al. 2009) for steelhead trout. The use of SNPs is becoming increasingly popular for population analyses that were previously dominated by (neutral) µSAT markers. Many studies have been conducted that compare the relative utility of both marker types (Liu et al. 2005; Morin et al. 2009, Smith et al. 2007, Hess et al. 2010), and in most observations SNPs perform on par with µSAT's for genetic distance (and nearly as well for genetic diversity analyses), though often a larger number of SNP loci are necessary to reach the same level of resolution as the more polymorphic µSAT markers. The use of SNPs provides many advantages over µSAT's, and their use offers great potential to identify fine scale or localized population differentiation that is valuable in monitoring and evaluation for conservation (Ryynanen et al. 2007; Narum et al. 2008). For example, SNPs are far more prolific in the genome, with greater coverage for linkage analyses (Moen et al. 2008). Moreover, because SNPs may be located within functional genes, they are candidates for detecting positive selection or selective divergence shaping population differences. SNPs are relatively easily amplified and scored, particularly with poor quality tissue source or DNA extract (Campbell and Narum 2008), and with advances in analysis platforms, they are currently amenable to superior high throughput capabilities. Although Chinook salmon in the CRB have been studied in great detail (Narum et al. 2004; Waples et al. 2004; Beacham et al. 2006; Matala et al. 2010), and steelhead to a similar degree (Narum et. al 2008; Nielson et al. 2009; Narum et al. 2010a), our efforts are likely to provide additional information that will benefit and expound on the characterization, productivity and management of these species.

Methods

Sample Collection

In the effort to expand on existing SNP genotypic baselines for Chinook salmon and steelhead trout in 2010, populations were included from throughout the CRB and were chosen to complement or overlap (when available) collections submitted to the standardized GAPS and SPAN consortium baselines for Chinook salmon and steelhead trout; this allows evaluation of multiple marker data sets and subsequent distribution of data to the identified consortiums. Our goal for coverage of Chinook salmon was to include all three lineages (lower Columbia, ocean-type, and stream-type) from all primary drainages and major subbasins in the CRB, providing both temporally stratified coverage of the existing baselines and/or represent regions of least coverage. Similar goals for summer-run, winter-run were also pursued, while maintaining the objective of testing the utility of the marker suites for differentiating anadromous from resident *O. mykiss* population components (Narum et al. 2008; Narum et al. 2010a). To date, collection expansions have been partitioned into 32 and 61 groups for analysis of Chinook salmon and steelhead trout respectively (Table 1). Further, priority collections for both species were identified as those relevant to Tribal fishery interests (particularly in the middle Columbia and Snake River regions). In year two of this project (2010) we are employing the same SNP marker panel for Chinook salmon that was implemented in year one (*see section 2, 2009 annual report*). For steelhead trout we have replaced some previously used but uninformative loci (approximately 27 of the original 96 identified in 2009). The remaining 69 loci have been incorporated into the creation of two panels of 96 loci for a total of 192 loci; of these we have constructed one panel of 96 optimized specifically for parentage based tagging (PBT). The total of 192 loci will be used in all genetic stock identification (GSI) analyses (appendix 1 & 2).

We have three primary goals for the application of the compiled SNP genotypic data. Objective one is annual genetic stock identification analyses that will be used for in-season monitoring of fishery returns through the migratory corridor, including harvest GSI in the lower Columbia River, and fish passage GSI at Bonneville and Lower Granite dams (*see sections 3 & 4 of this report*). In objective two, we will conduct PBT monitoring in cooperation with Idaho Department of Fish and Game (IDFG). The PBT effort entails genotyping all hatchery broodstocks on an annual basis in the Snake River Basin; this provides the ability to genetically assign all subsequently returning adult (hatchery) progeny to their respective hatcheries of origin. Lastly, our third objective is to maintain a ten year expanding baseline of SNP data for application in various analyses including population structure analyses, and investigations of landscape genetics and adaptation among populations for these and other *Oncorhynchid* species in the future.

Biological tissues for genetic analysis were sampled from rayed fins (juveniles), and either caudal fin, opercle punch or carcasses of adult fish. Tissue samples were originally stored in individually labeled vials containing either 95% non-denatured ethanol or a lysis buffer (0.5 M EDTA, pH 8.0, 2 M Tris, pH 7.5, 5 M NaCL, 20% SDS), or using a dry Whatman paper medium (LaHood et al. 2008). Many samples were contributed by outside agencies including NOAA Fisheries, Washington Department of fish and Game (WDFW), Oregon Department of Fish and Wildlife (ODFW), and IDFG.

Laboratory Protocol

Genomic DNA was extracted from digested tissue samples using a standard Qiagen® DNeasy™ protocol. Prior to amplification of SNP loci using primer-probe sets (fluorescent tags), an initial polymerase chain reaction (PCR) "pre-amp" step was implemented using whole genomic DNA to jumpstart SNP amplification via increased copy number of target DNA regions. The cycling regime and PCR conditions for the pre-amp step were as follows: one initial cycle of 95° C for 15 min, 14 cycles of 95° C for 15 seconds, 60 ° C for four minutes, and a final dissociation step. For each data collection run, a panel of 96 SNP loci were arrayed with 96 samples using a Fluidigm® microfluidic 96.96 chip (including one genotype indicator and one no-template control sample) to generate high throughput genotyping. Sample cocktails included: 3.4µl GTXpress Taqman (Applied Biosystems), 0.30µl GT load buffer (inclusing taq polymerase), 0.30µl H_2O and 2.0µl pre-amp DNA template. Single SNP assays were prepared in a 5.0µl reaction mix (per sample), containing the following reagents: 2.5µl DA load buffer, 0.25µl Rox dye, 1µl H_2O, and 1.25µl primer/probe. Microfluidic chips were loaded with assay cocktail dispensed at 4.5µl per well, and sample cocktail dispensed at 5.0µl per well. Chip loading and amplification was completed following standard manufacturers protocol on a Fluidigm IFC controller. Amplification conditions using a fast-cycling protocol were; 70° C for 30 min, 25° C for 10 minutes, and 95 ° C for one minutes, followed by 50 cycles of 95° C for 5 seconds, and 50° C for 25 seconds, and a final cool down step of 25° C for 10 minutes. Chips were imaged and scored on a Fluidigm EP1 imager using Fluidigm SNP Genotyping Analysis Software version 2.1.1. Carcass samples often provide poor quality and/or quantity of viable DNA relative to fresh tissue, and our final sample sizes were pared based on individual genotyping success. Successful genotyping for a given sample was defined proportionally as less than 10% missing data (i.e. fewer than nine SNP loci for Chinook salmon, and fewer than 18 SNP loci for steelhead trout).

Statistical Analysis

Allele frequencies were generated with the program CONVERT (Glaubitz 2004). Descriptive statistics including number of samples analyzed per collection per locus, the unbiased heterozygosity (H_E), observed heterozygosity (H_O) and index of inbreeding (F_{IS}) were generated using the analysis program GenAlEx version 6.41 (Peakall & Smouse 2006) . The Markov Chain Monte Carlo approach implemented in GENEPOP version 3.4 (Raymond and Rousset 1995) was used to test for deviations from Hardy-Weinberg equilibrium (HWE) expectation evaluated across SNP loci and collections; this was completed primarily as a test for non-random mating within collections or possible marker amplification problems (e.g. null alleles). It should be noted that these preliminary analyses proceeded without completion of tests for linkage disequilibrium (LD) for all pairs of loci across collections. This computationally demanding analysis is in progress using a simulated exact test in GENEPOP. For all pairs of loci with significant non-random association (linkage) we will select the most informative of the two, and drop the remaining locus in each pair from further analyses. Statistical significance (α) was adjusted for the number of simultaneous tests k (αk for $\alpha = 0.05$) for HWE tests by a sequential Bonferroni correction (Rice 1989); the same adjustment will apply to LD tests.

Table 1.) Inclusive Chinook salmon and steelhead trout collections in the 2010 SNP baseline expansion are identified by number. Refer to these numbers to identify collections in all figures and subsequent tables in this report. Chinook lineage is LC – Lower Columbia, OT – ocean type, and ST – stream type. Origins are identified as; wild or natural-origin (NOR) and hatchery-origin (HAT). In the Run column for steelhead, "Win" is winter-run and "Sum" is summer-run. All adult steelhead collections are presumed to be of anadromous lineage, while the resident and anadromous components within juvenile collections is unknown (?). Note collections are listed by lineage rather than numerical order (*)

CHINOOK SALMON

Collection (map) ID	BPA Region	(n)	Lat	Long	Lineage	Run	Origin	Year	Age
01). White Salmon R.	Big White Salmon	93	45.744	-121.525	LC	spring/fall	NOR	2008	juv
02). Cowlitz R.	Cowlitz	92	46.513	-122.635	LC	spring	HOR	2004	adult
03). North Fork Lewis R.	Lewis	85	45.867	-122.724	LC	late fall bright	NOR	2004	adult
04). North Fork Lewis R.	Lewis	94	45.867	-122.724	LC	early fall bright	NOR	2004	adult
05). Sandy R.	Sandy	92	45.563	-122.395	LC	spring	NOR	2006	adult
06). Sandy R.	Sandy	112	45.563	-122.395	LC	fall	NOR	2002	adult
07). Kalama R.	Kalama	90	46.017	-122.733	LC	spring	HOR	2004	adult
08). Elochoman R.	Elochoman	86	46.261	-123.298	LC	fall	NOR	1995-97	adult
09). Tumwater & Dryden	Wenatchee	93	47.542	-120.559	OT	summer	NOR	1993	adult
10). Lower Yakima R.	Yakima	62	46.312	-119.473	OT	fall	NOR	1998	adult
11). White Salmon R.	Big White Salmon	91	45.744	-121.525	OT	fall	NOR	2008	juv
12). Entiat R.	Entiat	64	47.696	-120.321	OT	summer	NOR	2008	adult
13). Little White Salmon R.	Little White Salmon	94	45.722	-121.641	OT	fall	HOR	2007	Juv
***32).** Lower Crab Creek	Crab	93	46.828	-119.874	OT	fall	NOR	2009	adult
14). Middle Fork John Day R.	John Day	91	44.913	-119.301	ST	spring	NOR	2006	adult
15). North Fork John Day R.	John Day	111	45.012	-119.007	ST	spring	NOR	2006	adult
16). Leavenworth-NFH	Wenatchee	93	47.559	-120.672	ST	spring	HOR	2005	adult
17). Cle Elum R.	Yakima	90	47.178	-120.999	ST	spring	HOR	1997	?
18). Shitike Creek	Deschutes	93	44.764	-121.238	ST	spring	NOR	2004	juv
19). Peshastin Creek	Wenatchee	87	47.558	-120.575	ST	spring	NOR	2005	juv
20). Entiat R.	Entiat	93	47.696	-120.321	ST	spring	NOR	2006	juv

21).	American R.	Yakima	78	46.976	-121.158	ST	spring	NOR	2003	adult
22).	Warm Springs R.	Deschutes	94	44.861	-121.244	ST	spring	HOR	2004	adult
23).	Little White Salmon R.	Little White Salmon	92	45.722	-121.641	ST	spring	HOR	2007	juv
24).	Chamberlain Creek	Salmon	45	45.454	-114.933	ST	spring	NOR	2009	juv
25).	Wenaha R.	Grande Ronde	48	45.946	-117.455	ST	spring	NOR	2006	juv
26).	John Day R.	John Day	119	44.760	-119.650	ST	spring	NOR	2000	juv/adult
27).	Chiwawa R.	Wenatchee	44	47.789	-120.659	ST	spring	HOR	2000	adult
28).	Lostine R. weir	Grande Ronde	56	45.535	-117.451	ST	early spring	HOR	2009	adult
29).	Lostine R. weir	Grande Ronde	56	45.535	-117.451	ST	late spring	HOR	2009	adult
30).	Lostine R. weir	Grande Ronde	57	45.535	-117.451	ST	early spring	NOR	2009	adult
31).	Lostine R. weir	Grande Ronde	52	45.535	-117.451	ST	late spring	NOR	2009	adult

STEELHEAD

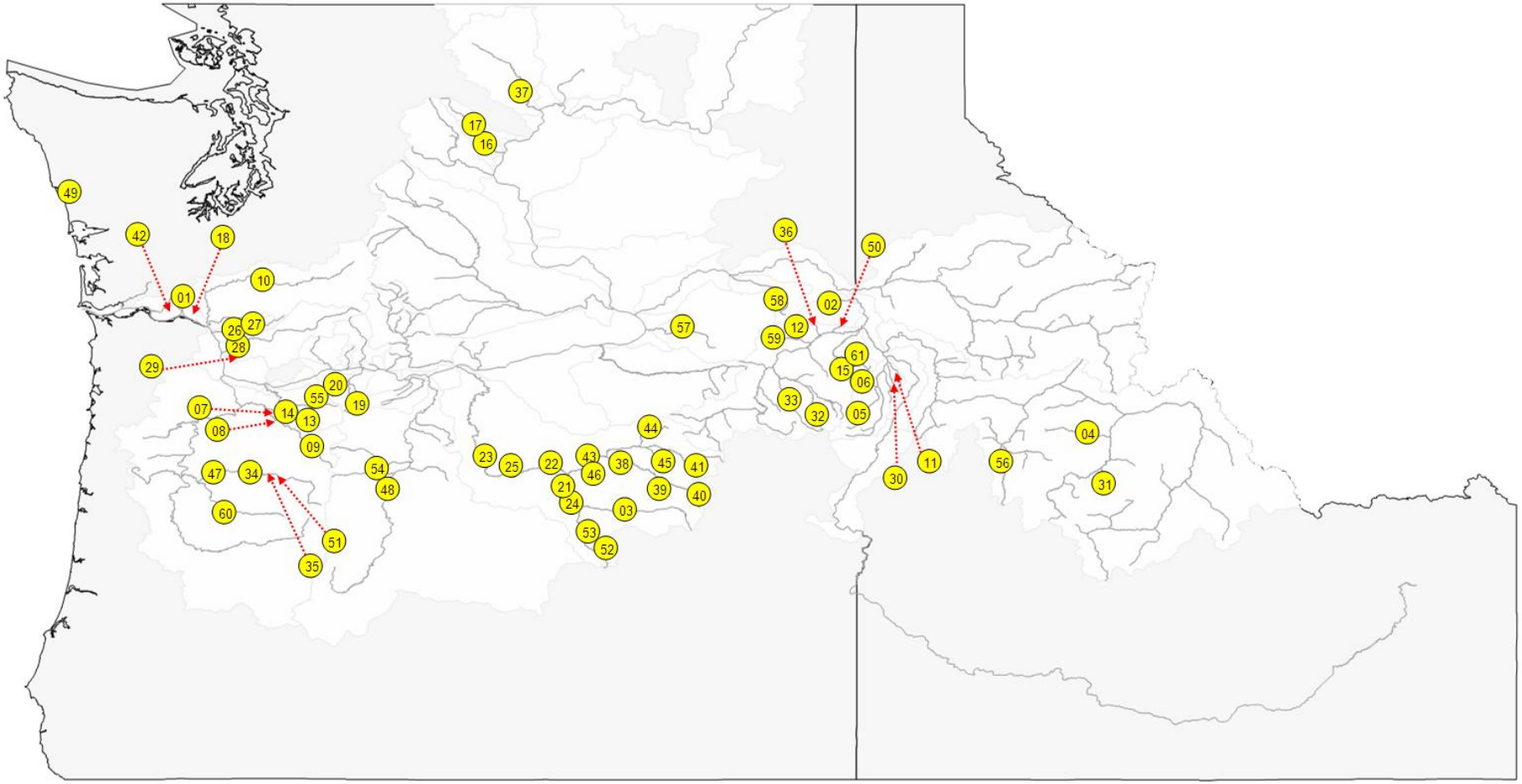
Collection (map) ID		**BPA Region**	**(n)**	**Lat**	**Long**	**Run**	**Origin**	**Year**	**Age**
01).	Abernathy Creek	Elochoman	170	46.238	-123.422	WIN	?	2007-2008	adult
02).	Asotin Creek	Asotin	49	46.344	-117.055	SUM	NOR	2000	juv
03).	Beech Creek	John Day	21	44.412	-119.116	SUM	NOR	2000	?
04).	Big Creek	Salmon	46	45.092	-114.730	SUM	NOR	2000	juv
05).	Big Sheep Creek	Imnaha	63	45.557	-116.834	SUM	NOR	2001	juv
06).	Camp Creek	Imnaha	25	45.557	-116.835	SUM	NOR	2001	juv
07).	Clackamas River	Willamette	58	45.372	-122.604	WIN	NOR	2005	juv/adult
08).	Clackamas River	Willamette	36	45.372	-122.604	WIN	HAT	2000	juv
09).	North Fork Dam	Willamette	60	45.372	-122.604	WIN	HAT	2006	adult
10).	Barrier Dam	Cowlitz	94	46.503	-122.588	?	NOR	2005	adult
11).	Cow Creek	Imnaha	44	45.768	-116.750	SUM	NOR	2000	juv
12).	Crooked Creek	Grande Ronde	98	45.977	-117.555	SUM	NOR	2001	juv
13).	North Fork Eagle Creek	Willamette	44	45.351	-122.384	WIN	NOR	2005	juv/adult
14).	Eagle Creek	Willamette	48	45.351	-122.384	WIN	NOR	?	adult
15).	Elk Creek	Grande Ronde	47	45.705	-117.153	SUM	NOR	2000	juv

16).	Entiat River	Entiat	94	47.664	-120.242	SUM	?	2006	juv
17).	Entiat Trap	Entiat	140	47.664	-120.242	SUM	NOR	2005	juv
18).	Germany Creek	Elochoman	48	46.190	-123.124	WIN	NOR	2005	smolt
19).	East/Middle Forks	Hood	59	45.574	-121.627	WIN	NOR	?	smolt
20).	West Fork	Hood	35	45.605	-121.633	SUM	NOR	?	smolt
21).	Baldy/Bridge creeks	John Day	30	44.361	-119.775	SUM	NOR	2006	juv
22).	Lower John Day River	John Day	34	44.474	-119.532	SUM	NOR	2000	juv
23).	Pine Creek	John Day	18	44.907	-120.445	SUM	NOR	2006	juv
24).	Rock Creek	John Day	10	44.528	-119.634	SUM	NOR	2006	juv
25).	Service Creek	John Day	11	44.797	-120.003	SUM	NOR	2006	juv
26).	Kalama River	Kalama	94	46.033	-122.870	SUM	NOR	2005	adult
27).	Kalama River	Kalama	94	46.033	-122.870	WIN	NOR	?	adult
28).	East Fork Lewis	Lewis	79	45.853	-122.780	WIN	NOR	2005	adult
29).	North Fork Lewis @ Merwin Dam	Lewis	94	45.957	-122.555	WIN	NOR	2006	adult
30).	Lightning Creek	Imnaha	45	45.655	-116.727	SUM	NOR	2000	juv
31).	Loon Creek	Salmon	41	44.598	-114.812	SUM	NOR	1999	juv
32).	Lostine River	Grande Ronde	45	45.552	-117.490	SUM	NOR	2000	juv
33).	Little Minam River	Grande Ronde	48	45.725	-117.785	SUM	NOR	2000	juv
34).	Little Rock Creek	Willamette	16	44.747	-122.395	WIN	NOR	1996	juv
35).	Mad Creek	Willamette	19	44.752	-122.398	WIN	?	1996	?
36).	Menatchee Creek	Grande Ronde	66	46.007	-117.365	SUM	NOR	1999	juv
37).	Methow River	Methow	92	48.049	-119.901	SUM	NOR	2007	smolt
38).	Middle Fork John Day	John Day	67	44.913	-119.296	SUM	NOR	1996	?
39).	Camp Creek	John Day	22	44.689	-118.797	SUM	NOR	2006	juv
40).	Clear Creek	John Day	39	44.589	-118.507	SUM	NOR	2005	juv
41).	Granite Creek	John Day	19	44.865	-118.562	SUM	NOR	2000	?
42).	Mill Creek	Elochoman	45	46.189	-123.177	WIN	NOR	2005	smolt
43).	Big Wall Creek	John Day	7	44.884	-119.414	SUM	NOR	2006	juv
44).	Camus Creek	John Day	19	45.021	-118.991	SUM	NOR	2005	juv
45).	Desolation Creek	John Day	19	44.994	-118.928	SUM	NOR	2005	juv
46).	Fox Creek	John Day	15	44.616	-119.294	SUM	NOR	2006	juv
47).	North Fork Santiam River	Willamette	39	44.687	-123.005	WIN	NOR	2005	adult
48).	Pelton Trap	Deschutes	45	44.694	-121.231	SUM	NOR	1998	adult
49).	Quinalt River	N/A	92	47.358	-123.994	WIN	HAT	2008	adult
50).	Rattlesnake Creek	Grande Ronde	17	46.042	-117.252	SUM	NOR	1999	juv

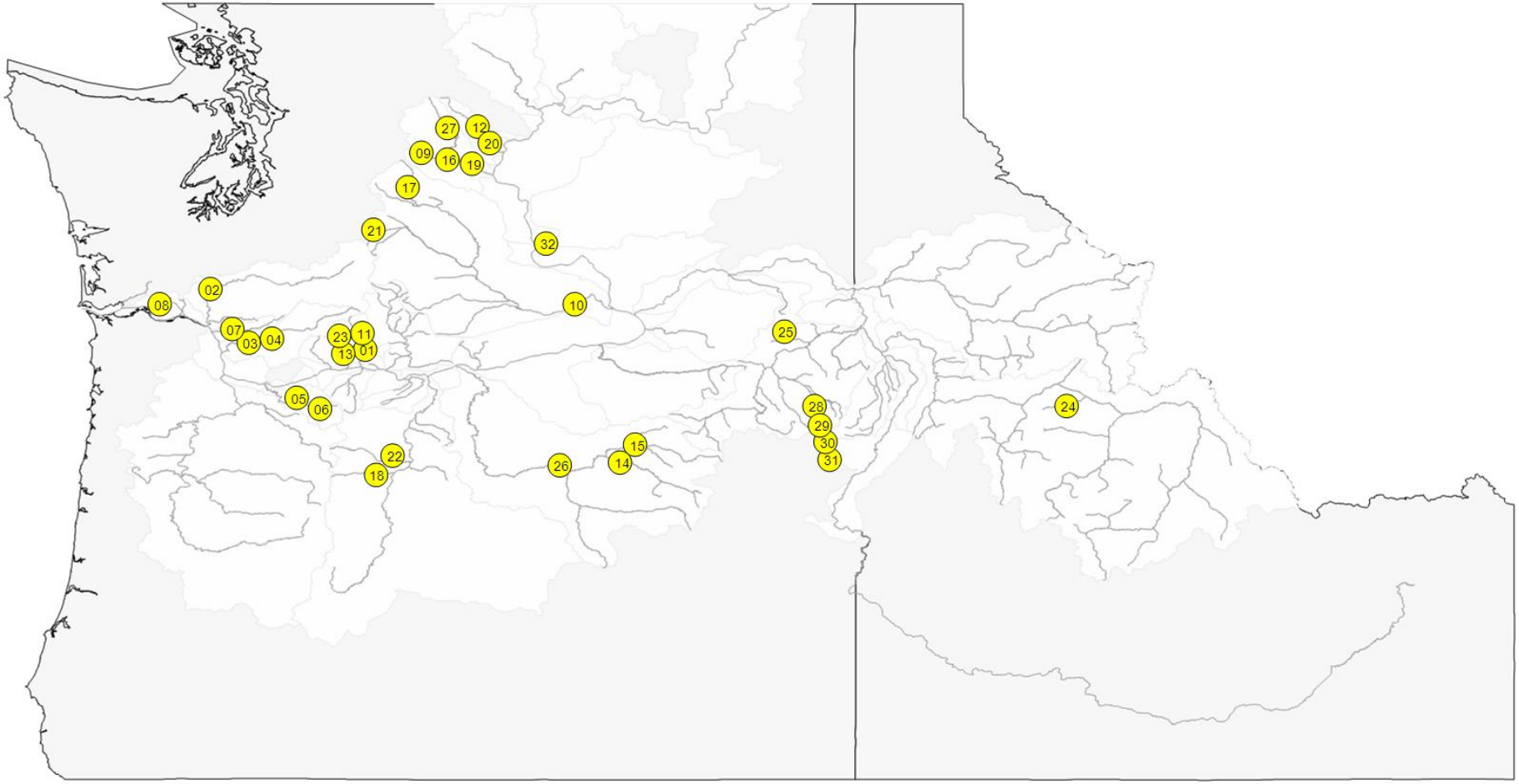
51).	Rock Creek	Willamette	17	44.755	-122.443	WIN	NOR	?	?
52).	Deer Creek	John Day	18	44.189	-119.514	SUM	NOR	2005	juv
53).	Murderer's Creek	John Day	18	44.317	-119.531	SUM	NOR	2005	juv
54).	Shitike Creek	Deschutes	31	44.762	-121.229	SUM	NOR	2000	adult
55).	Still Creek	Sandy	30	45.331	-121.916	?	?	1998	?
56).	Stolle Meadows	Salmon	45	44.607	-115.681	SUM	NOR	2000	juv
57).	Touchet River	Walla Walla	88	46.034	-118.684	SUM	NOR	1995	?
58).	Upper Tucannon River	Tucannon	44	46.205	-117.706	SUM	NOR	1995	juv
59).	Wenaha River	Grande Ronde	94	45.945	-117.451	SUM	NOR	2001	juv
60).	Wiley Creek	Willamette	93	44.415	-122.674	WIN	?	1997	?
61).	Cougar Creek	Grande Ronde	4	46.033	-117.320	SUM	NOR	2000	juv

Figure 1.) Map of study area showing: **a)** location of *O. mykiss* (steelhead) collections, and **b)** locations of *O. tshawytscha* (Chinook salmon) collections included in the 2010 baseline expansion effort.

a)



b)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The program LOSITAN (Antao *et al*. 2008) was used to evaluate the relationship between F_{ST} and H_e (expected heterozygosity) for all loci in an island model, to identify outlier loci (candidates for selection) having excessively high or low F_{ST} compared to neutral expectations. We used data simulations based on 50,000 replicates, a mean F_{ST} of 0.005, and a 0.99 confidence interval for all SNP loci under an infinite alleles model. Loci lying above or below these quantiles (outliers) may be under directional or balancing selection (respectively) in some populations. Interpreting patterns of neutral genetic diversity and drawing biological inferences from such comparisons (quantified by F_{ST}) is confounded by the need to discriminate between selected diversity and the underlying demographic processes (e.g. gene flow) that influence diversity among populations; we therefore excluded significant candidate loci under selection from further analyses of population structure.

GENEPOP (Raymond and Rousset 1995) was used to calculate global F_{ST} (θ of Weir and Cockerham 1984), which indicates the proportion of total variation attributed to differences among collections and a matrix of pairwise F_{ST} among all pairs of collections. Significance was determined by permutation tests based on 1000 individuals using ARLEQUIN version 3.5 (Excoffier *et al.* 2005) and critical values were adjusted for multiple tests following the method of Narum (2006). A pairwise matrix of Neis genetic distances (1972) and an un-rooted neighbor-joining (NJ) phylogram were generated using PHYLIP version 3.68 (Felsenstein 1992). The NJ tree indicates similarities (clusters) among groups identified by branch associations in the genetic distance topology of the tree. The SEQBOOT option was implemented to generate 1000 simulated data sets, and a consensus topology with bootstrap support was generated using the CONSENSE option in PHYLIP. The analysis program GenAlEx version 6.2 (Peakall and Smouse 2006) was used to conduct principle components analysis (PCA) to identify clusters or patterns of genetic similarity among populations. The multivariate PCA analysis reduces redundant variables into a smaller number of principal components that will account for most of the variance in the data without much loss of information. The first principal component accounts for the greatest amount of variability in the data, and each succeeding component accounts for as much of the remaining variability as possible.

Results

Descriptive Statistics

The mean expected heterozygosity (allelic variability) per locus ranged from 0.0003-0.4778, and 0.0000-0.4796 across collections for Chinook salmon and steelhead respectively (appendices1 & 2). For steelhead trout, SNP loci *Ocl_calT7RT2* was fixed; this is a species specific marker that is putatively diagnostic for identifying F1 hybrids of *O. mykiss /* cutthroat trout *(Oncorhychus clarkiI*). Two other hybrid markers, *Omy_myclarp404-111* and *Omy_Omyclmk438-96*, were not fixed . No loci were diagnostic for any particular lineage or life history type in the data sets for both species. Among 3073 total HWE tests for Chinook we observed 149 departures from expected genotypic proportions across groups. Among 11712 total HWE tests we observed 213 departures from expected genotypic proportions across groups for steelhead. For both species we identified some population specific and locus specific deviations; those with four or greater are identified in appendices 1 & 2. A plot of expected heterozygosity and genetic distance (F_{ST}) generated in LOSITAN identified several SNP outlier loci, or candidate loci under directional

selection (table 2). Eliminating non-neutral outlier loci is necessary before computing most population genetic parameters (e.g., F_{ST}, Nm, Ne) that require neutral loci (Luikart *et al*. 2003) in order to evaluate divergence as a function of demographic divergence.

Table 2

steelhead trout		Chinook salmon		
Coastal (8)	Inland (11)	Lower (3)	Ocean (0)	stream (2)
Omy_97954618	Omy_IL1102-163	Ots_Arnt	---	Ots_Ikaros-250
OMS00174	Omy_gadd45-332	Ots_MHC2	---	Ots_TAPBP
OMS00118	Omy_128996-481	Ots_C3N3	---	---
Omy_97660-230	Omy_109243-222	---	---	---
Omy_cd59-206	Omy_cox2-335	---	---	---
OMS00064	Omy_IL6-320	---	---	---
Omy_11201-359	Omy1011SNP	---	---	---
OMS00013	OMS00017	---	---	---
---	OMS00074	---	---	---
---	Omy_1143-438	---	---	---
---	Omy_pad-196	---	---	---

Numbers of candidate directional selection loci in parentheses.

Ultimately only neutral loci will be retained for subsequent population structure analyses, but exclusion of putative loci under selection did not occur prior to generation of results for this report. At this point in our analyses we have not determined the appropriate confidence level threshold for assuming positive selection since some loci fall near the line of neutrality and many among those shown (Figures1a & 1b) are lineage specific. Determining likelihood of selection requires a more thurough examination of the data (see Matala et al. 2010). The plots indicate the number of potential loci that may be valuable for landscape genetic analyses (based on selective divergence). One of the three potential candidate loci within both the lower and ocean-type lineages is a mitochondrial marker (C3N3) and therefore not of concern as an outlier.

Population Genetic Structure Analysis

For the 32 collections of Chinook salmon evaluated, we observed among-group variation (F_{ST}) that ranged from 0.0000 to 0.5244 across all loci and groups. The overall (global F_{ST}) estimate for each of the three lineages was significantly greater than zero (Figure 2). Patterns of pairwise among-group variation (F_{ST}) were mixed for comparisons among 6 ocean-type, 8 lower Columbia type and 18 stream-type collections (Figure 2). The least amount of among-group variation occurred within the ocean-type lineage, while the lower Columbia and stream-type lineages were comparable (note three exception in the stream-type lineage and one in the lower Columbia lineage). For the 61 collections of steelhead evaluated, we observed among-group variation (F_{ST}) that ranged from -0.0007 to 0.2262 across all loci and groups. The overall (global F_{ST}) estimate was significantly greater than zero (Figure 3). Patterns of pairwise among-

group variation (F_{ST}) were mixed for comparisons between winter-run, summer-run, interior type, coastal type, and probable resident and/or anadromous types (the latter distinction has not yet been evaluated or identified via these analyses). There was far less variability in F_{ST} values for steelhead than was observed among Chinook salmon lineages, although mean F_{ST} was generally greater among steelhead than was observed within Chinook salmon lineages.

We demonstrated genetic similarity among populations within each species through phylogenetic relationships in the topology of an unrooted NJ phylogram (Figure 4a & b), and in PCA cluster analyses to graphically display the relationship between collections; the latter is a spatial ordination of data in the context of the two axes that explain the greatest amount of total variation or differences among collections (Figure 5a & b). The confidence or concordance (>50%) of the NJ topology is indicated with bootstrap values at the nodes (Figures 4a & 4b) based on a tree structure. Results of the two analyses were complimentary, and revealed defined clustering of the most genetically similar collections for Chinook salmon (the three major lineages) and an intuitive association or distinction of interior vs. coastal *O. mykiss* forms (Figures 4a & 5a). In large part, the Chinook salmon and steelhead trout collections clustered accurately into geographic regions or major tributary (Figure 4a & b). The PCA for steelhead trout (Figure 5b) generally distinguishes inland groups from the Grande Ronde, Imnaha, and John Day rivers from those in the mid and upper Columbia River and Salmon River. The cluster patterns also resolve a distinction between Hood River at the eastern extreme of the winter-run range (but also the Kalama River winter-run fish), from all other coastal collections. Resident and anadormous components and the influence by either among collections has not been evaluated, but may have some bearing on the observed clustering; some loci may be useful in differentiating the components (Narum et al. 2010a).

Discussion

We have compiled extensive data sets of SNP genotypes for Chinook salmon and steelhead trout covering diverse regions in the Columbia River Basin (including the Snake River Basin) in this expansion effort that complements our first year results. Our goal was to construct SNP baselines of genotypes that will be expanded annually to provide continued evaluation of these species that is both spatially and temporally stratified. This strategy assures the greatest likelihood of discerning reproductively distinct aggregations of Chinook salmon and steelhead trout (Waples 1991) through time, while monitoring population variability related to demographic trends that occur locally and/or regionally. Philopatry (Quinn *et al.* 1991, Hendry et al. 2003) and hatchery supplementation activities (Ford et al. 2006; Hard & Heard 1999) will play a major role in how genetic divergence and differentiation is distributed geographically, and it will be important to evaluate such impacts on the ability to differentiate populations both qualitatively and quantitatively (e. g. genetic stock identification)

The results presented in this report substantiate and complement differentiation of groups of Chinook salmon (Waples et al. 2004; Narum 2008; Narum et al. 2010b) and steelhead trout (SPAN data set; in press). Result further suggest SNPs are a class of markers that perform at least as well as µSATs with great potential for monitoring population distinctions and composition in regard to migration and in-season fisheries.

Figure 2.) Pairwise F_{ST} comparisons between Chinook collections; the amount of total variation attributable to among-group differences. Among-lineage global F_{ST} is 0.239 (95% CI: 0.194-0.394).

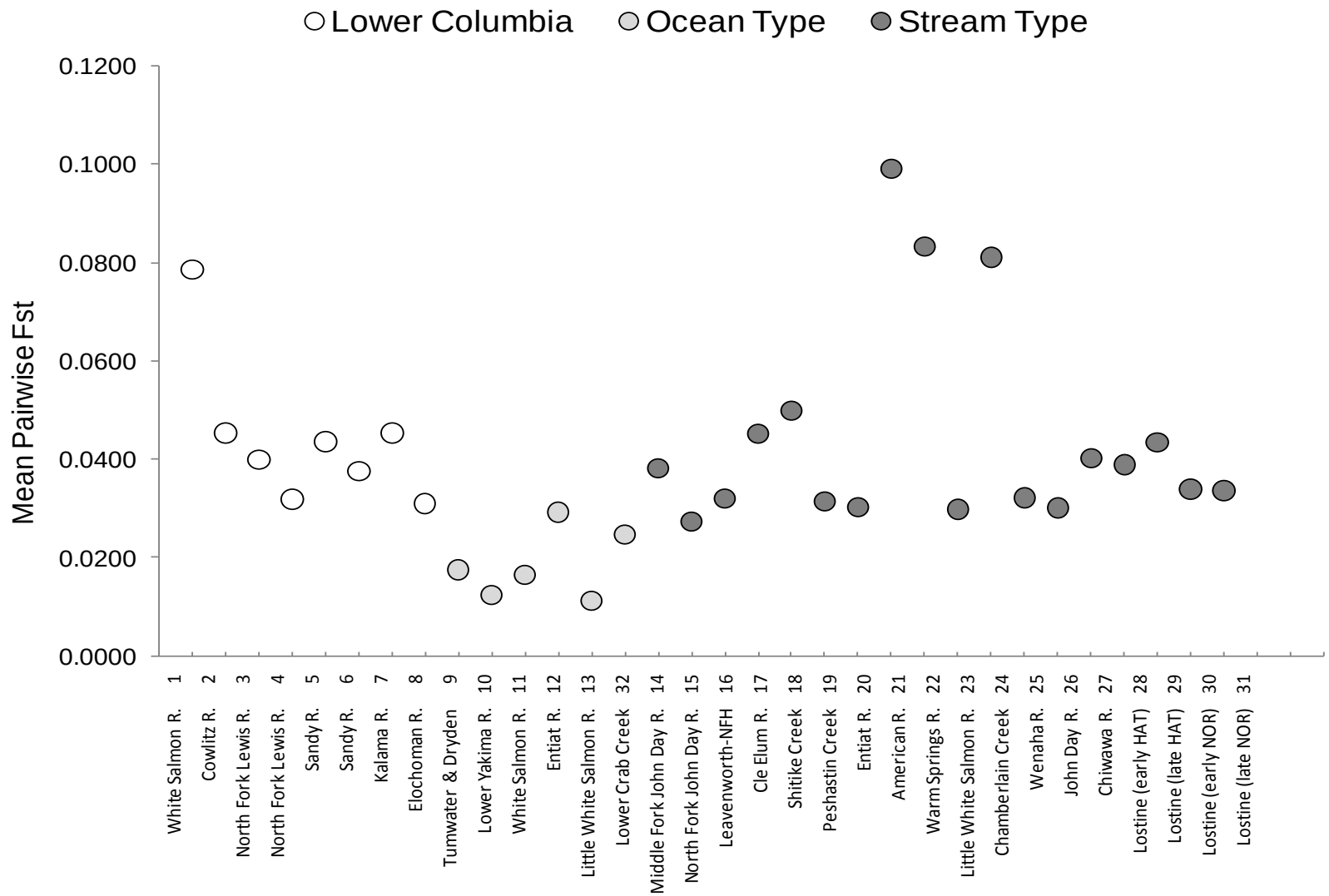
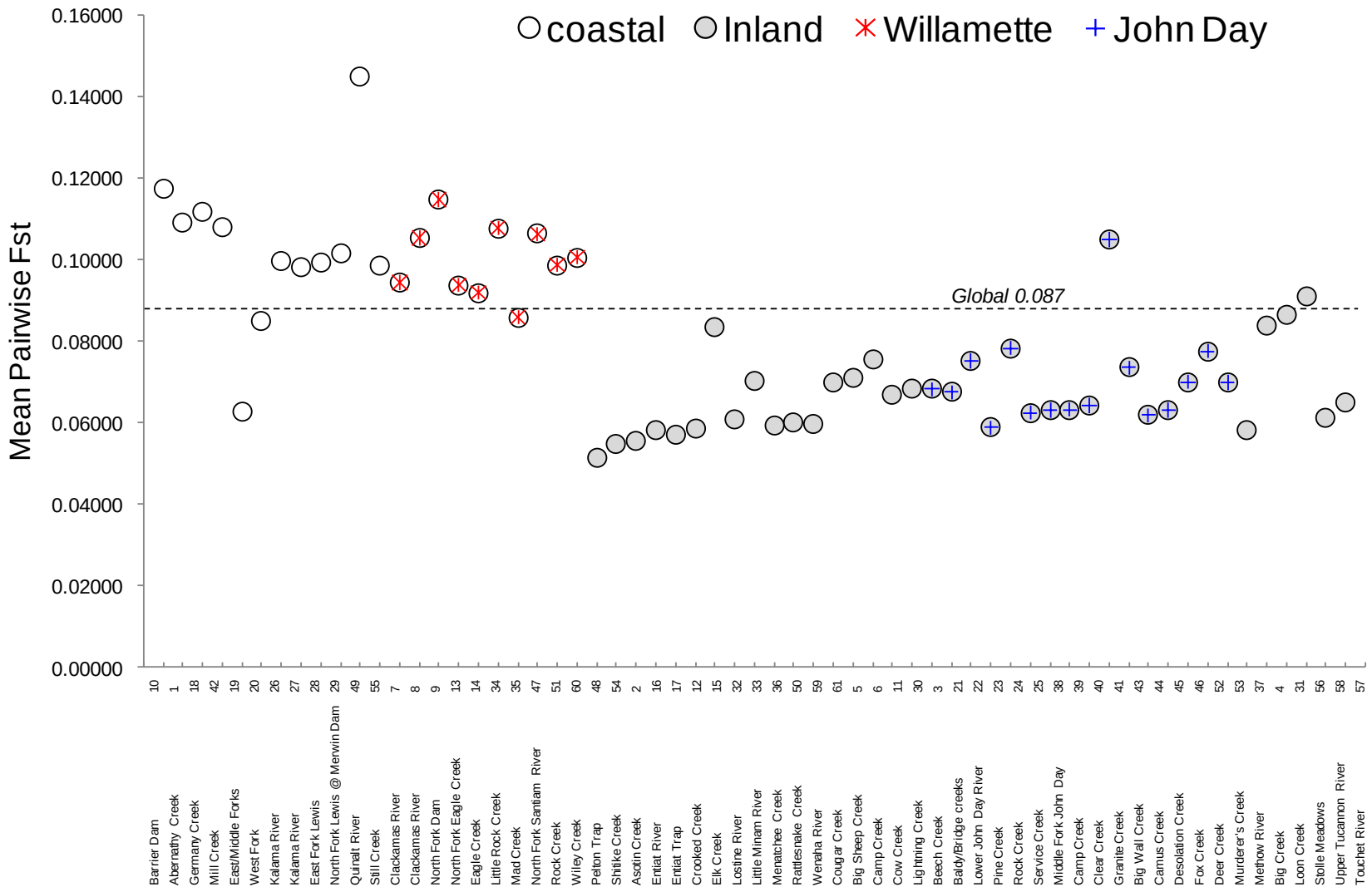


Figure 3.) Pairwise F_{ST} comparisons between steelhead collections; this is the amount of total variation attributable to among-group differences.



We will begin to examine more closely those populations that display unique attributes or differences in contrast to, or in agreement with expectations based on published information. In addition, the nature of SNPs as candidates for detecting positive selection (e.g. locations within functional genes) should provide more clarification of how population differences are shaped across landscapes (Matala et al. 2010). We will continue to investigate landscape genetics in greater detail by looking for correlations between environmental variables (e.g. temperature, migratory distance, elevation etc.) and genetic differences among populations of Chinook salmon, and compare these results to our initial findings from the 2009 data set. This will be our first year for investigating such population characteristics for steelhead trout in the CRB, but more importantly, our data will be implemented in current and ongoing application of PBT and GSI methods for both species. These additional and ongoing efforts will require further scrutiny of the genotypic data, as we have not yet identified significant selection candidate loci for the expanded data set. Although loci under positive selection have great utility for evaluations of adaptive variation, they are a confounding element that may violate basic population genetics assumptions when conducting structure analyses. Our future efforts include adding collections to the baseline to increase basin wide coverage of both species and to provide a temporally stratified view of populations (accounting for natural inter-annual population variation). We also intend to continually strive to increase the numbers of markers (SNP loci) employed. Existing HWE problems and technical laboratory issues will need to be resolved (see appendix 2) using QC measures before our baseline is complete and results are finalized.

Figure 4a.) Chinook salmon NJ-phylogram based on Neis distance (1972). Ocean and Lower Columbia types are shown in blue, and stream-type collections in red; note the 100% bootstrap support for the topology separating stream-type from the remaining two lineages.

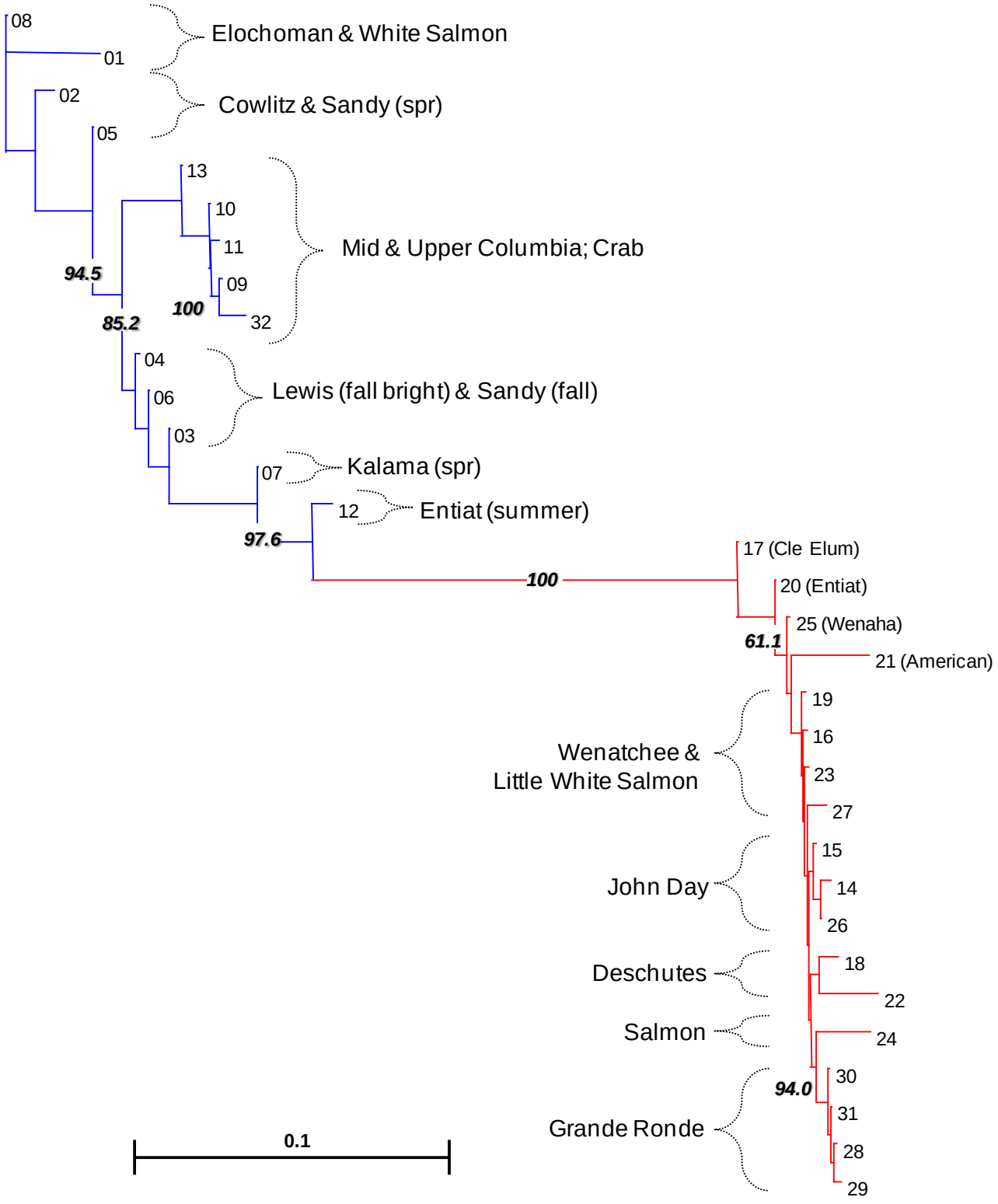


Figure 4b.) Steelhead NJ-phylogram based on Neis distance (1972). Coastal collections (blue) are left of the primary node marked with 100% bootstrap.

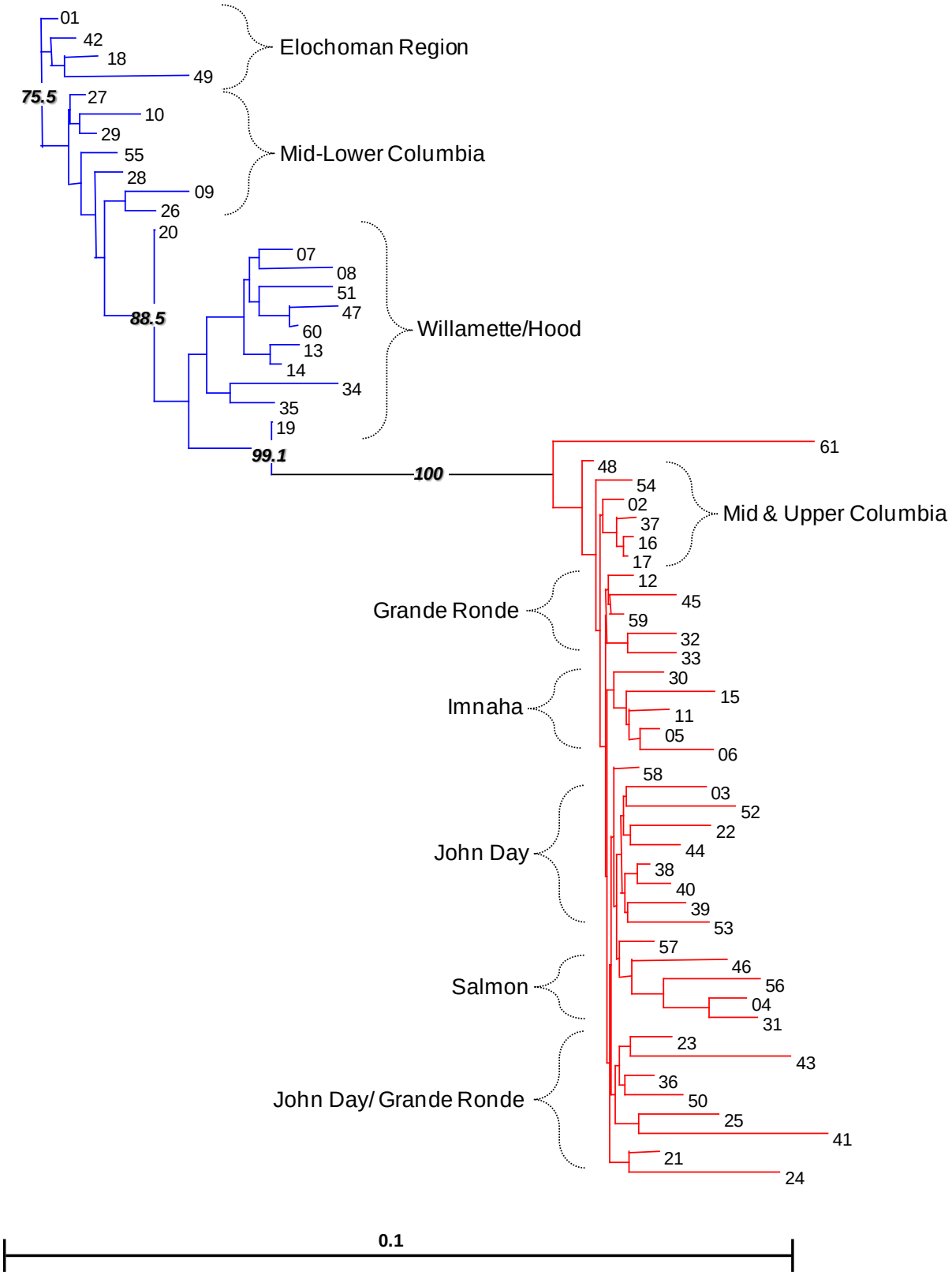


Figure 5a.) A PCA plot for Chinook showing the first two principle coordinate axes.

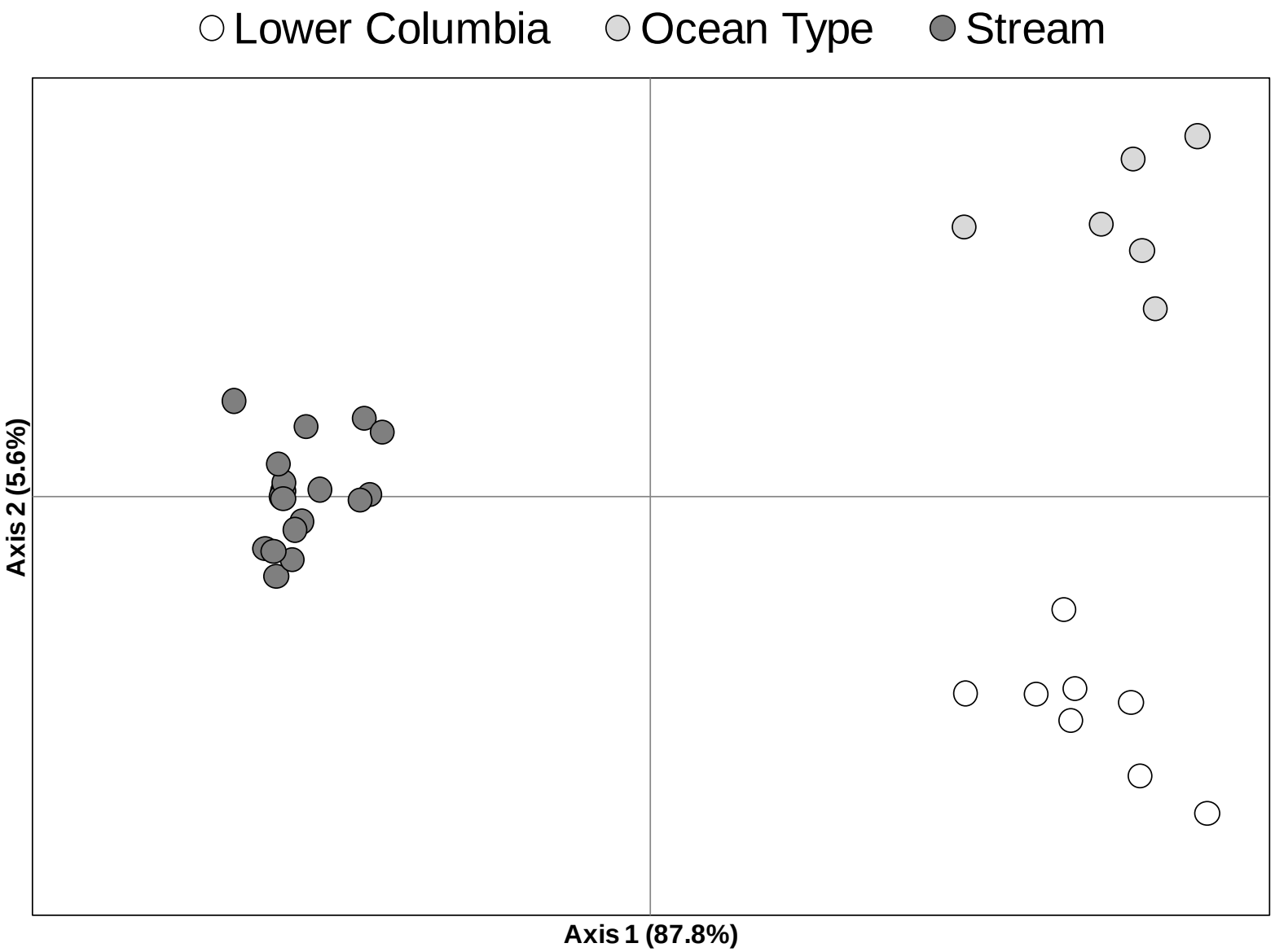
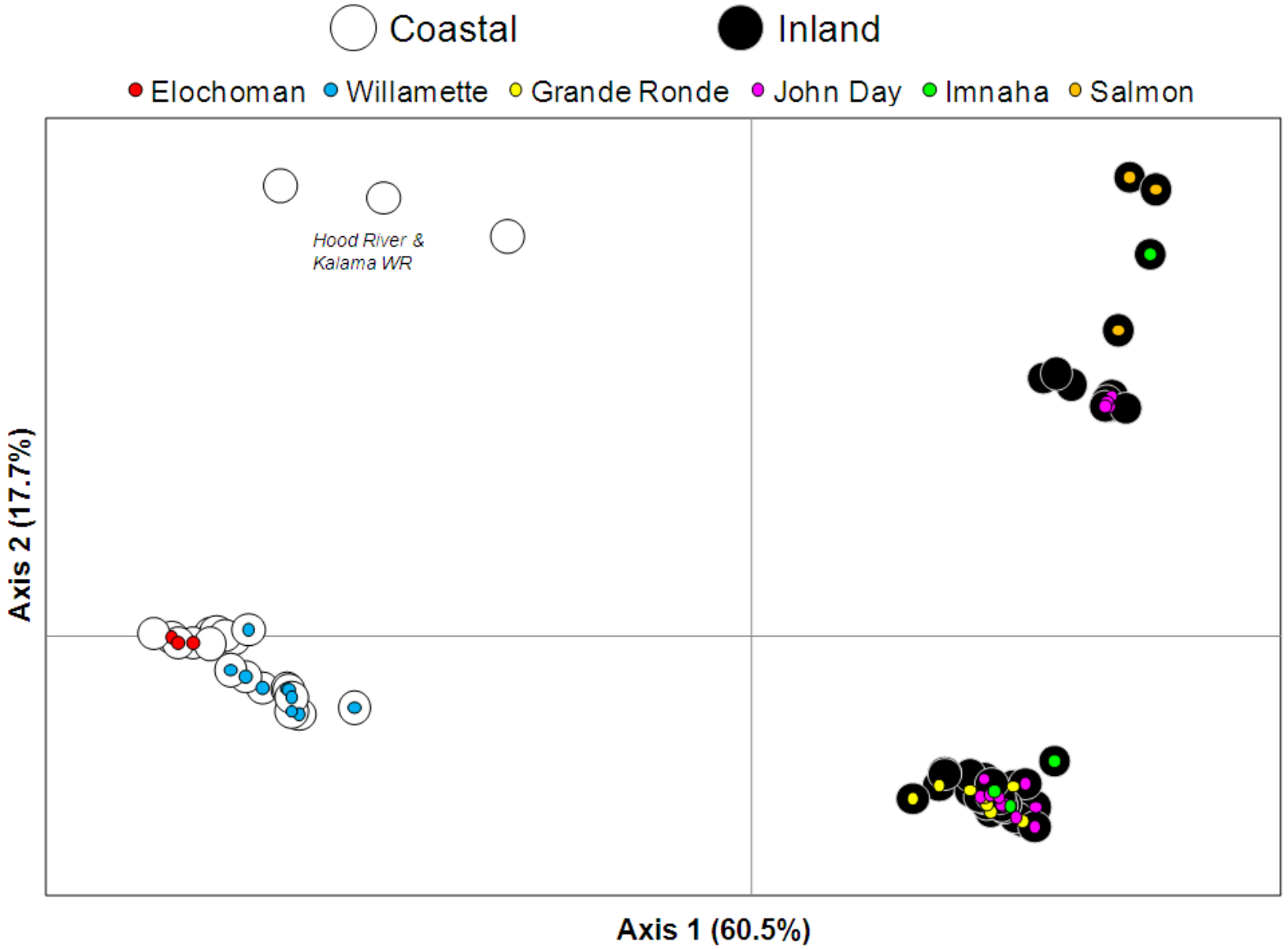


Figure 5b.) A PCA plot for steelhead showing the first two principle coordinate axes.



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Appendix 1.) Chinook salmon descriptive statistics from analysis of the Chinook salmon SNP baseline of 32 collections). Column headings are: (n) mean sample size, (He) Expected Heterozygosity, (Ho) Observed Heterozygosity, (F_{is}) Fixation Index, number of significant HWE deviations, proportion of polymorphic loci (%P) per collection, and ($F_{ST\ (mean)}$) among-collection variation per locus.

Chinook salmon by Locus

SNP locus	(n)	Ho	He	F_{is}	# Dev.	$F_{ST\ (mean)}$
Ots-102414-395	81.8	0.4854	0.4645	-0.0451		0.0685
Ots-105105-613	81.8	0.4018	0.3933	-0.0214		0.1950
Ots-106747-239	81.2	0.4762	0.4526	-0.0522		0.0619
Ots-110064-383	82.1	0.4092	0.4161	0.0165		0.1673
Ots-nramp-321	82.3	0.1211	0.1512	0.1989	(9)	0.6849
Ots-113242-216	81.3	0.3303	0.3286	-0.0053		0.1835
Ots-113457-40R	82.0	0.3080	0.3074	-0.0018		0.3366
Ots-123048-521	82.2	0.0977	0.0992	0.0151		0.0734
Ots-128757-61R	82.0	0.3130	0.3103	-0.0085		0.1543
Ots-94857-232R	81.9	0.4779	0.4567	-0.0464		0.0789
Ots-94903-99R	82.2	0.4528	0.4579	0.0112		0.0826
Ots-96222-525	82.4	0.2692	0.2818	0.0450		0.2229
Ots-96500-180	81.7	0.4226	0.4285	0.0139		0.1274
Ots-96899-357R	82.3	0.1170	0.1211	0.0338		0.0729
Ots-97077-179R	82.3	0.1901	0.1959	0.0299		0.2179
Ots-AldB1-122	81.8	0.2023	0.2097	0.0357		0.0569
Ots-aldb-177M	78.7	0.2312	0.2246	-0.0295		0.2604
Ots-ARNT	82.1	0.2361	0.2387	0.0109	(4)	0.5178
Ots-arp-436	79.4	0.2070	0.2059	-0.0054		0.3289
Ots-AsnRS-60	82.1	0.3065	0.3018	-0.0156		0.0425
Ots-aspat-196	82.2	0.1430	0.1412	-0.0130		0.1668
Ots-C3N3	82.3	0.0000	0.2380	1.0000		0.3972
Ots-Cath_D141	82.3	0.0377	0.0392	0.0386		0.0405
Ots-CCR7	82.3	0.0430	0.0431	0.0034		0.0739
Ots-CD59-2	82.2	0.4604	0.4589	-0.0033		0.0245
Ots-CD63	82.2	0.3034	0.2965	-0.0235		0.2461
Ots-cox1-241	82.1	0.2481	0.2695	0.0793		0.4510
Ots-CRB211	81.9	0.0409	0.0464	0.1192		0.0564
Ots-E2-275	82.0	0.3913	0.4084	0.0418		0.1800
Ots-EndoRB1-486	77.6	0.2195	0.2603	0.1568	(10)	0.0995
Ots-EP-529	82.1	0.1067	0.1037	-0.0291		0.0377
Ots-ETIF1A	82.1	0.3875	0.3820	-0.0146		0.2346
Ots-FARSLA-220	81.9	0.1246	0.1466	0.1500	(4)	0.6957

Ots-FGF6A	82.0	0.3850	0.3838	-0.0034		0.1278
Ots-FGF6B_1	81.8	0.4116	0.4104	-0.0031		0.1766
Ots-GDH-81x	82.0	0.3759	0.3936	0.0449		0.1063
Ots-GH2_1	82.4	0.0303	0.0298	-0.0175		0.0200
Ots-GnRH-271	82.1	0.0146	0.0143	-0.0219		0.0142
Ots-GPDH-338	82.4	0.0408	0.0419	0.0259		0.0249
Ots-GPH-318	82.4	0.2002	0.2050	0.0236		0.0720
Ots-GST-207	82.4	0.0916	0.0930	0.0149		0.0624
Ots-GST-375	82.5	0.0195	0.0205	0.0497		0.0371
Ots-GTH2B-550	81.9	0.3339	0.3522	0.0519		0.2443
Ots-hsc71-3'-488	81.8	0.2915	0.2993	0.0262		0.4010
Ots-hsc71-5'-453	81.9	0.2600	0.2696	0.0354		0.2491
Ots-hsp27b-150	82.3	0.2342	0.2357	0.0063		0.1555
Ots-HSP90B-100	82.3	0.2293	0.2467	0.0705		0.5021
Ots-IGF-I.1-76	82.5	0.1534	0.1529	-0.0034		0.1088
Ots-Ikaros-250	82.0	0.2126	0.2299	0.0752		0.5351
Ots-IL11	82.4	0.1067	0.1033	-0.0329		0.0831
Ots-IL8R_C8	82.2	0.2965	0.3024	0.0195		0.3036
Ots-mapK-3'-309	82.0	0.4484	0.4665	0.0389		0.0625
Ots-mapKpr-151	81.5	0.2803	0.2965	0.0547		0.1174
Ots-MHC1	82.2	0.2129	0.2375	0.1035	(4)	0.4646
Ots-MHC2	81.9	0.3735	0.3627	-0.0297		0.1167
Ots-mybp-85	82.2	0.3140	0.3185	0.0139		0.2615
Ots-Myc-366	82.3	0.0103	0.0106	0.0285		0.0185
Ots-myo1a-384	82.3	0.2005	0.2094	0.0429		0.1161
Ots-myoD-364	82.0	0.2928	0.2969	0.0137		0.3341
Ots-nkef-192	82.0	0.2766	0.2872	0.0371		0.3597
Ots-NOD1	81.7	0.3100	0.3138	0.0119	(4)	0.3588
Ots-LWSop-638	82.4	0.0471	0.0488	0.0355		0.0898
Ots-Ots311-101x	79.3	0.1849	0.1892	0.0228		0.3484
Ots-P450	82.4	0.1485	0.1724	0.1382	(6)	0.6403
Ots-P53	82.0	0.3893	0.3803	-0.0234		0.1468
Ots-PGK-54	82.3	0.2299	0.2432	0.0544		0.4321
Ots-Prl2	82.1	0.4348	0.4184	-0.0392		0.1532
Ots-RAG3	81.9	0.3280	0.3369	0.0264		0.3091
Ots-RAS1	82.3	0.0112	0.0115	0.0277		0.0248
Ots-RFC2-558	82.2	0.2264	0.2284	0.0089		0.3116
Ots-S7-1	81.9	0.4237	0.4309	0.0167		0.0910
Ots-SClkF2R2-135	82.0	0.4207	0.4202	-0.0011		0.0277
Ots-SL	82.3	0.1494	0.1751	0.1471		0.6253
Ots-SWS1op-182	82.1	0.4206	0.4055	-0.0374		0.1882

Ots-TAPBP	82.0	0.3313	0.3381	0.0202		0.3232
Ots-TGFB	82.3	0.1954	0.1941	-0.0066		0.0967
Ots-TLR3	82.0	0.3642	0.3714	0.0196		0.2556
Ots-TNF	79.5	0.0368	0.0344	-0.0676		0.6430
Ots-Tnsf	82.2	0.2949	0.3066	0.0383		0.1470
Ots-u07-07.161	81.7	0.4845	0.4778	-0.0140		0.0431
Ots-u07-17.135	82.3	0.1416	0.1423	0.0052		0.0576
Ots-u07-18.378	82.2	0.3046	0.3008	-0.0125		0.1852
Ots-u07-20.332	82.4	0.0522	0.0517	-0.0087		0.0652
Ots-u07-25.325	81.7	0.2779	0.2789	0.0035		0.2380
Ots-u07-49.290	81.9	0.4143	0.4232	0.0212		0.1199
Ots-u07-53.133	82.0	0.3057	0.3160	0.0326		0.2682
Ots-u07-57.120	81.8	0.1709	0.1907	0.1038	(5)	0.6124
Ots-u07-64.221	82.5	0.0053	0.0052	-0.0238		0.0206
Ots-u202-161	81.8	0.2304	0.2588	0.1099	(4)	0.4242
Ots-u211-85	82.3	0.2905	0.3055	0.0491		0.3141
Ots-u4-92	82.1	0.1093	0.1178	0.0719		0.0632
Ots-u6-75	82.1	0.1334	0.1359	0.0182		0.0334
Ots-unk526	81.8	0.2627	0.2636	0.0033		0.0512
Ots-zP3b-215	82.4	0.0003	0.0003	-0.0055		0.0053
Ots-ZR-575	81.9	0.2096	0.2495	0.1599	(8)	0.4933
Ots-SEXY1	77.9	0.4437	0.3331	-0.3320	(11)	0.0352
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All	81.9	0.2442	0.2502	0.0305		0.2079

Chinook salmon by population

Population	(n)	Ho	He	F_{is}	# Dev.	%P
01). White Salmon R.	92.8	0.2270	0.2300	0.0558	(7)	0.9063
02). Cowlitz R.	91.7	0.2680	0.2706	0.0137		0.8854
03). North Fork Lewis R.	84.3	0.2857	0.2897	0.0122		0.9063
04). North Fork Lewis R.	93.4	0.2859	0.2875	0.0116		0.9583
05). Sandy R.	91.1	0.2653	0.2893	0.0854	(9)	0.9479
06). Sandy R.	111.4	0.2853	0.2901	0.0131		0.9479
07). Kalama R.	89.1	0.3040	0.3152	0.0370	(4)	0.9583
08). Elochoman R.	85.2	0.2636	0.2751	0.0484	(5)	0.9375
09). Tumwater & Dryden	92.6	0.2439	0.2504	0.0114		0.9375
10). Lower Yakima R.	61.3	0.2550	0.2632	0.0312	(10)	0.9167
11). White Salmon R.	90.5	0.2546	0.2630	0.0193	(4)	0.9271
12). Entiat R.	62.9	0.2388	0.2994	0.1938	(22)	0.9271
13). Little White Salmon R.	93.9	0.2539	0.2752	0.1114	(15)	0.9583

14).	Middle Fork John Day R.	89.8	0.2278	0.2332	0.0461		0.8750
15).	North Fork John Day R.	109.4	0.2313	0.2353	0.0247		0.8854
16).	Leavenworth-NFH	92.4	0.2297	0.2234	-0.0077		0.8646
17).	Cle Elum R.	89.6	0.2744	0.2734	0.0006		0.8542
18).	Shitike Creek	92.8	0.2269	0.2231	0.0020		0.8229
19).	Peshastin Creek	86.8	0.2270	0.2267	0.0271	(6)	0.8125
20).	Entiat R.	92.9	0.2327	0.2609	0.1397	(15)	0.9271
21).	American R.	76.6	0.2155	0.2147	-0.0101		0.7917
22).	Warm Springs R.	93.6	0.2379	0.2351	-0.0022	(4)	0.8854
23).	Little White Salmon R.	91.8	0.2302	0.2319	0.0154		0.8333
24).	Chamberlain Creek	44.9	0.1843	0.1781	-0.0334		0.6458
25).	Wenaha R.	47.9	0.2475	0.2641	0.0838		0.8646
26).	John Day R.	118.3	0.2416	0.2490	0.0494	(7)	0.8646
27).	Chiwawa R.	43.7	0.2204	0.2176	-0.0105		0.8229
28).	Lostine R. weir	55.2	0.2279	0.2229	-0.0090		0.8125
29).	Lostine R. weir	55.9	0.2116	0.2178	0.0186		0.8229
30).	Lostine R. weir	56.6	0.2227	0.2216	0.0376	(6)	0.8229
31).	Lostine R. weir	51.9	0.2422	0.2301	-0.0138	(5)	0.8854
32).	Lower Crab Creek	89.8	0.2514	0.2492	-0.0135		0.8542
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	Mean	81.9	0.2442	0.2502	0.0326		0.8770

Appendix 2.) Steelhead descriptive statistics from analysis of the SNP baseline of 61 collections). Column headings are: (n) mean sample size, (He) Expected Heterozygosity, (Ho) Observed Heterozygosity, (F_{is}) Fixation Index, number of significant HWE deviations, proportion of polymorphic loci (%P) per collection, and ($F_{ST\ (mean)}$) among-collection variation per locus. Loci held over from the original 96 evaluated in 2009 are identified (*). Loci labeled n/a were not evaluated due to technical laboratory and scoring issues that have not be remedied.

Steelhead by Locus

SNP locus	Panel	(n)	Ho	He	F_{is}	# Dev.	$F_{ST\ (mean)}$
M09AAC.055	GSI	37	0.0782	0.0716	-0.0922		0.8537
M09AAD.076	PBT	49.9	0.3825	0.3771	-0.0142		0.1575
M09AAE.082	PBT	49.6	0.368	0.3641	-0.0106		0.0948
M09AAJ.163	PBT	49.8	0.4168	0.4087	-0.0198		0.0691
Ocl_calT7RT2	GSI	49.8	0	0	---		---
OMGH1PROM1-SNP1	GSI	49.8	0.1322	0.1265	-0.0447		0.0262
OMS00002	PBT	49.7	0.4264	0.4102	-0.0394		0.0428
OMS00003	GSI	49.7	0.31	0.2998	-0.0339		0.0866

OMS00006	PBT	49.8	0.4894	0.4794	-0.0207		0.0397
OMS00008	GSI	49.8	0.1992	0.2	0.004		0.1012
OMS00013	GSI	49.8	0.2762	0.2686	-0.0284		0.2665
OMS00014	GSI	49.8	0.0415	0.0454	0.0872	(4)	0.0318
OMS00015	GSI	49.6	0.1366	0.1318	-0.0365		0.0579
OMS00017	GSI	49.6	0.4132	0.3961	-0.0432		0.0913
OMS00018	GSI	49.8	0.2283	0.2274	-0.0042		0.0511
OMS00024	PBT	49.2	0.3848	0.3978	0.0327		0.0668
OMS00030	GSI	49.8	0.153	0.1546	0.0102		0.0302
OMS00039	PBT	49.4	0.4874	0.4652	-0.0478		0.0487
OMS00048	GSI	49.8	0.2587	0.2766	0.0646		0.1729
OMS00052	GSI	49.8	0.3093	0.3155	0.0194		0.0436
OMS00053	PBT	49.8	0.4176	0.4099	-0.019		0.1033
OMS00056	GSI	49.8	0.3468	0.3389	-0.0231		0.062
OMS00057	PBT	49.8	0.432	0.4272	-0.0112		0.0478
OMS00058	PBT	49.8	0.493	0.4535	-0.0872		0.0506
OMS00061	GSI	49.8	0.222	0.2123	-0.0459		0.1483
OMS00062	PBT	49.7	0.4042	0.388	-0.0418		0.0325
OMS00064	PBT	49.7	0.4517	0.4516	-0.0002		0.0787
OMS00068	PBT	49.8	0.3894	0.3857	-0.0096		0.0682
OMS00070	PBT	49.3	0.4794	0.4664	-0.0279		0.0667
OMS00071	PBT	49.8	0.4624	0.4551	-0.016		0.0897
OMS00072	PBT	49.8	0.492	0.4796	-0.0258		0.0393
OMS00074	PBT	49.9	0.4813	0.4609	-0.0444		0.0593
OMS00077	PBT	49.6	0.4203	0.4109	-0.0228		0.173
OMS00078	PBT	49.8	0.3809	0.3875	0.0169		0.0586
OMS00079	PBT	49.8	0.4898	0.4763	-0.0282		0.0458
OMS00087	GSI	40.6	0.1685	0.1809	0.0683	(5)	0.6446
OMS00089	PBT	49.7	0.4228	0.4144	-0.0202		0.058
OMS00090	PBT	49.7	0.4551	0.4616	0.0142		0.0704
OMS00092	GSI	49.8	0.2072	0.2071	-0.0006		0.0754
OMS00095	GSI	37	0.0234	0.0312	0.2485	(4)	0.9318
OMS00096	GSI	49.8	0.3748	0.3673	-0.0204		0.2426
OMS00101	PBT	49.8	0.4776	0.4558	-0.0477		0.07
OMS00105	PBT	49.7	0.4574	0.4455	-0.0269		0.1065
OMS00106	PBT	49.9	0.4172	0.4034	-0.034		0.0608
OMS00111	PBT	49.8	0.2449	0.2409	-0.0165		0.0506
OMS00112	PBT	49.8	0.2892	0.2688	-0.076		0.051
OMS00114	GSI	37	0.0615	0.0595	-0.0349		0.8759
OMS00118	PBT	49.3	0.4129	0.41	-0.0071		0.174
OMS00119	GSI	49.7	0.2627	0.257	-0.0225		0.0803

OMS00120	PBT	49.6	0.3509	0.3544	0.0098		0.0959
OMS00121	PBT	49.8	0.4801	0.4724	-0.0163		0.0551
OMS00129	GSI	49.5	0.2136	0.2521	0.1529	(5)	0.0748
OMS00132	PBT	49.9	0.4749	0.4583	-0.0362		0.0458
OMS00133	GSI	48.9	0.1791	0.1939	0.0762		0.1991
OMS00138	GSI	49.8	0.1514	0.1492	-0.015		0.0484
OMS00143	GSI	37	0.0852	0.0747	-0.1406		0.8493
OMS00149	GSI	49.9	0.1627	0.1571	-0.0354		0.078
OMS00151	GSI	49.9	0.2515	0.2529	0.0057		0.0467
OMS00154	PBT	49.8	0.3691	0.3546	-0.0408		0.181
OMS00169	GSI	49.9	0.0588	0.0592	0.0068		0.0986
OMS00173	GSI	49.9	0.1937	0.1872	-0.0347		0.0662
OMS00174	GSI	37	0.0623	0.0636	0.0201		0.869
OMS00175	PBT	49.9	0.4511	0.4467	-0.0098		0.0937
OMS00176	GSI	49.9	0.1298	0.1298	0.0003		0.0334
OMS00179	PBT	49.1	0.4092	0.428	0.044		0.0858
OMS00180	PBT	49.8	0.4165	0.4124	-0.01		0.1751
Omy_101832-195	PBT	49.8	0.4655	0.4494	-0.0359		0.0955
Omy_101993-189	PBT	49.8	0.3027	0.3007	-0.0066		0.049
Omy_102505-102	PBT	49.8	0.4127	0.4183	0.0133		0.0762
Omy_103705-558	GSI	49.9	0.2745	0.2667	-0.0294		0.0871
Omy_104519-624	PBT	49.8	0.4256	0.4303	0.0109		0.0284
Omy_105075-162	GSI	49.9	0.2608	0.2638	0.0115		0.0973
Omy_105105-448	PBT	49.8	0.4497	0.4526	0.0064		0.0575
Omy_105385-406	PBT	49.8	0.4767	0.4636	-0.0282		0.0718
Omy_105714-265	PBT	49.8	0.3955	0.3771	-0.0489		0.0638
Omy_107031-704	GSI	49.9	0.281	0.2743	-0.0245		0.4254
Omy_107285-69	GSI	49.8	0.2637	0.2485	-0.0612		0.0584
Omy_107806-34	PBT	49.7	0.4522	0.4651	0.0276		0.0631
Omy_108007-193	PBT	49.6	0.3881	0.3887	0.0014		0.2042
Omy_109243-222	PBT	49.8	0.3245	0.3323	0.0234		0.0887
Omy_109894-185	PBT	49.6	0.3918	0.3892	-0.0067		0.0534
Omy_110064-419	PBT	49.9	0.3555	0.3473	-0.0238		0.1063
Omy_110201-359	GSI	49	0.2592	0.2583	-0.0035		0.274
Omy_111383-51	PBT	49.7	0.494	0.4782	-0.0329		0.0429
*Omy_113490-159	PBT	49.7	0.4151	0.4159	0.002		0.1361
*Omy_114315-438	PBT	49.6	0.3876	0.3849	-0.0068		0.1512
Omy_114587-480	PBT	49.7	0.3628	0.3704	0.0204		0.0899
Omy_116733-349	PBT	49.8	0.3332	0.3348	0.0046		0.0642
Omy_128923-433	PBT	49.8	0.4198	0.4069	-0.0317		0.1435
Omy_128996-481	GSI	45.7	0.2593	0.2752	0.0577	(4)	0.325

Omy_129870-756	PBT	49.8	0.2452	0.2589	0.0529		0.0391
*Omy_130524-160	PBT	49.6	0.4681	0.4644	-0.0081		0.0385
*Omy_97077-73	GSI	49.9	0.0757	0.0722	-0.0489		0.0617
*Omy_97660-230	PBT	49.8	0.4069	0.3968	-0.0255		0.0963
*Omy_97865-196	GSI	49.8	0.0756	0.077	0.0179		0.0684
*Omy_97954-618	GSI	49.7	0.1827	0.19	0.0386		0.0733
Omy_99300-202	PBT	49.7	0.2874	0.296	0.0291		0.0507
Omy_ada10-71	PBT	49.7	0.3711	0.3482	-0.0658		0.0964
*Omy_aldB-165	PBT	49.6	0.4373	0.4334	-0.0088		0.1057
Omy_anp-17	PBT	49.8	0.4472	0.4331	-0.0325		0.0737
*Omy_aromat-280	GSI	49.5	0.3153	0.3406	0.0742		0.0584
*Omy_arp-630	PBT	49.8	0.4675	0.4511	-0.0363		0.0793
*Omy_aspAT-123	GSI	49.9	0.2777	0.2749	-0.0102		0.0875
*Omy_b1-266	PBT	49.3	0.409	0.4134	0.0107		0.1217
*Omy_b9-164	GSI	49.7	0.0879	0.1033	0.149	(10)	0.0464
*Omy_BAC-B4-324	PBT	49.7	0.3477	0.3387	-0.0264		0.2917
Omy_BAC-F5.284	GSI	49.7	0.1011	0.1012	0.0005		0.0389
Omy_BAMBI2.312	GSI	49.8	0.2085	0.2119	0.0161		0.061
Omy_bcAKala-380rd	PBT	49.7	0.4046	0.4204	0.0376		0.1592
Omy_ca050-64	GSI	49.8	0.3665	0.3664	-0.0002		0.0577
Omy_carban1-264	GSI	49.9	0.0889	0.0897	0.0088		0.0746
Omy_cd28-130	GSI	45.2	0.1702	0.1757	0.0312		0.4523
*Omy_cd59-206	PBT	49.9	0.3847	0.3928	0.0206		0.0951
*Omy_cd59b-112	GSI	49.8	0.2589	0.2547	-0.0162		0.0545
Omy_cin-172	GSI	49.7	0.3622	0.3508	-0.0326		0.0515
*Omy_colla1-525	PBT	49.8	0.4114	0.3998	-0.0292		0.062
*Omy_cox1-221	PBT	49.6	0.4479	0.4504	0.0055		0.0641
*Omy_cox2-335	GSI	49.7	0.2342	0.231	-0.0137		0.1032
*Omy_crb-106	PBT	49.1	0.3938	0.4443	0.1137	(4)	0.0668
Omy_CRBF1-1	GSI	44.7	0.1708	0.1907	0.1039	(8)	0.3501
*Omy_e1-147	GSI	49.8	0.1889	0.1892	0.0013		0.1152
*Omy_g1-103	GSI	49.4	0.1096	0.1166	0.0597		0.0816
*Omy_g12-82	PBT	49.8	0.4738	0.4615	-0.0265		0.0339
Omy_G3PD_2-371	GSI	49.8	0.2859	0.2882	0.0082		0.0394
*Omy_gadd45-332	GSI	49.8	0.1065	0.1026	-0.0376		0.1186
*Omy_gdh-271	GSI	49.8	0.2061	0.2066	0.0023		0.0489
*Omy_gh-475	GSI	49.8	0.1878	0.1975	0.0491	(4)	0.0502
*Omy_GHSR-121	GSI	49.8	0.1355	0.1353	-0.0017		0.0639
*Omy_gluR-79	PBT	49.9	0.4871	0.4779	-0.0193		0.0436
*Omy_hsc715-80	PBT	49.8	0.4781	0.4646	-0.0291		0.0681
Omy_hsf1b-241	GSI	44.6	0.1641	0.1661	0.0117		0.3734

*Omy_hsf2-146	PBT	49.5	0.2868	0.3039	0.0562		0.1625
*Omy_hsp47-86	GSI	49.8	0.3855	0.3896	0.0105		0.0509
*Omy_hsp70aPro-329	GSI	49.8	0.128	0.1245	-0.0278		0.0819
Omy_hus1-52	GSI	45.3	0.2197	0.2529	0.1314		0.3173
*Omy_IL17-185	PBT	49.7	0.5339	0.4685	-0.1397		0.059
Omy_Il-1b_.028	PBT	n/a	n/a	n/a	n/a		n/a
*Omy_IL1b-163	GSI	49.7	0.1708	0.1726	0.0105		0.6133
Omy_Il1b-198	PBT	n/a	n/a	n/a	n/a		n/a
*Omy_IL6-320	PBT	49.7	0.3656	0.3528	-0.0365		0.0737
Omy_impa1-55	GSI	45.9	0.0787	0.0782	-0.006		0.5534
*Omy_inos-97	GSI	49.8	0.073	0.0713	-0.0238		0.0411
*Omy_LDHB-1_i2	GSI	49	0.1264	0.1469	0.1399	(13)	0.0403
*Omy_LDHB-2_e5	GSI	49.8	0.3165	0.3126	-0.0127		0.0566
*Omy_LDHB-2_i6	GSI	49.9	0.0763	0.0773	0.0133		0.0689
Omy_lpl-220	GSI	49.9	0.292	0.2874	-0.016		0.0431
*Omy_mapK3-103	GSI	49.8	0.161	0.1641	0.0188		0.1417
*Omy_mcsf-268	GSI	49.8	0.0987	0.0965	-0.0229		0.0715
*Omy_metA-161	PBT	49.8	0.4349	0.4389	0.009		0.0545
*Omy_metB-138	GSI	49.8	0.2122	0.2096	-0.0126		0.0337
*Omy_myclarp404-111	GSI	49.8	0.006	0.0059	-0.0278		0.0241
*Omy_myoD-178	GSI	49.8	0.1969	0.1978	0.0047		0.0541
*Omy_nach-200	GSI	49.9	0.0279	0.0276	-0.0114		0.0209
*Omy_NaKATPa3-50	PBT	49.7	0.4014	0.4	-0.0034		0.0412
*Omy_ndk-152	GSI	49.8	0.1502	0.1472	-0.0208		0.6395
Omy_nips-299	GSI	45.9	0.0987	0.0947	-0.0428		0.5029
*Omy_nkef-241	PBT	49.8	0.4667	0.4646	-0.0045		0.0659
Omy_ntl-27	PBT	49.8	0.4458	0.4234	-0.053		0.0795
Omy_nxt2-273	GSI	49.5	0.1392	0.1377	-0.0105		0.0513
*Omy_Ogo4-212	PBT	n/a	n/a	n/a	n/a		n/a
*Omy_Omyclmk438-96	GSI	49.7	0.0101	0.0097	-0.0428		0.0362
*Omy_OmyP9-180	GSI	49.7	0.2504	0.2567	0.0249		0.1156
*Omy_Ots249-227	PBT	49.7	0.4386	0.439	0.0008		0.1053
*Omy_oxct-85	PBT	49.8	0.2394	0.2366	-0.012		0.1022
*Omy_p53-262	PBT	49.8	0.201	0.2038	0.0137		0.092
*Omy_pad-196	GSI	49.8	0.0406	0.0412	0.0129		0.0424
Omy_ppie-232	GSI	49.8	0.2068	0.209	0.0107		0.042
*Omy_rapd-167	PBT	49.3	0.3218	0.322	0.0005		0.0581
Omy_rbm4b-203	PBT	49.9	0.3439	0.3449	0.0028		0.1086
Omy_redd1-410	PBT	49.8	0.3355	0.32	-0.0485		0.0317
Omy_sast-264	GSI	49.8	0.3134	0.3081	-0.017		0.0908
*Omy_SECC22b-88	GSI	49.8	0.0517	0.0521	0.009		0.0548

Omy_SEXY1	PBT	43.5	0.4034	0.3077	-0.3111	(6)	0.2717
Omy_srp09-37	PBT	49.8	0.3474	0.3347	-0.0379		0.0779
*Omy_sSOD-1	GSI	49.8	0.175	0.1684	-0.039		0.205
*Omy_star-206	GSI	49.8	0.259	0.2525	-0.0259		0.2935
*Omy_stat3-273	PBT	49.8	0.3765	0.3736	-0.0078		0.0608
Omy_sys1-188	GSI	49.9	0.1482	0.14	-0.059		0.0406
*Omy_tlr3-377	GSI	49.9	0.1946	0.1942	-0.0018		0.0285
*Omy_tlr5-205	GSI	49.8	0.2305	0.2221	-0.0377		0.0681
Omy_txnip-343	PBT	49.8	0.383	0.3789	-0.0108		0.1122
*Omy_u07-79-166	GSI	49.8	0.2438	0.2519	0.0319		0.2677
Omy_u09-52.284	GSI	49.8	0.1312	0.1294	-0.0142		0.1039
Omy_u09-53.469	PBT	49.8	0.3849	0.3838	-0.003		0.0676
Omy_u09-54-311	PBT	49.9	0.3731	0.3658	-0.0199		0.07
Omy_u09-56.119	GSI	49.8	0.2773	0.2793	0.0072		0.2425
Omy_U11_2b-154	PBT	49.8	0.3957	0.3892	-0.0166		0.0494
Omy_UT16_2-173	GSI	49.9	0.1877	0.1838	-0.0209		0.0698
Omy_vamp5-303	GSI	49.6	0.3706	0.395	0.0618	(4)	0.1419
Omy_vatf-406	PBT	49.8	0.4784	0.4576	-0.0455		0.0824
Omy_zg57-91	GSI	49.8	0.1391	0.1372	-0.0142		0.0561
OMY1011SNP	PBT	49.7	0.4112	0.4187	0.0179		0.0846
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Mean	n/a	49.2	0.2967	0.2939	-0.0058	n/a	0.1281

Steelhead by population

Population	(n)	Ho	He	F_{is}	# Dev.	%P
01). Abernathy Creek	169.2	0.2992	0.3025	0.0198	(15)	0.9792
02). Asotin Creek	48.9	0.3028	0.3011	0.0003		0.9688
03). Beech Creek	20.3	0.2978	0.2920	-0.0112	(6)	0.8802
04). Big Creek	43.0	0.2717	0.2751	0.0093		0.8542
05). Big Sheep Creek	59.9	0.2817	0.2845	0.0178	(5)	0.9583
06). Camp Creek	24.6	0.2856	0.2769	*-0.0285*		0.8594
07). Clackamas River	57.8	0.3159	0.3140	-0.0038	(4)	0.9271
08). Clackamas River	35.9	0.3147	0.3106	-0.0156		0.9219
09). North Fork Dam	59.9	0.2877	0.2950	0.0190	(8)	0.9063
10). Barrier Dam	93.8	0.2992	0.2956	-0.0073	(6)	0.9271
11). Cow Creek	43.7	0.2854	0.2843	-0.0004		0.9323
12). Crooked Creek	97.7	0.2897	0.2997	0.0293		0.9844
13). North Fork Eagle Creek	43.9	0.3147	0.3147	-0.0010		0.9427
14). Eagle Creek	47.8	0.3034	0.3117	0.0293	(5)	0.9427
15). Elk Creek	46.7	0.2789	0.2738	-0.0219		0.8802

16).	Entiat River	93.9	0.3022	0.3015	0.0032	(4)	0.9792
17).	Entiat Trap	139.6	0.2983	0.3034	0.0215		0.9844
18).	Germany Creek	47.8	0.3068	0.3074	0.0004		0.9479
19).	East/Middle Forks	56.8	0.3225	0.3324	0.0374	(9)	0.9531
20).	West Fork	33.7	0.3087	0.3143	0.0133	(4)	0.9323
21).	Baldy/Bridge creeks	30.0	0.2870	0.2772	-0.0349		0.9479
22).	Lower John Day River	33.2	0.3020	0.2924	-0.0304		0.9271
23).	Pine Creek	17.3	0.2991	0.2921	-0.0236		0.9167
24).	Rock Creek	9.6	0.2793	0.2616	-0.0619		0.8177
25).	Service Creek	10.5	0.2895	0.2804	-0.0362		0.8698
26).	Kalama River	93.9	0.3102	0.3122	0.0132	(5)	0.9427
27).	Kalama River	90.7	0.3016	0.3106	0.0276	(6)	0.9323
28).	East Fork Lewis	78.4	0.2979	0.3044	0.0284	(7)	0.9375
29).	North Fork Lewis	93.9	0.3058	0.3129	0.0281	(8)	0.9583
30).	Lightning Creek	41.9	0.2753	0.2783	0.0022		0.8958
31).	Loon Creek	39.6	0.2840	0.2757	-0.0293		0.8698
32).	Lostine River	44.9	0.2957	0.2983	0.0143	(5)	0.9583
33).	Little Minam River	47.9	0.2888	0.2886	0.0046	(5)	0.9479
34).	Little Rock Creek	16.0	0.3163	0.2914	-0.0785		0.8698
35).	Mad Creek	19.0	0.3348	0.3287	-0.0259		0.9375
36).	Menatchee Creek	61.6	0.3050	0.3038	*-0.0022*		0.9219
37).	Methow River	88.9	0.2984	0.3025	0.0103		0.9583
38).	Middle Fork John Day	66.7	0.2930	0.2902	0.0069	(4)	0.9531
39).	Camp Creek	22.0	0.2956	0.2866	-0.0277		0.8958
40).	Clear Creek	38.9	0.2966	0.2875	-0.0247	(7)	0.9115
41).	Granite Creek	18.5	0.2833	0.2500	-0.0991		0.7760
42).	Mill Creek	44.9	0.3044	0.3048	-0.0015		0.9635
43).	Big Wall Creek	7.0	0.2922	0.2557	-0.1294		0.7604
44).	Camus Creek	18.9	0.2841	0.2824	-0.0057		0.9063
45).	Desolation Creek	18.9	0.2808	0.2793	-0.0063		0.9219
46).	Fox Creek	15.0	0.2774	0.2792	-0.0052		0.8542
47).	North Fork Santiam River	38.9	0.3073	0.3077	-0.0044		0.9115
48).	Pelton Trap	43.4	0.3031	0.3078	0.0163		0.9531
49).	Quinalt River	91.6	0.2841	0.2821	-0.0028	(6)	0.9063
50).	Rattlesnake Creek	16.2	0.3085	0.3008	-0.0160		0.9115
51).	Rock Creek	17.0	0.3265	0.3132	-0.0328		0.8802
52).	Deer Creek	17.9	0.2832	0.2821	*-0.0160*		0.8958
53).	Murderer's Creek	18.0	0.2942	0.2762	-0.0570		0.8854
54).	Shitike Creek	29.9	0.2935	0.3012	0.0262		0.9375
55).	Still Creek	29.9	0.3077	0.3135	0.0154		0.9427
56).	Stolle Meadows	42.0	0.2667	0.2699	0.0078		0.8490
57).	Touchet River	87.7	0.2860	0.2891	0.0119	(6)	0.9583
58).	Upper Tucannon River	42.5	0.2960	0.2876	-0.0167		0.9375

59).	Wenaha River	93.6	0.2833	0.2918	0.0295	(6)	0.9635
60).	Wiley Creek	92.6	0.3018	0.3096	0.0326	(7)	0.9427
61).	Cougar Creek	4.0	0.3108	0.2760	-0.1310		0.7448
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	Mean	49.1	0.2967	0.2939	-0.0068		0.9153

Section 3: Genetic Stock Identification of Chinook Salmon Mixtures in the Mainstem Columbia River

Introduction

Genetic Stock Identification (GSI) methods have proven to be effective in determining the proportion of stock origin in several mixed stock applications of Chinook salmon (Shaklee et al. 1999, Beacham et al. 2006). These methods have been demonstrated to be useful even at relatively fine geographic scales such as within the Columbia River Basin (CRB) (Hess et al. 2011). Within the CRB, Chinook salmon consist of three major genetic lineages, which can be further broken into populations that are genetically structured on a finer spatial scale (e.g., Waples et al. 2004). Partitioning of CRB Chinook salmon populations into twelve reporting groups has been documented for informativeness and accuracy in various types of GSI applications (Hess et al. 2011, Hess et al. in press), and these reporting groups were also used in the analyses of this study.

Chinook salmon fisheries in the mainstem of the lower Columbia River provide an ideal and important application of GSI because the fish harvested consist of mixtures of stocks from the entire CRB. In addition, fisheries in this location represent a majority of the CRB harvest of this species taken by the commercial, sport, and tribal fishermen. In order to help establish sustainable fisheries, GSI can be used to address two primary questions: 1) how are Chinook salmon stocks temporally and spatially distributed in the mainstem lower Columbia River; and 2) how are these stocks temporally and spatially distributed in the harvests of fisheries. This information would then have the potential to be used by fisheries managers to shape the various fisheries in a sustainable way that would protect less abundant Chinook salmon stocks, while targeting the stocks of adequate abundance.

Thus, our study had two primary objectives: 1) utilize GSI techniques to estimate stock composition of Chinook salmon passing Bonneville Dam; and 2) determine stock composition of Chinook salmon harvested in sport, commercial, and tribal fisheries in the mainstem Columbia River. Specifically for this study, we represented the total fish present in the mainstem Columbia River by sampling fish at a fixed point at Bonneville Dam and we represented both spring- and fall-run Chinook salmon harvested above and below Bonneville Dam by sampling sport, commercial, and tribal fisheries in the 2010 season. To estimate temporal stock distributions, we obtained sample sizes that were large enough to analyze Chinook salmon stocks from these fisheries and Bonneville Dam across weekly strata. We characterized spatial distributions of Chinook salmon stocks by pooling fishery samples into two regions located within the downriver and upriver side of Bonneville Dam.

In this report, we utilized 92 previously developed single nucleotide polymorphism (SNP) loci to genotype unknown mixture samples to estimate stock composition of these mixtures, by fishery, location, and weekly strata. Although we have recently shown that SNPs are nearly as powerful as microsatellite markers for performing GSI using simulations and statistical power analyses (Hess et al. 2011), it is important to assess the true accuracy of this method. Therefore, here we validate the accuracy of the GSI analysis by comparing the known origins of subsets of fish that contain coded wire tags and our estimated origins of these same fish using GSI. Identified weaknesses of the baseline and the power of these 92 SNP markers are discussed as well as the strategy we will employ to improve the overall power of GSI for our specific fisheries

applications. The use of these high-throughput SNP markers could make in-season GSI applications possible in future years.

Methods

Tissue collection

Tissues were sampled from a total of five different mixture sources: 1) Bonneville Dam (entire run), and the spring- and fall-run seasons of the following fisheries: 2) sport, 3) test, 4) commercial, and 5) tribal. Chinook salmon were non-lethally sampled at the Bonneville Adult Fish Facility (AFF) throughout the run from April until October of 2010 (statistical weeks 16-43).

Biological data such as species, date, length, presence/absence of adipose fin, were recorded for each individual that was tissue-sampled for genetic analysis. We collected tissue samples, in the form of fin punches from each Chinook salmon. After non-lethal sampling is completed, all fish were released to a recovery pond and then to the fish ladder to continue upstream migration. Tissues samples were preserved in ethanol or dried on Whatman filter paper (Lahood et al. 2008) before being shipped to the Hagerman Fish Culture Experiment Station for GSI and estimation of stock composition. This sampling effort is covered under Scientific Research Permit #1379 under Section 10 of the ESA (permit included in PISCES attachments).

Table 1. Details of strata of spring-run Chinook salmon mixture sources. Week 8 equals 2/15/2010-1/21/2010 and week 20 equals 5/10/2010-5/16/2010.

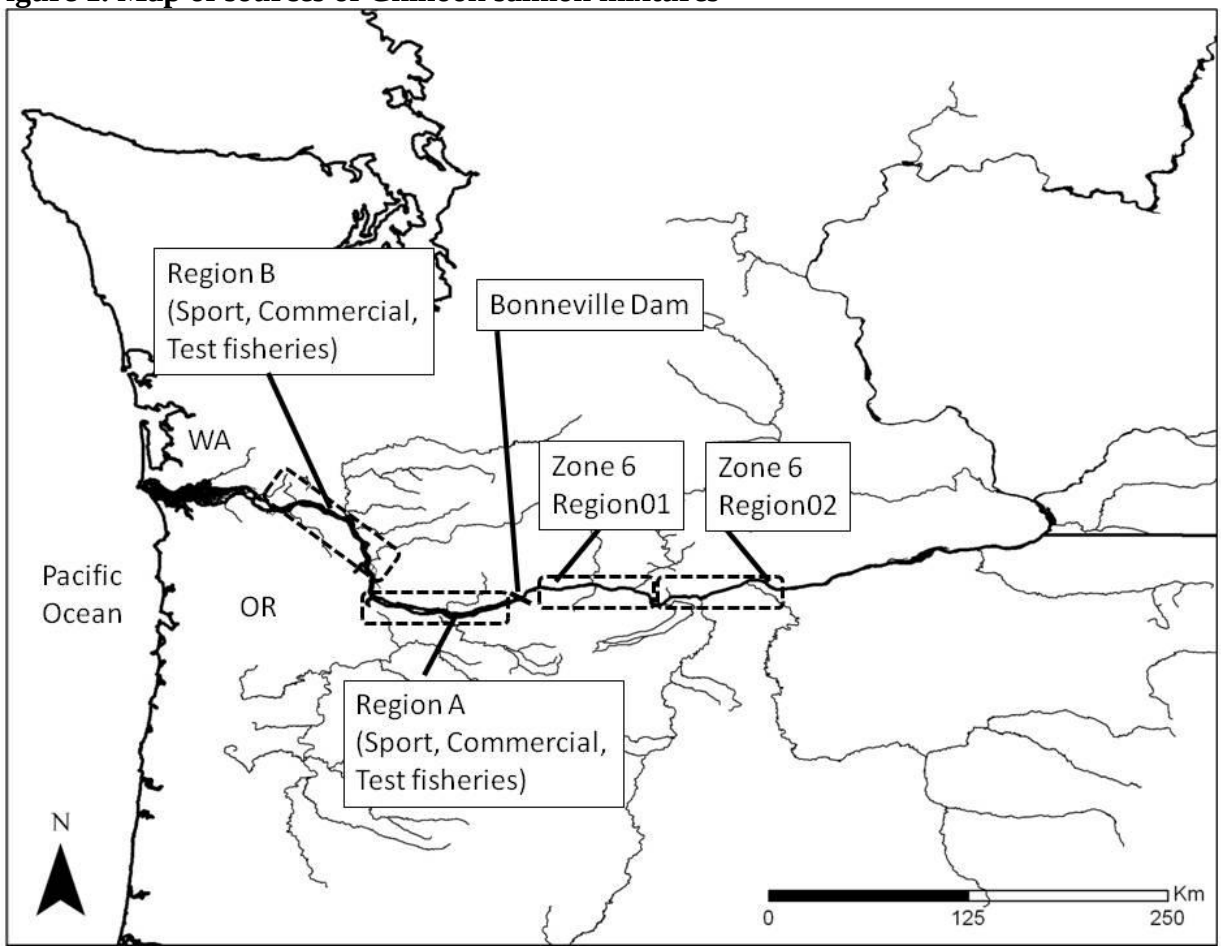
Spring Fishery			Statistical week													
Source	Region	Type	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Sport	A	CWT	0	0	0	5	10	17	19	45	34	0	0	0	0	130
		regular	0	0	6	21	73	83	80	55	66	0	0	0	0	384
	B	CWT	0	1	4	8	16	21	2	8	38	0	0	0	0	98
		regular	1	2	16	42	80	77	15	55	61	0	0	0	0	349
Commercial	A	CWT	0	0	0	0	0	0	0	76	0	0	0	0	0	76
		regular	0	0	0	0	0	0	118	6	0	0	0	0	0	124
	B	CWT	0	0	0	0	0	0	32	178	0	0	0	0	0	210
		regular	0	0	0	0	0	0	117	101	0	0	0	0	0	218
Onboard	A	regular	0	0	0	0	0	0	0	5	0	0	0	0	0	5
	B	regular	0	0	0	0	0	0	11	16	0	0	0	0	0	27
Test	A	regular	0	0	0	0	0	0	0	0	60	24	0	0	4	88
	B	regular	0	0	1	5	0	44	0	63	0	26	0	0	79	218
Bonneville Dam	--	regular	0	0	0	0	0	0	0	0	40	149	-	-	-	189
Ceremonial	01	regular	0	0	0	0	0	0	9	182	223	177	0	0	0	591

Table 2. Details of strata of fall-run Chinook salmon mixture sources. Week 32 equals dates 8/2/2010-8/8/2010 and week 43 equals 10/18/2010-10/24/2010.

Fall Fishery			Statistical week												
Source	Region	Type	32	33	34	35	36	37	38	39	40	41	42	43	Total
Sport	A	CWT	3	1	2	3	2	1	4	7	1	0	0	0	24
		regular	2	4	22	49	55	41	55	65	35	0	0	0	328
	B	CWT	1	0	6	12	18	5	0	0	0	0	0	0	42
		regular	0	22	50	88	131	94	0	0	0	0	0	0	385
Commercial	A	CWT	9	12	14	81	0	0	0	34	1	11	1	3	166
		regular	42	88	86	68	0	0	0	116	6	55	60	60	581
	B	CWT	9	52	0	0	0	0	0	0	0	1	11	1	74
		regular	91	95	0	0	0	0	0	0	0	99	89	13	387
Bonneville Dam	--	regular	6	18	0	93	203	178	164	179	151	94	37	0	1123
Tribal	01	regular	0	0	0	118	282	282	240	0	67	0	0	0	989
	02	regular	0	0	0	198	186	192	187	0	223	0	0	0	986

Tissues were sampled in 2010 from Chinook salmon fisheries with existing programs in place with Washington Department of Fish and Wildlife (WDFW), Oregon Department of Fish and Wildlife (ODFW), Warm Springs tribe, and Yakama Nation. The spring-run fisheries were sampled below Bonneville Dam in the sport, commercial, and test fishery (regions A and B), and sampled above Bonneville Dam in region 01 as part of the Warm Springs tribal ceremonial fishery (Figure 1, Table 1). The fall-run fisheries were sampled above Bonneville Dam (Zone 6 Yakama Nation tribal fishery) and below Bonneville Dam (regions A and B via the sport and commercial fishery, Table 2). The tribal fall-run and the sport spring-run Chinook salmon fisheries were sampled in large enough numbers over multiple weeks that made it possible to characterize these fisheries by weekly strata in two regions - the sport and tribal fisheries were divided into regions A and B and regions 01 and 02, respectively. Region A, corresponds to our grouping of pre-existing Oregon and Washington state sport fishing zones 1-4 (or commercial zones 4-5) and region B corresponds to our grouping of sport zones 5-10 (or commercial zones 1-3). Region 01 and region 02 in the Zone 6 fishery correspond to pre-existing Oregon and Washington state fishing zone 61 and a grouping of zones 62 and 63, respectively. These sets of groupings were established for this study in order to achieve balanced sampling for analysis of these fishery datasets, as well as to set an appropriate spatial scale of analysis to minimize variance of our estimates of stock proportions over temporal strata.

Figure 1. Map of sources of Chinook salmon mixtures



Fisheries on spring-run Chinook salmon have the advantage of widespread hatchery markings to distinguish hatchery fish from natural origin fish based on the absence or presence of the adipose fin, respectively. These adipose markings make it possible to have a mark selective sport and commercial fishery, in which only fish with missing adipose fins (hatchery-origin) are legally retained. In addition to sampling hatchery-origin fish from the mark selective commercial and sport fisheries, we were able to obtain samples from natural-origin fish before they were released from commercial and sport fishing vessels via the monitoring program of WDFW and ODFW. Samples of natural-origin fish were also obtained from the Test fishery operated by WDFW and ODFW. However, only the "onboard monitoring" mixture contained all natural-origin fish samples and in sufficient numbers to compare the other fishery mixtures from the spring-run fisheries to determine whether there was a significant effect of "origin" on stock proportions.

Molecular data

The following 92 SNP loci were used for genotyping Chinook salmon: Ots_102414-395, Ots_105105-613, Ots_106747-239, Ots_110064-383, Ots_nramp-321, Ots_113242-216, Ots_113457-40R, Ots_123048-521, Ots_128757-61R, Ots_94857-232R, Ots_94903-99R, Ots_96222-525, Ots_96500-180, Ots_96899-357R, Ots_97077-179R, Ots_AldB1-122,

Ots_aldb-177M, Ots_ARNT, Ots_arp-436, Ots_AsnRS-60, Ots_aspat-196, Ots_C3N3, Ots_Cath_D141, Ots_CCR7, Ots_CD59-2, Ots_CD63, Ots_cox1-241, Ots_CRB211, Ots_E2-275, Ots_EndoRB1-486, Ots_EP-529, Ots_ETIF1A, Ots_FARSLA-220, Ots_FGF6B_1, Ots_GDH-81x, Ots_GH2_1, Ots_GnRH-271, Ots_GPDH-338, Ots_GPH-318, Ots_GST-207, Ots_GST-375, Ots_GTH2B-550, Ots_hsc71-3'-488, Ots_hsp27b-150, Ots_HSP90B-100, Ots_IGF-I.1-76, Ots_Ikaros-250, Ots_IL11, Ots_IL8R_C8, Ots_mapK-3'-309, Ots_mapKpr-151, Ots_MHC1, Ots_MHC2, Ots_mybp-85, Ots_Myc-366, Ots_myo1a-384, Ots_myoD-364, Ots_nkef-192, Ots_NOD1, Ots_OPLW173_1, Ots_Ots311-101x, Ots_P450, Ots_P53, Ots_PGK-54, Ots_Prl2, Ots_RAG3, Ots_RAS1, Ots_RFC2-558, Ots_S7-1, Ots_SClkF2R2-135, Ots_SL, Ots_SWS1op-182, Ots_TAPBP, Ots_TGFB, Ots_TLR3, Ots_TNF, Ots_Tnsf, Ots_u07-07.161, Ots_u07-17.135, Ots_u07-18.378, Ots_u07-20.332, Ots_u07-25.325, Ots_u07-49.290, Ots_u07-53.133, Ots_u07-57.120, Ots_u07-64.221, Ots_u202-161, Ots_u211-85, Ots_u4-92, Ots_u6-75, Ots_unk526, and Ots_ZR-575. In addition, a single locus linked to the Y chromosome (Ots_SEXY1) was used to determine the sex of fish from the fishery mixtures as well as Bonneville Dam. See previous sections on SNP marker and baseline development for details of laboratory protocols involved in using these DNA markers for genotyping fish.

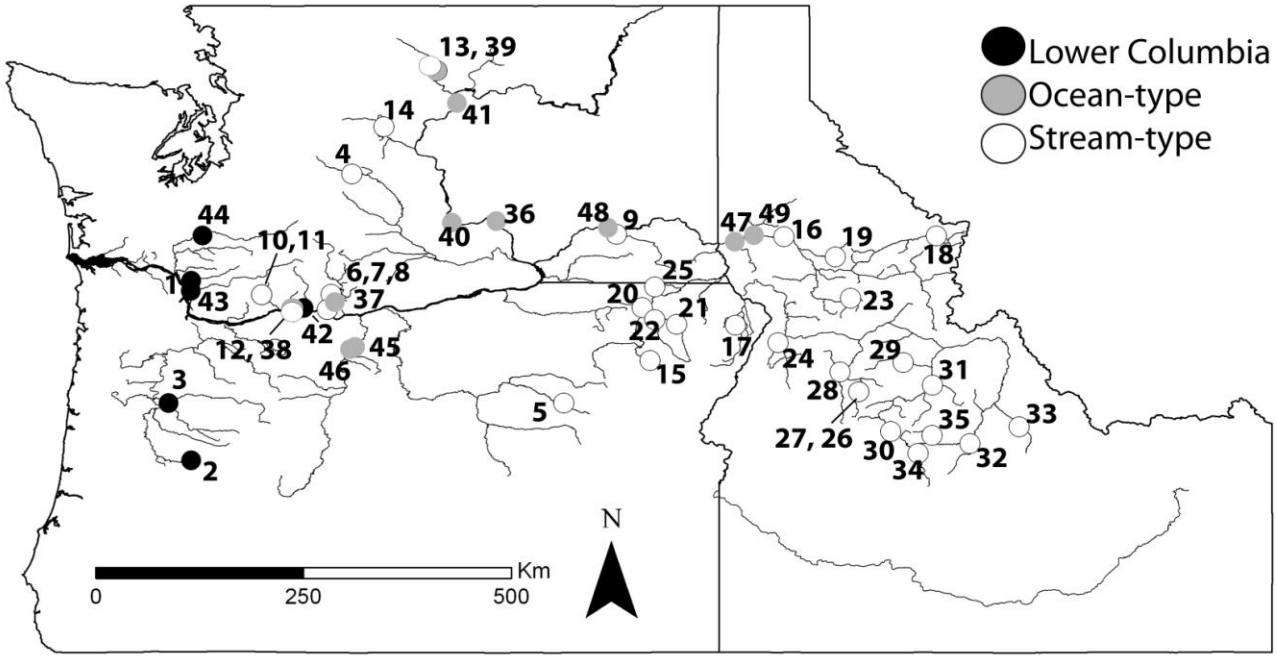


Figure 2. Map of the Columbia River Basin and collection locations of baseline samples. The circles on the map indicate the collection locations, and shading corresponds to the membership of these collections to the three major Chinook salmon lineages present in the Columbia River basin.

Statistical Analyses

SNP genotype data was utilized to estimate stock composition using the pre-expansion version of the Chinook salmon SNP baseline. We grouped 51 baseline populations (Figure 2, Table 3) into twelve reporting groups that were determined primarily by the relative genetic similarity among populations according to a phylogenetic analysis. Genetic distances were computed from allele

frequencies according to Cavalli-Sforza and Edwards (1967), with the PHYLIP v 3.69 (Felsenstein 1989) executable "Gendist", after processing of dataset for bootstrapping (1000 replicates), using PHYLIP executable "Seqboot". Distances were clustered using the Neighbor – Joining method (Saitou and Nei, 1987), and a consensus tree was built with the PHYLIP executable "Consense"). The Phylip package is available at the following link: http://evolution.genetics.washington.edu/phylip/

Mixture simulations were examined with the program ONCOR v1.0 (available at http://www.montana.edu/kalinowski) using the "100% simulations" feature to evaluate the power of the baseline to analyze mixture samples at the reporting group level (Anderson et al. 2008). A threshold of 90% correct assignment has been demonstrated to be an appropriate criterion for assessing the power to assign back to reporting groups (Beacham et al. 2006; Seeb et al. 2007). For these 100% simulations we set the parameters of mixture sample size and number of iterations to the values of 200 and 1000, respectively. Genotypes from fisheries mixtures were also analyzed in ONCOR to estimate stock composition by fishery and strata. We analyzed all strata that had $n > 20$ samples. These mixture proportions were generated with 95% confidence intervals using 1000 bootstraps.

Table 3. Sample sizes and reporting groups of baseline populations. Chinook salmon baseline collections (n=4014). Lineages are: ST- stream type, OT – ocean type, and LC – Lower Columbia. See Figure 2 for location by population (pop) number. Origin indicates whether fish were produced from a hatchery (HAT) or from natural spawning (NOR).

Population ID	Pop #	(N)	Reporting Group	Latitude	Longitude	Origin	Run	Lineage
Kalama R.	1	78	L_Columbia_R_sp	46.022	-122.734	HAT	Spring	LC
McKenzie R.	2	82	Willamette_R	44.117	-123.086	HAT	Spring	LC
North Santiam R.	3	79	Willamette_R	44.697	-122.983	HAT	Spring	LC
Cle Elum R.	4	76	Mid_Columbia_R_sp	47.180	-121.002	HAT	Spring	ST
John Day R.	5	66	Mid_Columbia_R_sp	44.698	-118.713	NOR	Spring	ST
Klickitat R.	6	145	Mid_Columbia_R_sp	45.710	-121.270	HAT	Spring	ST
Klickitat R.	7	169	Mid_Columbia_R_sp	45.710	-121.270	NOR	Spring	ST
Klickitat R.	8	125	Mid_Columbia_R_sp	45.710	-121.270	BrSt	Spring	ST
Tucannon R.	9	64	Mid_Columbia_R_sp	46.526	-118.142	NOR	Spring	ST
Carson-NFH	10	91	U_Columbia_R_sp	45.872	-121.977	HAT	Spring	ST
Winthrop-NFH	11	69	U_Columbia_R_sp	48.476	-120.187	HAT*	Spring	ST
Little White Salmon R.	12	92	U_Columbia_R_sp	45.713	-121.639	HAT	Spring	ST
Methow R.	13	80	U_Columbia_R_sp	48.299	-120.065	HAT	Spring	ST
Chiwawa R.	14	83	U_Columbia_R_sp	47.593	-120.656	NOR	Spring	ST
Catherine Cr.	15	77	RapidR_Clearwater_sp	45.158	-117.779	NOR	Spring	ST
Dworshak-NFH	16	83	RapidR_Clearwater_sp	46.501	-116.329	HAT	Spring	ST
Imnaha R.	17	67	RapidR_Clearwater_sp	45.561	-116.834	NOR	Spring	ST
Lochsa R.	18	74	RapidR_Clearwater_sp	46.508	-114.681	HAT	Spring	ST
Lolo Cr.	19	77	RapidR_Clearwater_sp	46.279	-115.775	NOR	Spring	ST
Lookingglass Cr.	20	89	RapidR_Clearwater_sp	45.731	-117.864	HAT	Spring	ST
Lostine R.	21	74	RapidR_Clearwater_sp	45.537	-117.478	Unknown	Spring	ST
Minam R.	22	79	RapidR_Clearwater_sp	45.600	-117.729	NOR	Spring	ST
Newsome Cr.	23	74	RapidR_Clearwater_sp	45.831	-115.608	NOR	Spring	ST
Rapid R.	24	93	RapidR_Clearwater_sp	45.353	-116.394	HAT	Spring	ST
Wenaha R.	25	43	RapidR_Clearwater_sp	45.956	-117.728	NOR	Spring	ST
Johnson Cr. (weir)	26	88	SF_Salmon_sp	44.899	-115.492	NOR	Spring	ST
Johnson Cr.	27	65	SF_Salmon_sp	44.958	-115.499	HAT	Spring	ST
Secesh R.	28	77	SF_Salmon_sp	45.033	-115.722	NOR	Spring	ST
Big Cr.	29	91	MF_Salmon_sp	45.138	-115.038	NOR	Spring	ST
Cape Horn Cr.	30	77	MF_Salmon_sp	44.388	-115.174	NOR	Spring	ST
Camas Cr.	31	43	MF_Salmon_sp	44.892	-114.721	NOR	Spring	ST
East Fork Salmon R.	32	91	Upper_Salmon_sp	44.259	-114.317	NOR	Spring	ST
Pahsimeroi R.	33	86	Upper_Salmon_sp	44.441	-113.787	HAT/NOR	Spring	ST
Sawtooth Hatchery	34	90	Upper_Salmon_sp	44.152	-114.881	HAT	Spring	ST
W. F. Yankee Fork	35	58	Upper_Salmon_sp	44.349	-114.727	NOR	Spring	ST
Hanford Reach	36	81	U_Columbia_R_su/fa	46.713	-119.481	NOR	Sum/Fall	OT
Klickitat R.	37	87	U_Columbia_R_su/fa	45.870	-121.098	NOR	Sum/Fall	OT
Little White Salmon R.	38	91	U_Columbia_R_su/fa	45.713	-121.639	HAT	Sum/Fall	OT
Methow R.	39	75	U_Columbia_R_su/fa	48.293	-120.065	NOR	Sum/Fall	OT
Priest Rapids Hatchery	40	76	U_Columbia_R_su/fa	46.629	-119.872	HAT	Sum/Fall	OT
Wells Dam	41	83	U_Columbia_R_su/fa	47.946	-119.867	HAT	Sum/Fall	OT
Spring Cr.	42	75	L_Columbia_R_fa	45.728	-121.521	HAT	Fall	LC
Lewis R.	43	84	L_Columbia_R_fa	45.953	-122.584	NOR	Fall	LC
Cowlitz R.	44	81	L_Columbia_R_fa	46.466	-122.740	HAT	Fall	LC
lower Deschutes	45	74	Deschutes_R_fa	45.280	-121.020	NOR	Fall	OT
upper Deschutes	46	86	Deschutes_R_fa	44.878	-121.048	NOR	Fall	OT
Clearwater R._GAPS	47	60	Snake_R_fa	46.520	-116.610	NOR	Fall	OT
Lyons Ferry Hatchery	48	84	Snake_R_fa	46.589	-118.220	HAT	Fall	OT
Nez Perce Tribal Hatchery	49	82	Snake_R_fa	46.519	-116.665	HAT	Fall	OT

Under origin, "BrSt" is broodstock, and under run, "sum" is summer. **Carson National Fish Hatchery*.

Results

Power analysis of baseline

The 51 collections were grouped into 12 reporting groups based on the clustering we observed in the phylogenetic analysis (Figure 3). We used ONCOR to simulate 100% mixtures from each of the baseline collections, and a majority (45 of 51; 88.2%) of the simulated mixtures were estimated to be composed of greater than 90% proportion of the correct reporting group (Figure 3). However, six collections showed less than 90% proportions, two were significantly below 90% (as indicated by their upper 95% confidence interval). These two collections were John-Day R. (middle Columbia R. sp) and Dworshak Hatchery (Rapid R./Clearwater R. sp). These collections may be affected by high straying rates (e.g. John-Day R.) or out-of-basin hatchery stock sources (e.g. upper Columbia R. fish in Dworshak Hatchery).

ONCOR was also used to assign individuals from the baseline in a "leave-one-out" analysis to estimate correct individual assignment (Figure 3). This accuracy measure showed much poorer performance of baseline power, where only a half of the total baseline collections produced greater than 75% correct individual assignment. Among the worst reporting groups in terms of average percent correct individual assignment of the baseline collections were lower Columbia R. spring (52%), Upper Columbia R. spring (61%), and Snake R. fall (61%) reporting groups.

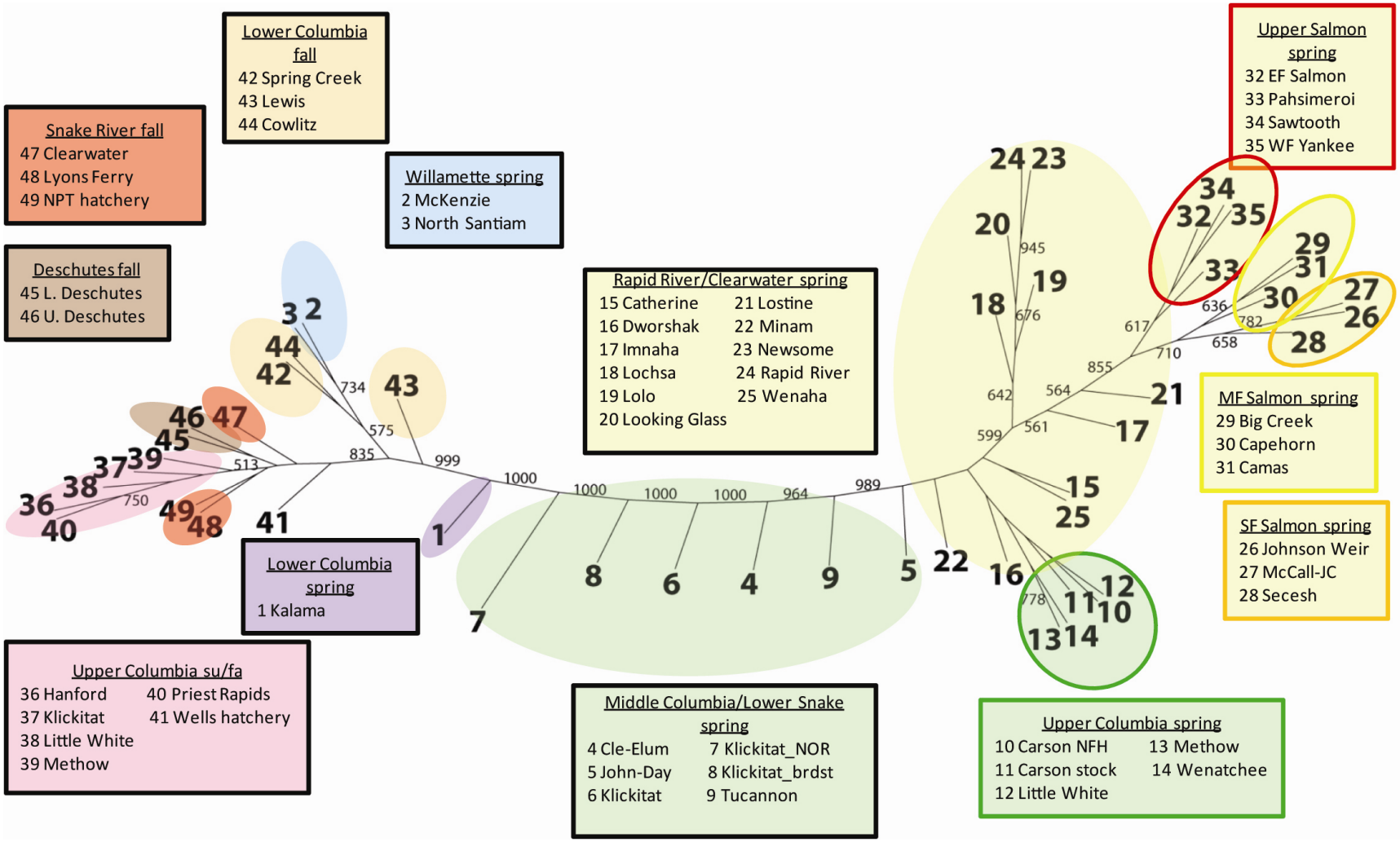


Figure 3. Neighbor Joining tree of Chinook salmon baseline populations based on Cavali-Sforza genetic distance of SNP loci. The boxes indicate the twelve reporting groups that were used to group populations based on genetic similarity, life history, and geographic proximity.

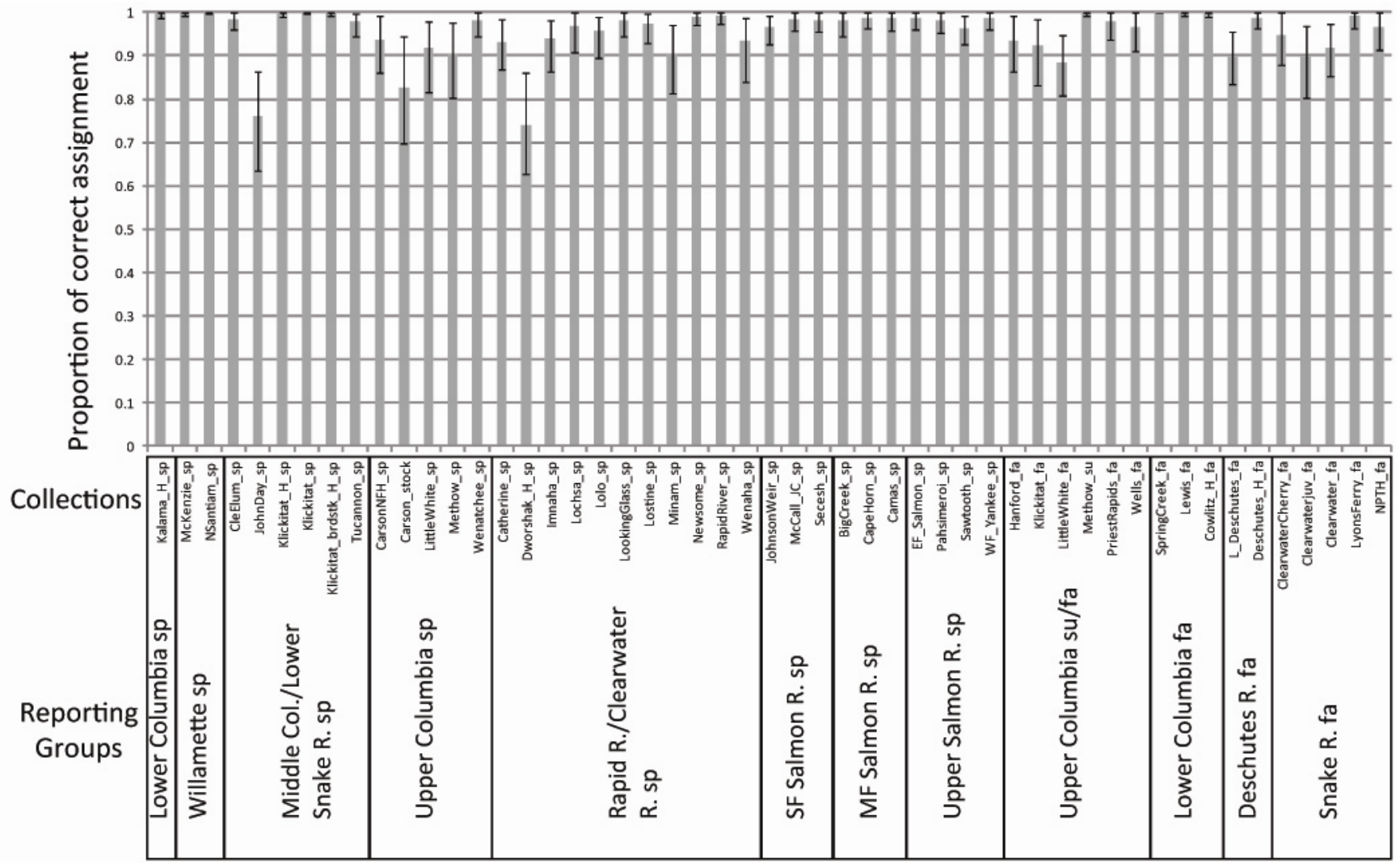


Figure 4. Stock proportion results from 100% mixture simulations of reporting groups using ONCOR.

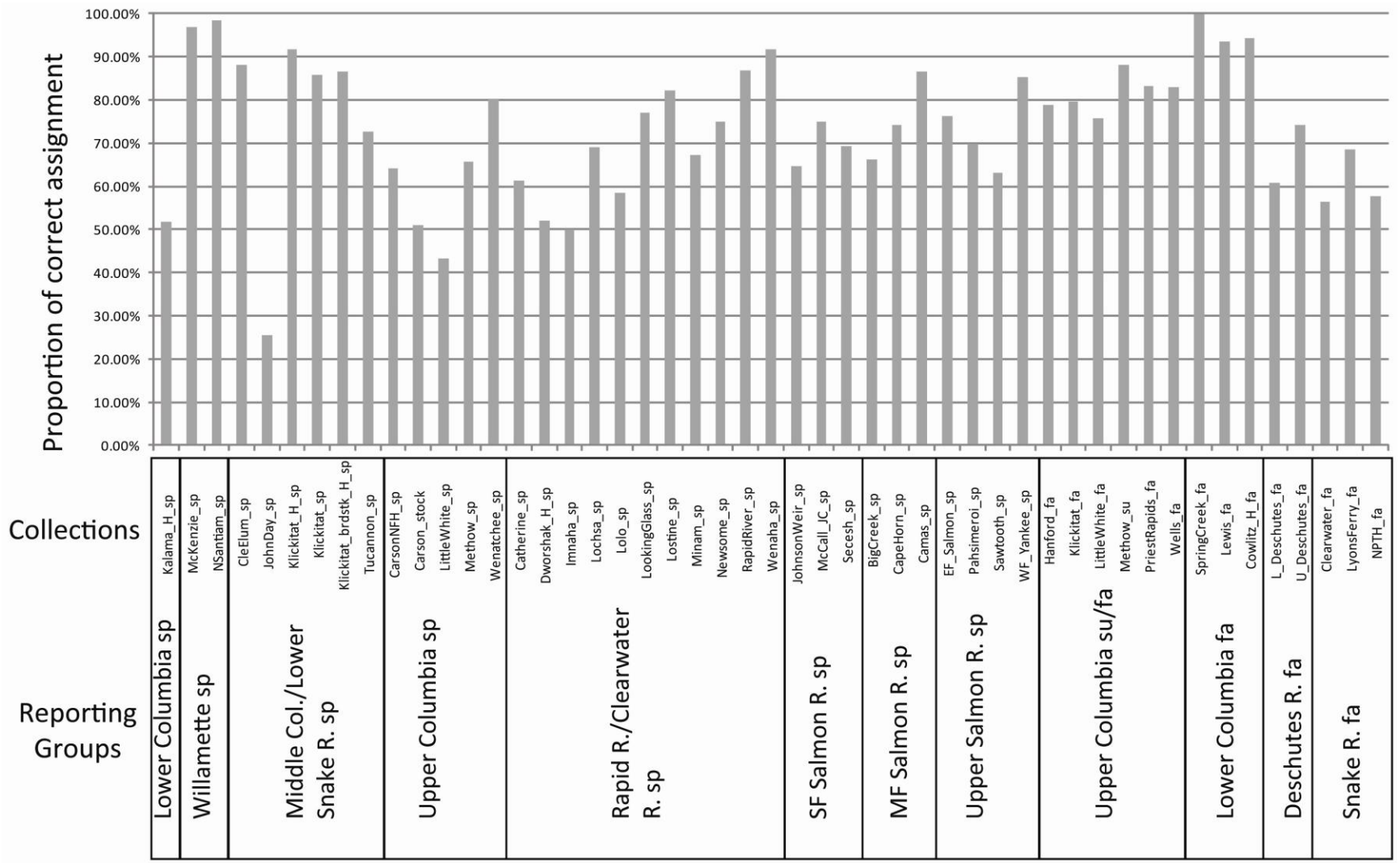


Figure 5. Correct assignment results from leave-one-out analysis of baseline individuals assigned to reporting groups using ONCOR.

Table 4. Concordance between GSI assignment of spring-run Chinook salmon and coded wire tag data.

Reporting group	CWT	L_Columbia_R_sp	Willamette_R	Mid_Columbia_R_sp	U_Columbia_R_sp	RapidR_Clearwater_sp	SF_Salmon_sp	MF_Salmon_sp	Upper_Salmon_sp	U_Columbia_R_su/fa	L_Columbia_R_fa	Deschutes_R_fa	Snake_R_fa	Deschutes_R_sp	Umatilla_R_sp
DNA	**N**	**21**	**94**	**71**	**110**	**156**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**60**	**2**
L_Columbia_R_sp	12	9	1	0	0	0	0	0	0	0	0	0	0	0	0
Willamette_R	102	5	86	1	1	2	0	0	0	0	0	0	0	2	0
Mid_Columbia_R_sp	77	0	2	58	3	2	0	0	0	0	0	0	0	10	0
U_Columbia_R_sp	172	0	2	9	83	32	0	0	0	0	0	0	0	40	1
RapidR_Clearwater_sp	161	0	3	3	21	119	0	0	0	0	0	0	0	8	1
SF_Salmon_sp	3	0	0	0	2	1	0	0	0	0	0	0	0	0	0
MF_Salmon_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Upper_Salmon_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U_Columbia_R_su/fa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L_Columbia_R_fa	7	7	0	0	0	0	0	0	0	0	0	0	0	0	0
Deschutes_R_fa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Snake_R_fa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Concordant assignment		76.19%	91.49%	81.69%	75.45%	76.28%								†66.67%	‡50.00%

CWT source hatcheries
L_Columbia_R_sp: Friends of the Cowlitz,Fallert Creek H.,Kalama Falls H., Lewis R. H.
Willamette_R: Clackamas H., Dexter ponds, Gnat Cr. H., Leaburg H., Marion Forks H., McKenzie H., S. Santiam H., Willamette H.
Mid_Columbia_R_sp: Cle Elum H., Klickitat H.
U_Columbia_R_sp: Carson NFH, Chiwawa H., Leavenworth H., Little White Salmon NFH, Ringold H., Winthrop NFH
RapidR_Clearwater_sp: Clearwater H., Dworshak H., Kooskia H., Lookingglass H., NPT H., Rapid R. H., Wallowa H.
†Deschutes_R_sp: Round Butte H., Warm Springs NFH; Concordance based on most similar reporting group- U_Columbia_R_sp
‡Umatilla_R_sp:Umatilla H.; Concordance based on most similar reporting group- U_Columbia_R_sp

Concordance of GSI results and CWT data

In addition to the 100% mixture simulations and leave-one-out analysis, we used coded wire tag (CWT) recoveries from the Chinook salmon harvest mixture to assess the accuracy of our GSI methods. As many as 514 and 306 CWTs were recovered from the spring-run and fall-run sport/commercial fisheries, respectively (Tables 1, 2). The percentage of individuals that were assigned to a reporting group using GSI and were confirmed accurate with CWT recovery data was used to quantify concordance between these data types. Not every reporting group could be examined in this way, however, data was available to analyze five reporting groups for the spring-run harvest (Table 4). It was first necessary to group CWT source hatcheries into similar reporting groups as used by the GSI analysis. Most CWT source hatcheries were compatible with the GSI reporting groups with the exception of the Deschutes R. and Umatilla R. spring-run groups that were missing representation in the genetic baseline. The five reporting groups that were represented by both CWT source hatcheries and genetic baseline collections resulted in concordance levels above 75%, and the Willamette R. spring group achieved highest

concordance (91%). For the lower Columbia spring group concordance score, we pooled individuals genetically assigned to the lower Columbia fall group, because the genetic baseline was missing Lewis and Cowlitz R. spring-run collections.

Table 5. Concordance between GSI assignment of fall-run Chinook salmon and coded wire tag data

Reporting group	CWT	L_Columbia_R_sp	Willamette_R	Mid_Columbia_R_sp	U_Columbia_R_sp	RapidR_Clearwater_sp	SF_Salmon_sp	MF_Salmon_sp	Upper_Salmon_sp	U_Columbia_R_su/fa	L_Columbia_R_fa	Deschutes_R_fa	Snake_R_fa	Rogue_R	Umatilla_R_fa
DNA	**N**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**60**	**55**	**0**	**144**	**4**	**28**
L_Columbia_R_sp	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Willamette_R	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Mid_Columbia_R_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U_Columbia_R_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RapidR_Clearwater_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SF_Salmon_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF_Salmon_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Upper_Salmon_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U_Columbia_R_su/fa	159	0	0	0	0	0	0	0	0	57	1	0	62	0	18
L_Columbia_R_fa	58	0	0	0	0	0	0	0	0	0	51	0	1	1	0
Deschutes_R_fa	9	0	0	0	0	0	0	0	0	0	0	0	5	2	1
Snake_R_fa	93	0	0	0	0	0	0	0	0	3	0	0	76	1	9
Concordant assignment										95.00%	92.73%		52.78%	†NA	‡64.28%

CWT source hatcheries
U_Columbia_R_su/fa: Carlton Rearing Pond, Klickitat H., Little White Salmon NFH, Priest Rapids, Similkameen H., Wells H., Wenatchee
L_Columbia_R_fa: Big Cr. H., Clackamas H., Cowlitz H., Elochoman H., Kalama Falls H., Kalama H., N. Toutle H., Spring Cr. H., Washougal H.
Snake_R_fa: Lyons Ferry H., Nez Perce H., Oxbow H.
†Rogue_R: Clatsop Economic Development Council (CEDC) Youngs Bay, Klaskanine H.; Outside-Columbia-River-Basin stock
‡Umatilla_R_fa: Broodstock from U_Columbia_R_su/fa group but collection not represented in genetic baseline.

For the fall-run harvest, the following three reporting groups were represented by both CWT source hatcheries and genetic baseline collections: Upper Columbia R. summer/fall, lower Columbia R. fall, and Snake R. fall reporting groups. The Snake R. fall group produced the lowest concordance score (53%), whereas the other two groups were above 90%. Two groups were not represented in the genetic baseline, Rogue R. (an out-of-basin derived stock in lower Columbia hatcheries) and the Umatilla R. fall group. The majority of misassigned fish were those assigned to the Upper Columbia R. summer/fall group using GSI, but originated from Snake R. fall hatcheries according to CWT information. Therefore, this misassignment may generally inflate the numbers of Upper Columbia R. summer/fall, while incorrectly downward biasing estimates of Snake R. fall fish. This error is likely a result of the close phylogenetic relationship observed for these two groups (Figure 3).

Stock proportions of the spring-run Chinook mixture sources

Comparisons of total stock composition across the different sources of fishery mixtures revealed that these various mixtures are distinguished from each other in the following two ways: 1)Some of the mixture sources contain significant proportions of stocks that are absent from other sources, or 2) A specific stock was present in all mixture sources but in significantly different proportions.

For the spring-run Chinook salmon collected from five mixture sources (harvests from four types of fisheries and non-lethal sampling at Bonneville Dam), we found that the Willamette River spring-run was one of the stocks present in some mixtures but lacking in others. This stock was present in all the fisheries below Bonneville Dam, but absent at both Bonneville Dam and the Ceremonial fishery in Zone 6 (Figure 6).

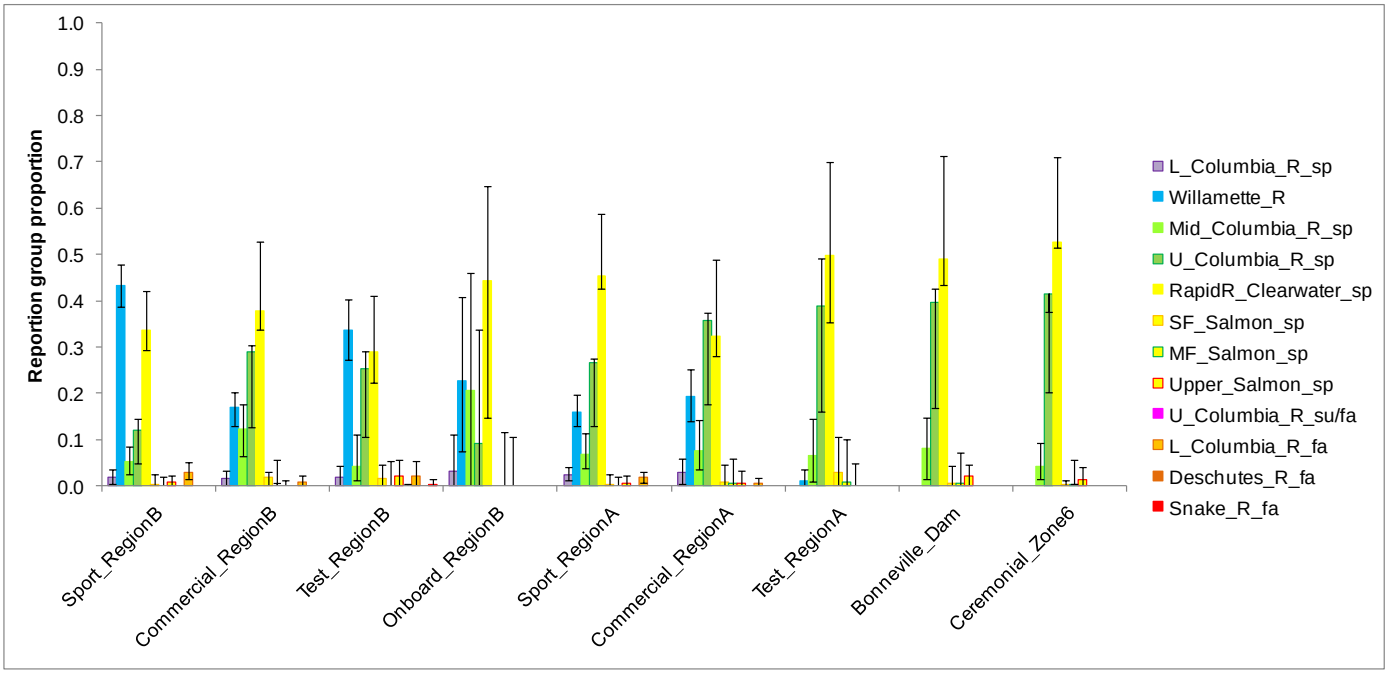


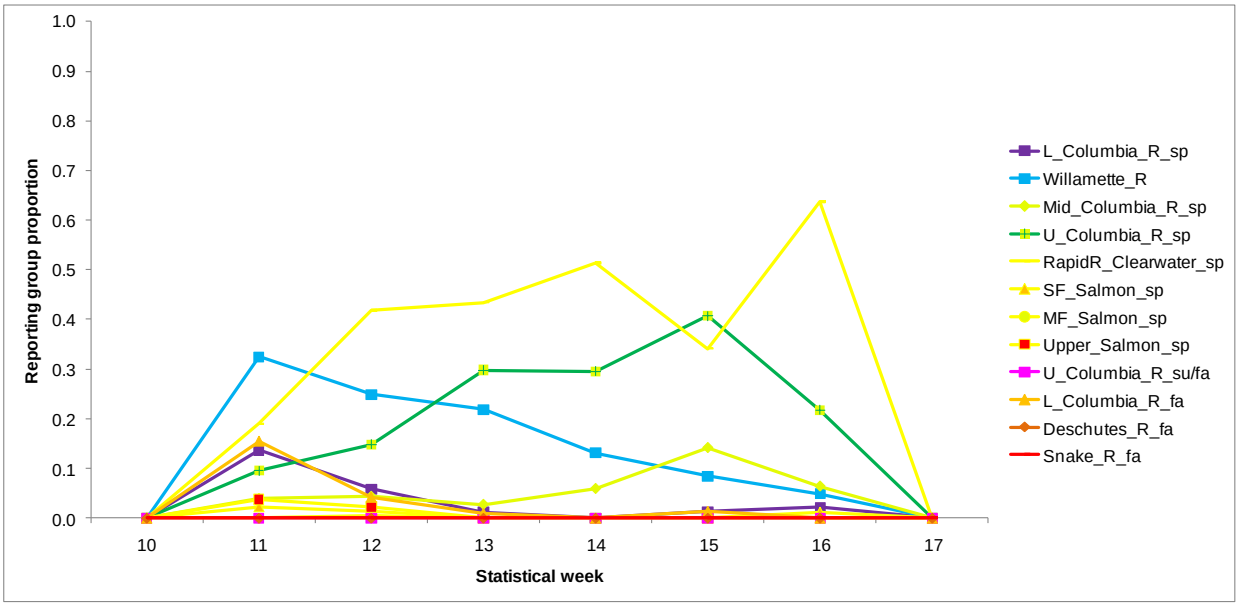
Figure 6. Comparison of stock proportions across all spring-run Chinook salmon mixture sources. Only comparable weeks (weeks 16-17) from Bonneville Dam were included.

The temporal and spatial distribution of the Willamette River spring stock could be examined in more detail by using the sport fishery harvest, which was sampled across a greater number of weeks compared to other spring-run Chinook salmon mixture sources. We found that the Willamette R. stock had the greatest stock proportion in Region B of the sport fishery (Figure 7) and this proportion appeared to peak around the 10th or 11th statistical week (early March). This temporal and spatial distribution explains the diminished proportion of this stock in all fisheries from Region A, but particularly from the Test fishery which was comprised of fish harvested primarily during weeks 16-17. This relatively late harvest is very different in composition from the harvest obtained by the commercial fishery that has significantly greater proportion of Willamette fish, because the commercial fish were harvested earlier during weeks 14-15 (Table 1) and near the border between regions A and B. The early run-timing and "region B" spatial-

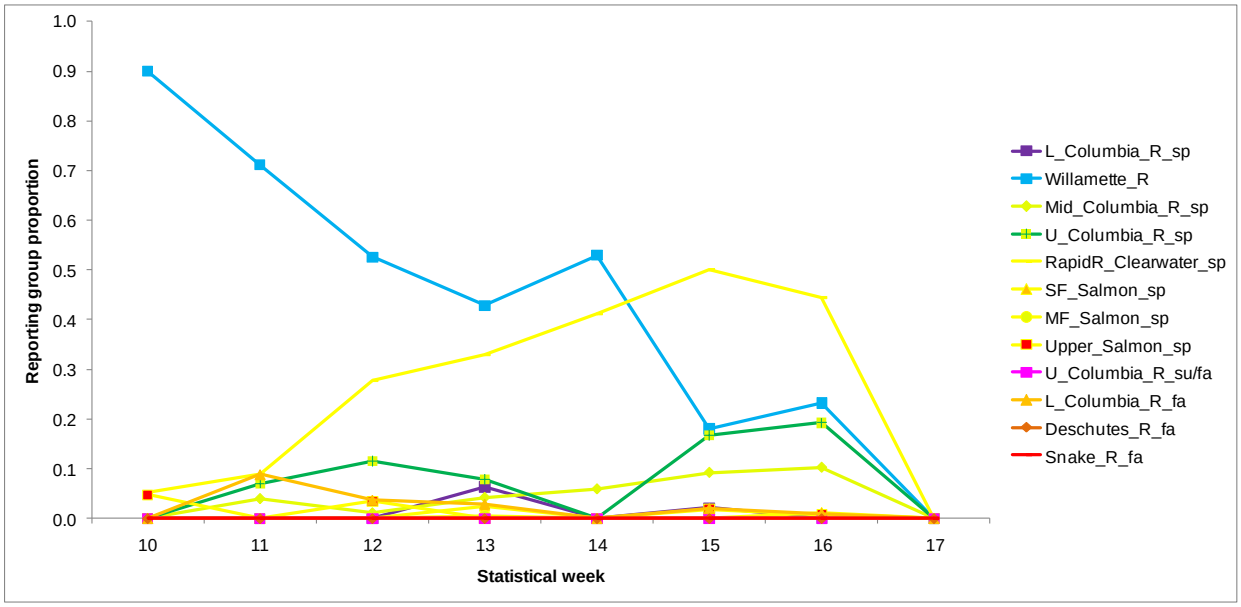
association that characterized the Willamette R. spring Chinook salmon group explains most of the differences that appear among the estimated stock composition of these various fishery mixtures. Not surprisingly, Chinook salmon that passed above Bonneville Dam and were harvested in the Ceremonial fishery were very similar to the total adult fish that were non-lethally sampled at the dam in terms of stock compostion. Both of these latter sources were comprised almost exclusively by the following three reporting groups: Rapid R./Clearwater R., Upper Columbia R., and middle Columbia R. spring-run Chinook salmon.

Figure 7. Sport spring-run Chinook salmon fishery by time strata and region. Region A has a smaller proportion of Willamette R. stocks than Region B due to the relative location of each region.

Region A



Region B



Stock proportions of the fall-run Chinook salmon mixture sources

Proportions of the lower Columbia R. fall-run stock showed large variation among fall-run Chinook salmon fishery mixtures (Figure 8). This stock represented a relatively large proportion of the mixtures from Region B of the sport and commercial fisheries (20-50%) and Region 01 of the Zone 6 Tribal Fishery (>50%), however it represented a much smaller proportion of the mixtures from Region A, Bonneville Dam, and Region 02 of the Zone 6 Tribal Fishery (5-20%). All four of the fall-run Chinook reporting groups (Upper Columbia R. summer/fall, lower Columbia fall, Deschutes R. fall, and Snake R. fall stocks) were represented in proportions with confidence intervals significantly greater than zero in all the mixture sources. In addition, the proportion of Upper Columbia summer/fall stock was greater than all other stocks for all mixture sources, except commercial fishery in region B and tribal fishery in region 01, in which the lower Columbia R. fall stock represented a significantly greater proportion. It is surprising that the harvests from the sport and commercial fisheries in region B contained significantly different proportions of the lower Columbia R. fall stock. This difference cannot be explained by the timing of these particular harvests. For example, the sport mixture was obtained during the first half of the season (weeks 32 – 37, Table 2) which should include the run-timing peak of the Lower Columbia fall stock (week 36-37, see Section 4). In contrast, the commercial harvest excluded the run-timing peak since it did not occur during the entire mid-season weeks (34-40 in region B, Table 2), therefore harvest timing does not explain why there was significantly greater proportion of lower Columbia fall stock in commercial as compared to the sport harvest.

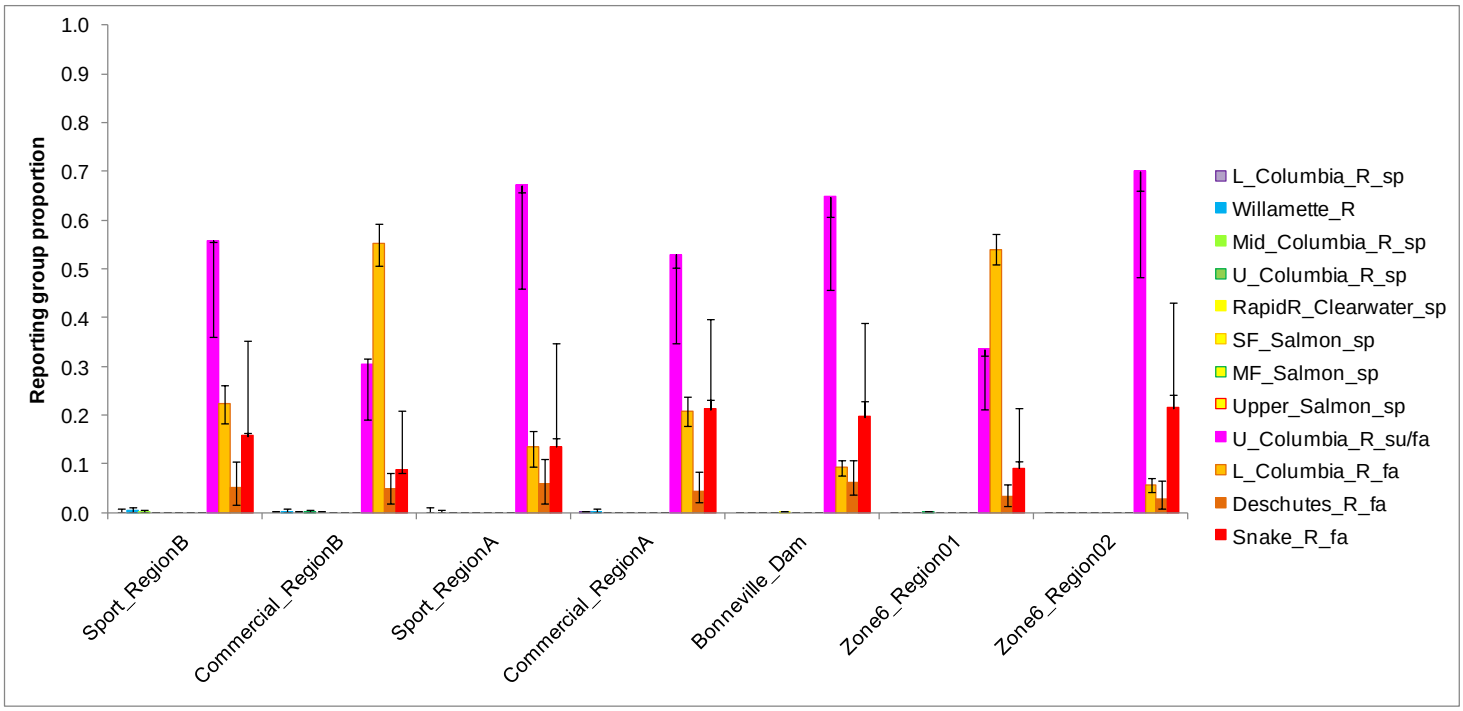
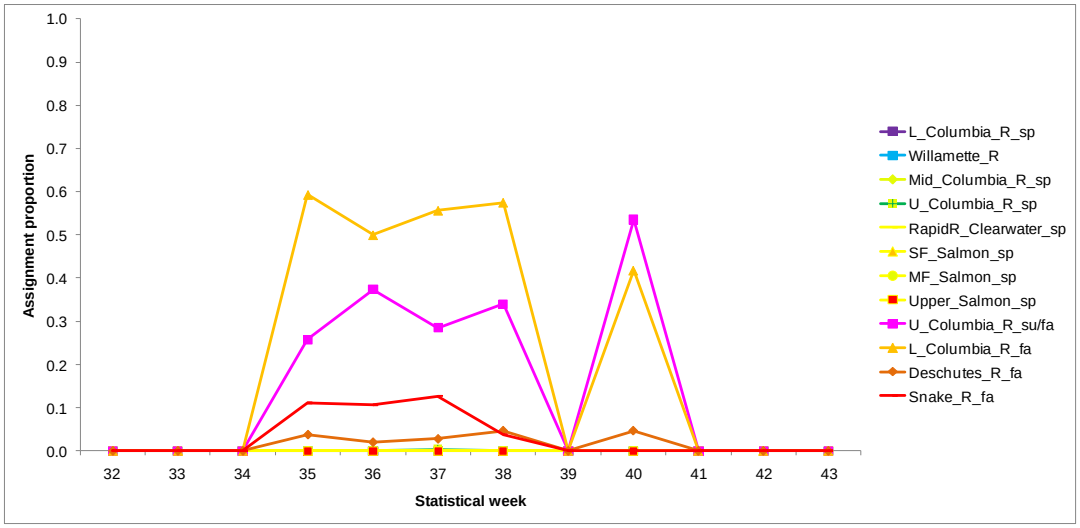


Figure 8. Comparison of stock proportions across all fall-run Chinook salmon mixture sources. The Sport/Commercial Zones and Zone 6were divided into two regions (Regions A and B and Regions 01 and 02, respectively). Only comparable weeks (weeks 32-43) from Bonneville Dam were included. 95% Confidence intervals were generated using 1000 bootstraps.

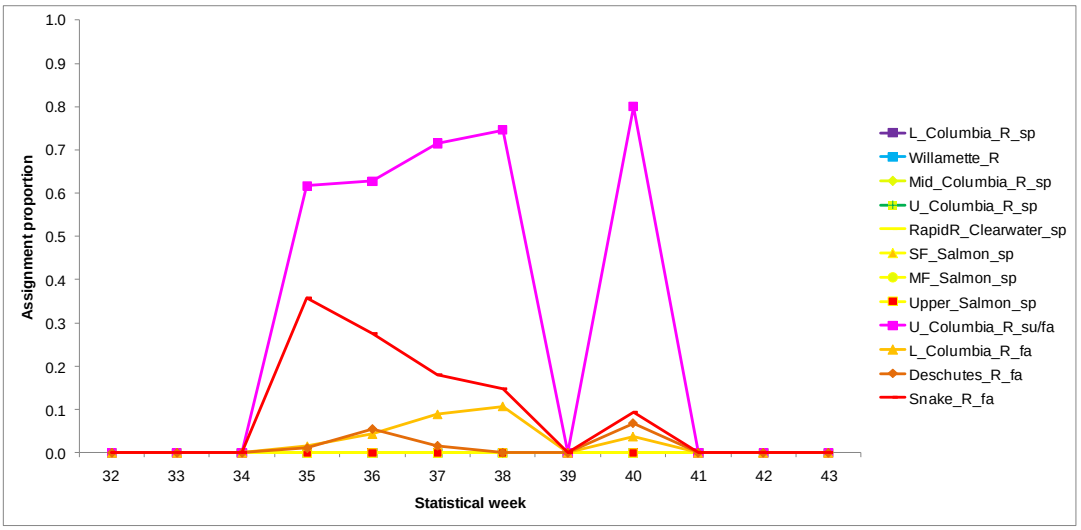
Since the fish that assign to the lower Columbia R. fall stock and pass Bonneville Dam are most likely part of the Spring Creek National Fish Hatchery, their final destination is a tributary that empties into Region 01 of the Zone 6 area. This trend is best seen in Region 01 of the Zone 6 Tribal Fishery, in which we examined the temporal trends of using a weekly time strata series (Figure 7). We found that the Lower Columbia fall stock represents a larger proportion of the mixtures during weeks 34 to 38, and then subsides, while the Upper Columbia summer/fall stock then becomes dominant.

Figure 9. Tribal Zone 6 fall-run Chinook salmon fishery by time strata and region. In Region 01, the Lower Columbia fall Chinook salmon reporting group predominates throughout most of the season but becomes less abundant by week 40. In Region 02, the Snake R. fall Chinook salmon peak in week 35 and decline in subsequent weeks. For reference, week 32 equals dates 8/2/2010-8/6/2010 and week 43 equals dates 10/18/2010-10/24/2010.

Region01



Region02



Discussion

Management implications

This study demonstrates great potential for the application of genetic stock identification in the management of mainstem Columbia River Chinook salmon fisheries evidenced by high accuracy of stock assignment, and the ability to discriminate Chinook salmon fishery mixtures by their relative stock proportions. The spring-run Chinook salmon harvested in commercial, sport, and test fisheries were primarily composed of three adipose-clipped stocks (in descending order of stock composition): Rapid River Hatchery/Clearwater R., Upper Columbia R. (i.e., Carson stock), and Mid-Columbia R stocks. These Chinook salmon stocks were also the most strongly represented at Bonneville Dam and within the harvest of the Ceremonial fishery above the dam. The fisheries below the dam also contained a fourth stock, Williamette R., which was found in highest abundance during the earlier part of the season and locations closer to the mouth of the Columbia R.

For fall-run Chinook salmon fisheries, the sport and commercial fisheries below Bonneville Dam contained large proportions of Lower Columbia fall stocks (20-55% composition), as well as the following stocks (in descending order): upper Columbia R. summer/fall, Snake R. fall, and Deschutes R. fall. Even though the sport and commercial fisheries occurred in both regions A and B, there were significantly smaller proportions of lower Columbia fall-run stock in the sport compared to the commercial fishery. This difference did not appear to be due to any difference in timing of the fisheries, but may be caused by differences in catch retention practices. For example, the commercial fishery is required to retain all adipose clipped Chinook salmon, but the sport fishermen may exercise preference for retaining only larger, brighter Chinook salmon. If this preference were widely practiced in the sport fishery, the darker fish from the lower Columbia R. fall-run ("tules") may be avoided and would explain the reduced stock proportions in the harvest. The entire Zone 6 tribal Chinook fishery was heavily comprised of Upper Columbia R. summer/fall stock (30-70% depending on region), but Region 01 (closest region to Bonneville Dam) of Zone 6 contained far greater proportion of Lower Columbia R. fall stock (~54%) than Region 2 (< 6%), whereas Snake R. and Deschutes R. fall stocks were similar in both regions (9-20% and ~3%, respectively). The Snake R. stock appeared to peak early in the fall, based on the weekly strata analysis in Zone 6.

Future directions

Validation of accuracy was conducted using CWT data which revealed the reporting groups with highest accuracy but also exposed weaknesses in the current baseline. The greatest misassignment was observed between Upper Columbia R. and Rapid River spring-run stocks, and between Upper Columbia R. summer/fall and Snake R. fall-run stocks, which decreased concordance between GSI and CWT data. In addition, fish with CWTs from hatcheries using Deschutes R. and Umatilla R. spring-run and Rogue R. and Umatilla R. fall-run stocks were not represented in the genetic baseline. Expansion of the baseline will help improve on these weaknesses; discussed in Section 2. For example, collections from Shitike Cr. and Warm Springs R. are now included to represent the Deschutes R. spring-run Chinook. Also the lower Columbia R. spring-run baseline collections now include Cowlitz R. stock, which will help decrease misassignment between lower Columbia R. spring- and fall-run reporting groups. Finally, additional collections were added to the Upper Columbia R. and Rapid R. spring-run

reporting groups and should improve their assignment accuracy. Adding a second set of 96 SNP markers are part of our plans for next year's expansion, and this substantial change is expected to benefit all reporting groups with improved accuracy and spatial resolution.

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Section 4: Characterization of Chinook salmon and steelhead run-timing

Introduction

The Columbia River Basin supports ESA listed wild stocks of Chinook salmon and steelhead as well as hatchery supplemented populations. Both Chinook salmon and steelhead have been declining in the Columbia River Basin for several reasons including climate change, hatchery practices, habitat degradation, over harvesting, and hydropower. Along with abundance estimates, basic information related to the way in which stocks of salmonids are distributed both spatially and temporally are needed by fisheries managers to achieve sustainable fisheries.

As evident from the analyses of stock composition of Chinook salmon fisheries harvests in Section 3, certain stocks seem to have strong spatial and temporal associations. However, because the type of fishery gear, harvest regulations, and locations targeted varies considerably among fisheries, it is necessary to establish a study that samples a representative mixture of all hatchery- and natural-origin stocks at a fixed location to accurately characterize abundance and run-timing distributions of stocks.

Here we analyze fish across the entire run of Chinook salmon from April to October to estimate weekly proportions of stocks and use total Chinook salmon abundance data to estimate abundance of each stock. We restrict the focus of this study to one species, Chinook salmon, and genotyping of 92 single nucleotide polymorphism (SNP) loci, but we also describe efforts to analyze a second species, steelhead, and genotype over twice the number of SNP loci (192). The methods we describe for analyzing Chinook salmon in this report will be adapted to an analysis of steelhead in future reports. In our 2009 PISCES Annual Report (Section 4), we demonstrated how we can exploit the highly structured populations of steelhead in the Columbia River Basin (Nielsen et al. 2009, Narum et al. 2006a,b), and use genetic stock identification (GSI) to assign unknown fish to the population they originated. We anticipate that the high number of SNP loci (n=192) and the number of baseline collections that have recently been genotyped (Section 2) will provide equivalent and for some reporting groups, finer resolution and higher accuracy, as compared to previous baselines that employed microsatellite DNA markers (Blankenship et al. in press).

The aim of this study was to use GSI to discriminate Columbia River Chinook salmon stocks according to their peak run-timing. Since Bonneville Dam is the most downstream dam on the Columbia River, the mixtures obtained here represent a majority of Columbia River Basin stocks. Our study offers a rare opportunity to monitor a broad geographic scale of salmonid populations over several years. This long-term study will allow us to characterize trends in run timing and provide information regarding the consistency of anadromous steelhead and Chinook salmon across years to fisheries managers.

Methods

Sample Collection

Tissue samples were obtained from adult Chinook salmon in 2010 and steelhead in 2009 during migration runs at Bonneville Dam (n = 2648 and 1760, respectively). Samples were pooled into weekly strata (Chinook salmon mean n = 95, range 0-235 per week) spanning the majority of the run-year from April to October (Table 1). Biological data such as species, date, length, presence/absence of adipose fin, were recorded for each individual that was tissue-sampled for genetic analysis. We collected tissue samples, in the form of fin punches from each fish. After non-lethal sampling was completed, all fish were released to a recovery pond and then to the fish ladder to continue upstream migration. Tissues samples were preserved in ethanol or on Whatman filter paper (Lahood et al. 2008) before being shipped to the Hagerman Fish Culture Experiment Station for GSI and estimation of stock composition. This sampling effort is covered under Scientific Research Permit #1379 under Section 10 of the ESA (permit included in PISCES attachments).

Table 1. Sample numbers by weekly strata for Chinook salmon and steelhead that were DNA sampled or tallied for abundance at Bonneville Dam.

		Statistical week																												
Species	Sample type	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	Total
Chinook salmon	DNA	40	149	235	233	147	109	101	109	114	60	54	69	43	25	16	15	6	18	0	93	203	178	164	179	151	94	37	6	2648
	Total abundance	31813	55880	54747	48598	31299	9421	13588	21918	13374	20755	22386	15338	10830	5428	3526	3461	3235	5593	11772	50730	124834	116757	81046	82034	31269	14271	5185	2267	891355
Steelhead	DNA	5	4	5	13	15	13	22	26	55	31	36	62	89	180	286	180	130	135	0	77	63	63	35	116	60	35	19	5	1760
	Total abundance	383	375	224	370	538	662	725	1282	1824	2111	3176	7575	10385	19919	47799	48050	22191	154014	103109	54148	40087	26849	20385	12737	8680	6431	3756	2020	599805

Chinook salmon and steelhead numbers are based on 2010 and 2009, respectively. "DNA" numbers indicate the samples available for genotyping and inclusion in our GSI analyses. Total abundance was based on tallies provided by the Fish Passage Center (http://www.fpc.org) as observed at their fish counting window. For reference, week 32 equals dates 8/2/2010-8/6/2010 and Week 43 equals dates 10/18/2010-10/24/2010

Molecular markers

The same 92 SNP loci described in Section 3 were used to genotype Chinook salmon mixtures.

Statistical analyses

SNP genotype data was utilized to estimate stock composition using the baseline described in Section 3, Figure 2).

Genotypes from Bonneville Dam mixtures were also analyzed with the program ONCOR v1.0 (available at http://www.montana.edu/kalinowski) to estimate stock composition weekly strata. We analyzed all strata that had n > 5 samples. These mixture proportions were generated with 95% confidence intervals using 1000 bootstraps.

Results

Run-timing of Chinook salmon stocks in 2010

Variation in stock proportions across weekly strata within the migrating season was relatively large (Table 2). The largest run-timing distributional differences for the major spring-run stocks (stocks arriving before June 30th;Ordinal day 180), were observed between relatively early peak run-timing stocks Upper Columbia R., Rapid R./Clearwater R., and middle Columbia R. spring-run stocks (median dates May 1st, May 1st, and May 6th, respectively), versus the relatively late peak run-timing stocks from the Upper and South Fork Salmon R. (median dates June 6th and 13th, respectively). The major fall-run stocks showed minimal differences in peak run-timing, however the stocks can be ordered by median date as follows: Snake R. fall (Sep 5th), lower Columbia R. fall (Sep. 7th), Upper Columbia R. summer/fall (Sep. 8th), and Deschutes R. fall (Sep. 11th).

Table 2. Basic information on run-timing distributions of Chinook salmon stocks passing Bonneville Dam.

Reporting group	Individual assignment 2010	Median ordinal day	Mean ordinal day	1st quartile ordinal day	3rd quartile ordinal day	5th percentile ordinal day	95th percentile ordinal day	Median date	Mean date	Interquartile range (d)
L_Columbia_R_sp	0	-	-	-	-	-	-	-	-	-
Willamette_R	0	-	-	-	-	-	-	-	-	-
Mid_Columbia_R_sp	113	132	133	120	141	108	160	12-May	12-May	21
U_Columbia_R_sp	480	126	129	118	137	111	161	6-May	9-May	19
RapidR_Clearwater_sp	506	125	131	117	145	111	162	5-May	10-May	28
SF_Salmon_sp	66	154	151	141	162	118	173	3-Jun	31-May	22
MF_Salmon_sp	6	135	133	119	145	111	155	15-May	13-May	26
Upper_Salmon_sp	106	152	146	132	161	113	175	1-Jun	26-May	29
U_Columbia_R_su/fa	1031	252	242	236	267	171	280	9-Sep	29-Aug	31
L_Columbia_R_fa	106	251	250	244	256	238	271	8-Sep	7-Sep	12
Deschutes_R_fa	48	264	261	251	272	239	280	21-Sep	17-Sep	21
Snake_R_fa	186	252	251	243	265	211	280	9-Sep	7-Sep	22
	Estimated abundance									
L_Columbia_R_sp	189	158	147	133	161	130	164	7-Jun	26-May	28
Willamette_R	0	-	-	-	-	-	-	-	-	-
Mid_Columbia_R_sp	29572	126	128	115	137	105	165	6-May	8-May	22
U_Columbia_R_sp	102045	121	123	113	130	106	153	1-May	3-May	17
RapidR_Clearwater_sp	127169	121	126	112	131	105	163	1-May	5-May	19
SF_Salmon_sp	16513	164	160	153	169	135	173	13-Jun	6-Jun	16
MF_Salmon_sp	2653	132	134	120	149	110	158	12-May	13-May	29
Upper_Salmon_sp	23427	157	154	145	170	113	178	6-Jun	2-Jun	25
U_Columbia_R_su/fa	375858	251	244	242	263	175	275	8-Sep	31-Aug	21
L_Columbia_R_fa	58900	250	251	246	255	241	266	7-Sep	7-Sep	9
Deschutes_R_fa	27804	254	257	248	267	243	277	11-Sep	14-Sep	19
Snake_R_fa	113207	248	249	242	258	235	269	5-Sep	6-Sep	16

These summary statistics of run-timing distributions are based on two methods. One method characterized run-timing distributions of fish that were sampled for DNA analysis and individually assigned to reporting groups (top half). The second method (bottom half) estimated abundance of each stock based on stock proportions and total numbers of Chinook salmon that were observed passing Bonneville Dam at the fish counting window. This information is also plotted in Figure 3.

Estimated abundance of Chinook salmon stocks in 2010

There were nine major stocks passing Bonneville Dam that we estimated abundance greater than 10,000 (10k) fish in the season (Table 3). The five major stocks of the spring-run in order of magnitude were South Fork Salmon R. (16k), upper Salmon R. (23k), middle Columbia R. (29k), upper Columbia R. (102k), and Rapid R./Clearwater R. (127k). The four major stocks of the fall-run in order of magnitude were Deschutes R. (28k), lower Columbia R. (59k), Snake R. (113k), and upper Columbia R. summer/fall (376k). These stock abundance estimates were based on the stock proportions that were estimated in ONCOR across weekly strata (Figure 1), and were multiplied with the total abundance of Chinook salmon that was tallied on a daily basis at the Bonneville Dam fish counting window. This calculation was used to generate the stock abundance on a weekly basis to visualize peak run-timing (Figure 2) and to characterize the distributions of run-timing for each stock (Figure 3).

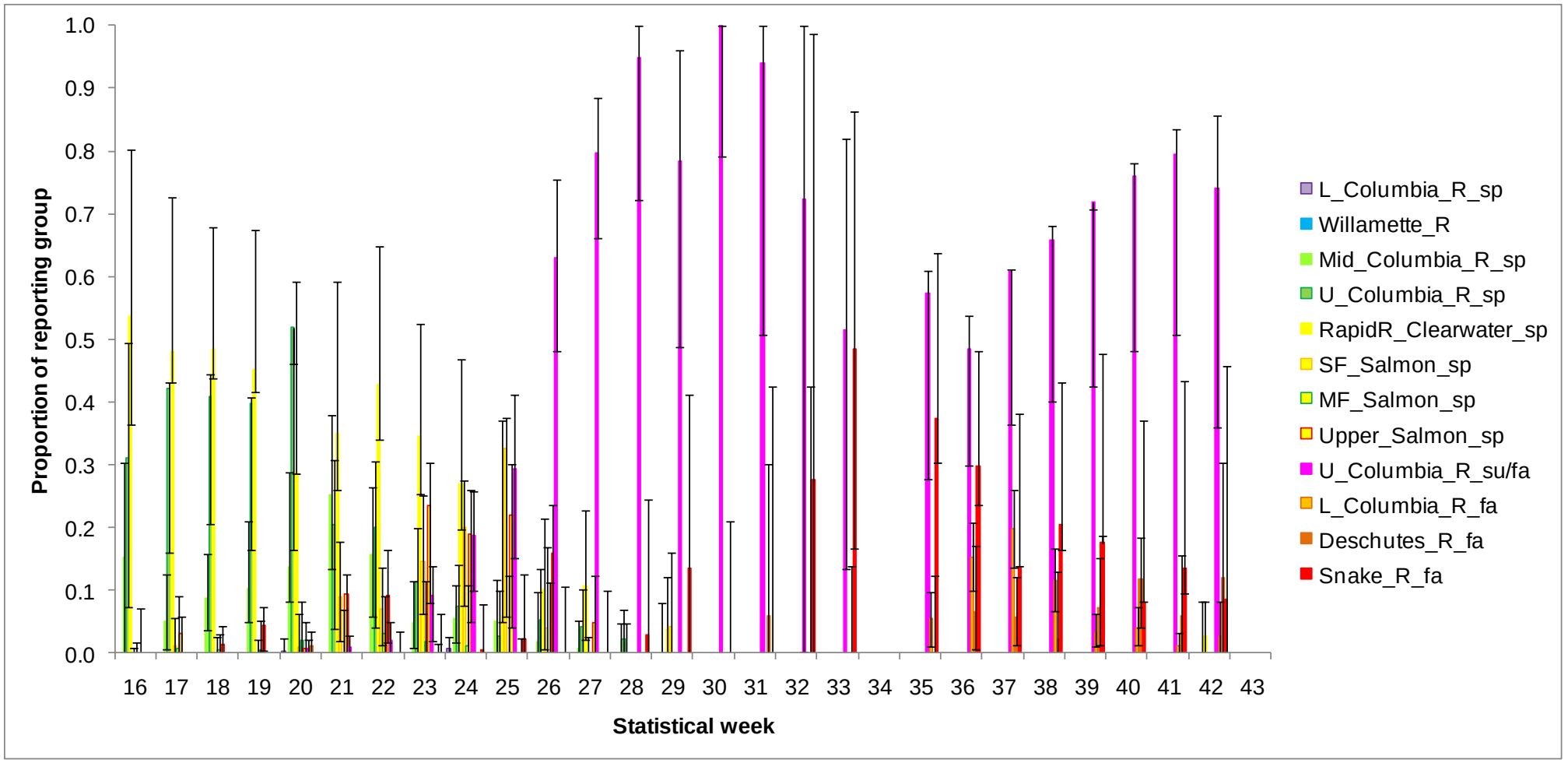

Figure 1. Estimated weekly proportions of reporting groups for Chinook salmon passing Bonneville Dam in 2010. Estimated reporting group proportions for weeks 34 and 43 were unavailable due to insufficient sample numbers of Chinook salmon.

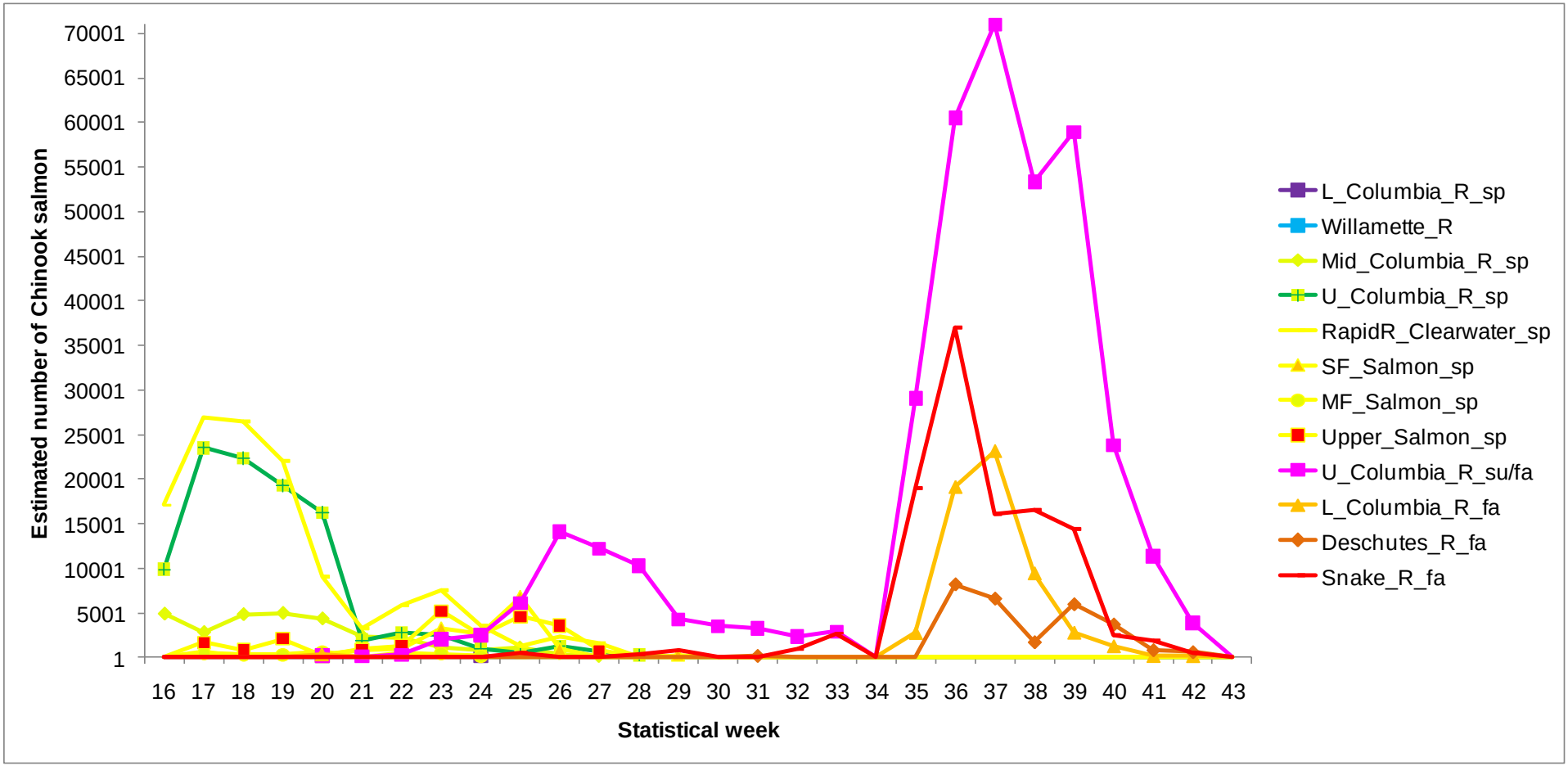

Figure 2. Estimated weekly abundance of Chinook salmon reporting groups passing Bonneville Dam in 2010. This data was generated by first estimating reporting group proportions of weekly pooled mixtures of Chinook salmon passing Bonneville Dam and then multiplying the proportions with weekly tallies of Chinook salmon at the Bonneville Dam fish counting window. Estimated abundance for weeks 34 and 43 were unavailable due to insufficient sample numbers of Chinook salmon.

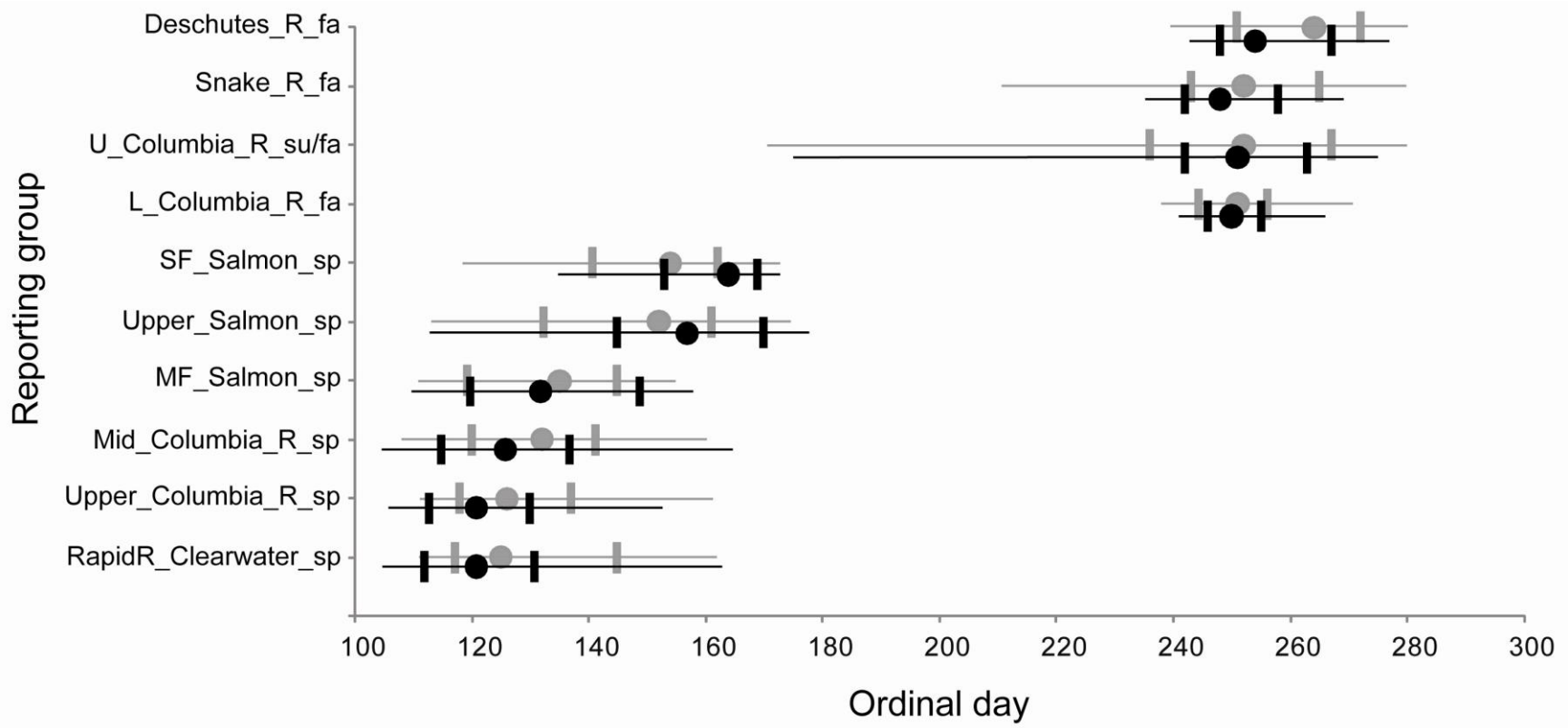

Figure 3. Columbia River Chinook salmon stock timing distributions at Bonneville Dam in 2010, including median (circles), inter-quartile (bars), and 5th and 95th percentile dates (horizontal lines). The distributions were either based solely on the Chinook salmon that were sampled at Bonneville Dam and individually-assigned to reporting groups (in grey), or were based on the weekly estimated reporting group proportions that were applied to the total abundance of Chinook salmon tallied at the Bonneville Dam fish counting window (in black). This latter method for estimating run-timing distributions may be more accurate because it corrects bias imposed by uneven sampling..

Discussion

Management Implications

This study demonstrates great potential for the application of genetic stock identification in the management of Columbia River Chinook salmon fisheries evidenced by the ability to estimate stock abundance, characterize run-timing distributions, and discriminate some of the major stocks by peak run-timing. Results indicate the following nine stocks of Chinook salmon were estimated to have greater than 10,000 (10k) fish pass Bonneville Dam in 2010: the South Fork Salmon R. (16k), upper Salmon R. (23k), middle Columbia R. (29k), upper Columbia R. (102k), and Rapid R./Clearwater R. (127k) spring-run stocks and the Deschutes R. (28k), lower Columbia R. (59k), Snake R. (113k), and upper Columbia R. summer/fall (376k) stocks. The most dramatic difference in run-timing among groups of genetically-related stocks was observed among the spring-run stocks which were separated into early- and late- run-timing distributions. In fact, the peak timing of the two "late" stocks from the South Fork Salmon R. and upper Salmon R. occurred around 30 days after the peak timing of the earlier spring-run stocks (middle Columbia R., upper Columbia R., and Rapid R./Clearwater R.).

Although we did not include an analysis of steelhead in this report, we will use similar methods to characterize run-timing distributions of the Columbia River steelhead stocks in the future. Given our ability to analyze Chinook salmon stocks with 92 SNP markers, we expect we will have even greater power to finely discriminate steelhead stocks by using the 192 SNP markers that are currently available as discussed in Section 2. In addition, one of the advantages that genetic stock identification has over other methods for estimation of stock composition (e.g. coded wire tagging) is the ability to estimate proportions for both hatchery and wild fish. We intend to incorporate information on hatchery and natural-origin steelhead to help characterize run-timing of less abundant or "minor" Columbia River stocks.

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