

2012 Annual Report

GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS

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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 and other anadromous fishes; 2) expand and create genetic baselines for multiple species including Chinook salmon, steelhead (*O. mykiss*), sockeye salmon and kokanee (*O. nerka*), and coho salmon; 3) implement Genetic Stock Identification (GSI) programs for mainstem Chinook salmon and steelhead fisheries and 4) GSI of fish passing Bonneville Dam (steelhead, sockeye, and Chinook salmon). In the third year of this project, SNP discovery and evaluation goals (Objective 1) were achieved with two completed projects on *O. mykiss* and one project on Pacific lamprey which identified SNP markers using restriction associated DNA sequence (RAD-seq) technology. For genetic baseline expansion (Objective 2), the Chinook salmon and steelhead GSI baselines continue to expand using a total of 192 SNP markers for each species which includes a 96-SNP panel optimized for parentage based tagging (PBT) and a 96-SNP panel optimized for GSI. In addition, a 96-SNP baseline for *O. nerka* has become available for assigning individuals to the three major Columbia River sockeye stocks. We have now compiled genotypes from 192 SNP markers in 79 Chinook salmon collections, 192 SNP markers in 145 steelhead collections, and 96 SNP markers in 22 *O. nerka* 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 identified loci in all three species that may be candidate markers and showed selective divergence across the study collections. The third year of the project included two broad applications of GSI; namely, stock composition of Chinook salmon and steelhead fisheries (Objective 3), and stock composition of Chinook and sockeye salmon and steelhead passing Bonneville Dam (Objective 4). Chinook salmon and steelhead fishery applications of GSI were integrated with the new genetic technology of parentage based tagging (PBT). The challenge imposed by long histories of exogenous stock transfers from specific hatchery programs often prevents effective application of GSI in assigning hatchery fish. However, as the role of PBT is expanding to tag all hatchery fish, the role of GSI is smaller but still essential for filling in information gaps that remain after PBT has been used to identify hatchery-origin fish. Objective 3 addressed a recent concern of fishery managers related to an expansion of the Chinook salmon sport fishing boundary around the mouth of the Wind R. A comparison of stock composition among spring Chinook salmon samples from below Bonneville Dam in the sport and commercial fishery, non-lethal interrogation at the dam, and above the dam in the tribal ceremonial fishery in Zone 6, demonstrated that the Wind R. sport fishery continues to primarily target its intended stock despite the boundary change. Spring-run Chinook salmon from these sources in the 2012 were primarily composed of two adipose-clipped stocks: Rapid River Hatchery/Clearwater R. and Upper Columbia R. (i.e., Carson stock). PBT-assignments made it possible to further discriminate fish by their hatchery-of-origin (ten total hatcheries represented), which is a vast improvement over the GSI assignments that would have mostly been to the Rapid River Hatchery/Clearwater R. stock (a very broadly distributed reporting group). A third spring-run stock, Willamette R., was found primarily in harvests taken closer to the mouth of the Columbia R. For fall-run Chinook salmon fisheries, we tested accuracy of the Chinook salmon baseline using known-origin mixture samples based on coded wire tags and observed greater than 90% reporting group concordance for the three main fall-run stocks. We demonstrated that we could in fact use PBT to assign a steelhead caught at high-seas in Alaskan waters to Snake River

hatchery parents, which may warrant a more thorough examination of high-seas harvest of Snake River steelhead in the future. Stock composition of unclipped steelhead harvested in the tribal fishery in zone 6 was a quarter hatchery-origin fish from the Snake R. For Objective 4, fish were sampled as they migrated past Bonneville Dam. We used a combination of GSI and PBT to estimate run-timing distributions and abundance of hatchery and wild Chinook salmon and steelhead stocks in 2012. Our results indicate there were seven hatchery stocks and seven wild stocks of spring-run Chinook salmon estimated to have greater than 2,000 fish pass Bonneville Dam in 2012. It may interest fisheries managers to know that the run-timing of these stocks contributed to the total abundance of Chinook salmon that pass through the Columbia River mainstem in two management periods, spring and summer. In fact, we observed more than 4,000 natural-origin and 8,000 hatchery-origin fish from spring-run Chinook salmon reporting groups (mostly Snake R. origin) that are estimated to return during the summer management period (June 16 – July 31). There were some consistent run-timing results with those from previous analyses, e.g. Salmon R. and Klickitat R. spring-run Chinook salmon have relatively late runs compared to other spring stocks. There were seven wild steelhead and five hatchery steelhead stocks with an estimated abundance greater than 1000 fish passing Bonneville Dam in 2012. We described three run-timing categories which were most distinctive among hatchery stocks and included an early Skamania summer-run (and Yakima R.), an intermediate run-timing category that contains most wild and hatchery steelhead stocks, and a late run-timing category that arrives after August 25th and includes South Fork Clearwater R., and upper Clearwater R. Three sockeye populations were estimated with the following abundances and median run-timing dates: Okanogan (415,500, Jul. 3rd), Wenatchee (98,900, Jun. 27th), and Redfish Lake (500, Jul 25th). Examination of stock composition of PIT-tagged sockeye that were never detected at their terminal dam revealed differential survivorship of these stocks, with Redfish Lake and Wenatchee sockeye salmon having lower survivorship relative to the Okanogan stock.

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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 salmon 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 older genetic marker technology such as microsatellite markers. Over the past few years, our lab has contributed to the increasing numbers of SNP markers that are available for salmonids, and we have reached a point where rigorous stock composition and assessment goals for timely management of fisheries and highly accurate, precise stock assignments can be achieved using one or two panels of 96 SNP markers independently of any other marker-type.

Objective 2) Baseline Expansion

Currently, genetic baselines of microsatellite markers are in place for Chinook salmon across the coastwide range (Seeb et al. 2007), steelhead (Blankenship et al. 2011), and *O. nerka* 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 be 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 (Narum et al. 2008b, Hess et al. 2011, Hess and Narum 2011). This proposal includes two GSI projects that will utilize genetic baselines: 1) GSI to Evaluate Harvest; and 2) GSI of fish passing Bonneville Dam.

This study includes GSI analysis of Chinook salmon collected from commercial, recreational, and tribal fisheries in the Columbia River and GSI analysis of steelhead collected from the tribal fishery above Bonneville Dam. (Subsequent years of the study will include sockeye 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), Oregon Department of Fish and Wildlife (ODFW), Yakama Nation Fisheries Program (YNFP) and Warm Springs Confederated Tribes. 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.

Finally, we continue to utilize a new genetic technology, parentage based tagging (PBT), in combination with GSI to help augment and refine our stock identification results. PBT is an efficient approach for mass tagging fish. The method is carried out by first genotyping a set of potential parents which then provides the opportunity to assign a set of genotyped offspring to their true parent pair. PBT is currently being utilized on a broad scale in the Columbia River Basin to tag all Chinook salmon and steelhead Snake River hatchery broodstock (Steele et al. 2011). This application has effectively tagged all Snake River hatchery Chinook salmon and steelhead starting with the 2008 brood years. When parent pairs of a Snake River hatchery fish are identified with PBT, we can provide accurate information including age of the fish and the source hatchery in which its parents were spawned. We can now use PBT in both Chinook salmon and steelhead GSI applications to identify all Snake River hatchery-origin fish, and then we estimate stock-of-origin of all other hatchery fish that were not assigned with PBT (i.e. non-Snake River hatchery-origin) and all wild fish using GSI. In this way PBT and GSI are very complimentary, and using them in combination takes full advantage of the strengths of each method, while resolving their limitations. Exogenous stock transfers by hatcheries have made hatchery-origin fish challenging to assign with GSI and represents a main limitation that is addressed with PBT.

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, steelhead, and *O. nerka* to create genetic baselines. The third section contains GSI estimates of stock composition of Chinook salmon and steelhead sampled in mainstem fisheries in 2012. The fourth section includes analysis of run-timing distributions and estimated abundance of adult Chinook and sockeye salmon and steelhead stocks migrating over Bonneville Dam in 2012.

Section 1: SNP Discovery

Introduction

The discipline of conservation genetics relies on data derived from sets of genetic markers for analysis. Discovery of appropriate genetic variations and development of assays to interrogate those variations has long been an expensive and time consuming endeavor. However, techniques utilizing new sequencing technologies allow the generation of billions of bases of sequence data at ever decreasing costs and now allow genotyping of individuals directly from sequencing data as well as the ability to generate genotyping assays from the aligned sequences. In our laboratory we've employed the RAD (Restriction-site Associated DNA) sequencing technique to identify and genotype SNP (Single Nucleotide Polymorphism) variations from sequence data generated on an Illumina HiSeq instrument (technique described in Miller *et al.* 2007 and Baird *et al.* 2008). Utilizing a restriction enzyme (Sbf1) with an eight base recognition site we have been able to generate genotypes for greater than 10,000 SNPs for up to 96 individuals from a single lane of sequence data. These genotypes have been used in our laboratory directly for population genetic analysis as well as for association with physical traits (Hess *et al.* 2012; Hecht *et al.* 2012b; Narum *et al.* 2013). Moreover, paired end sequencing data has been used to create sequence alignments long enough for Taqman assay development. One hundred twenty Taqman™ assays were created and evaluated for the selection of a 96-SNP panel for the study of Pacific lamprey (a species for which no such markers had been developed previously).

Methods

O. mykiss SNP discovery using RAD sequencing

In 2012 several projects were undertaken using the RAD sequencing method of genotyping by sequencing (GBS). Two of these projects examined the genetic basis of physical traits in the species *Oncorhynchus mykiss* (Hecht *et al.* 2012b and Narum *et al.* 2013). Detailed methods and analysis are available in each of the respective publications. Briefly, in Hecht *et al.* 2012b samples were taken of out-migrating fish (smolts) and from resident fish in two separate river systems and their genotypes were examined for association with the trait. While in Narum *et al.* 2013, samples were taken from mortalities and survivors of a 6 week thermal stress regime and their genotypes were analyzed for association with survival. Each study utilized the same library preparation method (described in Baird *et al.* 2008) and bioinformatic genotyping pipeline (described in Miller *et al.* 2011 and Hecht *et al.* 2012b). Association testing for SNP loci associated with heat stress hardiness was carried out using the program PLINK (Purcell *et al.* 2007). The TASSEL program (Bradbury *et al.* 2007) was used for association of genotypes with smolting behavior.

Lamprey RAD sequencing and SNP assay development

RAD sequencing was used in this study to genotype 518 fish representing a comprehensive sample of life histories, coexisting species, and adult spawning locations in order to illuminate genetic structure in these sparsely studied fishes. Full analysis and results are available in Hess

et al. 2012. A quality filtered set of 4,439 SNP sites were identified in the study and 162 of the SNPs were revealed to be adaptive through outlier testing. A separate analysis of the sequencing data was also done to discover species diagnostic SNP markers that would allow the genetic identification of coexisting lamprey species. This set of SNP sites was analyzed and filtered for the most informative 160 markers whose SNP site was located at position 24 or higher to accommodate the assay primer site. A custom PERL script was written that used the sequences from each marker as input, searched through read 1 data, identified reads that contained that marker sequence, and retrieved paired end sequences from the read 2 data. The program output was a fasta file that contained 100 base sequences of both forward and reverse orientation reads for each given SNP locus. The files were then imported into SEQUENCHER v4.7 and assembled into contigs. The assembled contigs were then edited and ambiguities (aside from the intended SNP site) were masked with “N”. The completed contigs were then exported and concatenated into a single fasta file. Another custom PERL script was then used to convert the fastq file to a text file with the SNP variation identified by square brackets for uploading to the LifeTechnologies website for Taqman™ assay design. One hundred thirty-five (135) assays passing design on the LifeTechnologies automated design site were trimmed down to 120 for ordering. A set of 190 lamprey samples were used to evaluate the new Taqman™ assays using our Fluidigm EP1 platform. Assays were evaluated for cluster scoring, genotype concordance to RAD data, redundancy of information between assays, and general utility for parentage and population dynamics. A filtered set of 96 assays was chosen based on this data.

Results

O. mykiss SNP discovery using RAD sequencing

Analysis of the sequencing data from each of the *O. mykiss* RAD projects resulted in genotypes for over 20,000 SNP loci among the individuals tested. As would be expected, most of the loci matched previously reported sequences for RAD sequencing using the SbfI enzyme (Miller *et al. 2012* and Hecht *et al. 2012a*). However, 6,911 SNP loci were gleaned from the sequencing data from in these studies that had not been previously reported by other RAD studies using the SbfI enzyme in this species. These sequences with their SNP sites are reported in the electronic attachment (Supplemental Table 1).

Lamprey RAD sequencing and SNP assay development

Roughly 8,000 SNP sites were identified within the set of Pacific lamprey used in the SNP discovery pipeline. After strict filtering to remove loci with poor genotyping, potential genotyping errors, and non-informative loci a set of 4,439 loci remained for analysis. Rigorous testing described in Hess *et al. 2012* revealed several sites associated with coast-wide population structure and life history variants. Putatively species diagnostic SNP sites were selected from a pool of roughly 1,000 identified in the sequencing data. A filtered set of 160 of the top performing loci (plus 5 species diagnostic loci) were used for Taqman™ assay development. Of

the 165 loci selected, eight produced poor assemblies due to nearby repeats or large indels and did not produce adequate consensus sequences for assay design. One hundred twenty of the assays passing in-silico design were ordered and tested on 190 lamprey extracts to evaluate accuracy and ease of scoring. Of the 120 assays ordered, 112 produced accurate genotypes for a conversion rate of 93% which is very high for assays designed from this type of data. From these results, a set of 96 assays was selected as our Pacific lamprey panel (Table 1). The panel includes 2 species diagnostic assays (LampSD-478 and LampSD-802) which distinguish the Pacific lamprey from the genus *Lampetra* and further differentiate *L. richardsoni* from *L. pacifica*. Another 9 assays target SNPs identified as adaptive and the remaining 85 assays are putatively neutral with high minor allele frequency.

Discussion

New sequencing technologies now allow for rapid and efficient identification and development of genetic markers. Techniques such as RAD sequencing, which reduce the complexity of a given genome by over 300 fold, now allow for the sequencing of more individuals for less cost with simpler data analysis than has been previously possible. This has now made it possible to conduct studies requiring a high density of markers across a genome, such as our *O. mykiss* association studies. Or alternatively, to quickly identify, genotype, and design informative markers in species without extensive genomic sequence data as we were able to do in our Pacific lamprey study.

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Section 2: Genetic Baseline Expansion

Introduction

Distinct population aggregates of Chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*Oncorhynchus mykiss*), and the species *Oncorhynchus nerka* (including sockeye salmon), have evolved through the cumulative effects of selection and genetic drift (Waples 1991; Nielsen et al. 2009). Philopatry, or homing to natal rearing grounds in order to spawn is a characteristic behavior of Pacific salmon species that is well documented. Philopatry can significantly restrict gene flow and shape productivity limitations among naturally reproducing populations (Hasler and Scholz 1983; McIssac and Quinn 1988; Quinn *et al.* 1991). Population distinctions may be more readily resolved on a large geographic scale where gene flow and reproductive restrictions are often well defined by migration distance, yet the distribution of suitable spawning habitat and local adaptations may produce fine scale genetic structure between closely adjacent stream sections or watersheds (Beacham *et al.* 2006; Matala et al. 2012). Homing miscues (straying) are thought to be necessary to buffer loss of genetic diversity in salmon (Milner and Bailey 1989), particularly in small populations. However, the rate of straying among wild fish is generally low (Quinn 1993; Heard *et al.* 1995) and despite the resulting moderate gene flow, genetic structure between populations may persist (Neville *et al.* 2007). There is some evidence that the incidence of straying is higher among hatchery origin fish. This may be an artifact of changes in fish passage protocols, transport through the hydro system, or artificial rearing practices. An elevated rate of immigration between populations may erode local adaptations, and lead to changes in spatial and temporal variability within and/or among populations (Hess and Matala *in review*).

Objective two of the BPA project 2008-907-00 (Genetic Assessment of Columbia River Stocks) involves the collection, analysis and distribution of single nucleotide polymorphism (SNP) genotypic data. These data serve as species specific baselines to characterize Chinook salmon, steelhead trout, and *O. nerka* population structure throughout the Columbia River Basin. Our 2012 annual report provides a description of SNP baseline expansion efforts, building from preceding microsatellite (μ SAT) baselines founded by multi-agency consortiums for Chinook salmon (Seeb et al. 2007) and steelhead trout (Stephenson et al. 2009). These include coast-wide development of SNP baselines (e.g., the expansion of GAPS under the Chinook Technical Committee LOA), establishment of standardized SNP scoring protocols among fisheries management entities, and specific directed baseline applications including parentage based tagging (PBT). In comparison to more polymorphic μ SAT markers that have dominated past studies (e.g. ~200 SNPs needed for Chinook salmon GSI, Hess et al. 2010), it has been shown that a larger number of SNP loci are necessary to reach the same level of resolution in genetic structure analyses. However, large numbers of highly informative SNP assays are indeed available, and their abundance stands to increase with the advent of new technologies such as Restriction site Associated DNA (RAD) sequencing (Miller et al. 2007; Baird et al. 2008; Hecht et al. 2013). SNPs are highly prolific in the genome, with substantial coverage for linkage analyses (Moen et al. 2008). Moreover, because SNPs may be located within functional genes, they are candidates for detecting positive (adaptive) selection or selective divergence, a force instrumental in shaping population differences. SNPs are relatively easily amplified and scored, even with poor quality tissue source or DNA extract (Campbell and Narum 2008). With

advances in analysis platforms, SNPs are currently amenable to superior high throughput capabilities.

In the Columbia River Basin, Chinook salmon have been studied in great detail (Narum et al. 2004a; Waples et al. 2004; Beacham et al. 2006; Narum et al. 2008b; Matala et al. 2011), and steelhead to a similar degree (Narum et al. 2004b; Nielson et al. 2009; Blankenship 2011; Narum et al. 2011). Genetic analysis of *O. nerka* (hereafter intended to represent both sockeye salmon and kokanee) in the Columbia River Basin has been relatively limited in scope. For these three species our efforts are likely to provide additional information that will benefit and expound on their characterization, productivity and features of effective management. The additional information that SNPs provide represents advanced potential for identifying fine scale or localized population differentiation that may prove valuable in monitoring for conservation purposes (Ryynanen et al. 2007; Narum et al. 2008a). For example, criteria for defining populations are somewhat ambiguously defined among management agencies. They include distinct population segments (DPS), evolutionarily significant units (ESU) and major population groups (MPG). These definitions may rely heavily on geography, records of stock transfer, and basic biology or behaviors observed across locales or subbasins. Although genetic structure also influence the delineation of defined populations, such characterizations are likely to change coincident with improvements in genetic tools (particularly SNPs associated with adaptive selection). This additional information may ultimately influence criteria for identifying populations on the basis of both gene flow and adaptive potential across diverse landscapes in the Columbia River Basin (Matala et al. 2011, Narum et al. 2011). Currently our SNP baselines are being used in archival or temporal studies, population structure analyses in unsupplemented watersheds, efforts to reintroduce fish into extirpated regions within historic ranges, and finally broad implementation of genetic stock identification (GSI), including GSI of tribal harvest fisheries and GSI to evaluate migration timing through the hydro system.

Methods

Baseline sampling: expanding coverage

Each project year, populations (collections) of *O. tshawytscha*, *O. mykiss*, and *O. nerka* will be chosen for baseline expansion based on availability, novelty, and in accordance with our goal of including all extant stocks from throughout the Columbia River Basin. Further, priority collections for all three species have been identified as those relevant to basin-wide management and tribal fishery interests (particularly in the middle Columbia and Snake River regions). Priority collections include major supplementation stocks for all three species. Complete coverage for Chinook salmon will include lower Columbia, ocean-type, and stream-type lineages. Complete coverage for steelhead trout will include inland and coastal lineages as well as summer-run and winter-run ecotypes. For the long-term, we intend to include resident *O. mykiss* populations to test the utility of the SNPs for differentiating anadromous from resident *O. mykiss* life histories (Narum et al. 2008a; Narum et al. 2011). Complete baseline coverage for *O. nerka* will include the anadromous form (sockeye salmon) and the land-locked form (Kokanee). For each species the baselines may include life history variants (e.g., precocial fish), and temporally stratified samples (e.g., archival) contingent on availability. Some collections will be complementary to, or overlap those submitted to the standardized GAPS and SPAN consortium baselines for Chinook salmon and steelhead trout thus allowing evaluation of multiple marker data sets in some cases. The existing baselines completed through 2011 are defined by

collections described in detail in Hess et al (2011a) and Hess et al. (2012), which are comprised of 40 Chinook salmon collections, 90 steelhead trout collections, and 14 *O. nerka* collections. For the 2012 baseline expansion efforts, no additional collections were genotyped at the CRITFC laboratory for *O. mykiss*; however, based on further structure analyses that were conducted following submission of our 2011 annual report, some collections were combined based on genetic similarity, resulting in a total of 73 baseline populations from the original 90. With the addition of 72 updated collections (i.e. all 192 SNP loci) from the Snake River DPS contributed by IDFG in April 2012, our final baseline data set for project year 2012 included 145 discrete populations. A thorough genetic analysis was conducted and prepared for publication based on this current composite baseline (2010-2012; Appendix 1a). We expanded the Chinook salmon baseline by an additional 11 populations in 2012 (Appendix 1b), and IDFG contributed a Snake River component of 28 collections. This brings the total number to 79 populations in the current composite baseline for Chinook salmon. For *O. nerka*, we added five Sawtooth Valley lakes collections from the upper Salmon River region, Wallowa Lake kokanee in the Grande Ronde system, and a sample of captive broodstock for the Red Fish Lake Sockeye Salmon supplementation program in Idaho (Appendix 1c). Additional *O. nerka* baseline collections have been gathered but not yet genotyped, representing various kokanee hatchery stocks and several lakes in the upper Deschutes River system. Our two primary goals for these species specific genotypic baselines are: 1) annual genetic stock identification (GSI) analyses that will be used for 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), 2) 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.

Laboratory Protocol

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. 2006?). Many samples were contributed by outside agencies including NOAA Fisheries, Washington Department of fish and Game (WDFW), Oregon Department of Fish and Wildlife (ODFW), United States Fish and Wildlife Service (USFWS) and IDFG. 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, each 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 (including taq polymerase), 0.30µl H₂O 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 H₂O, 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 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 3.1.1. 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 ten missing SNP genotypes per individual for *O. nerka*, and 18 missing SNPs for steelhead trout and Chinook salmon).

Statistical Analysis

For the steelhead trout baseline, all samples were screened for species ID and hybridization between *O. mykiss* and *O. clarkii* congeners using three species specific SNP markers: *Ocl_gshpx-357*, *Omy_myclarp404-111*, and *Omy_Omyclmk438-96* (Hess & Narum 2011). Our protocol is to remove all individuals identified as hybrids and congeners from the data set prior to analyses. For each baseline data set respective of species, allele frequencies (i.e. minor allele frequency at each locus; MAF), mean among-group variation (F_{ST}) per locus, and number of samples analyzed per population per locus were calculating using the program GenAIEx version 6.2 (Peakall and Smouse 2006). The Markov Chain Monte Carlo (MCMC) approximation of Fisher's exact test implemented in GENEPOP v. 3.3 (1000 batches with 1000 iterations; Raymond and Rousset 1995) was used to test for deviations from Hardy-Weinberg equilibrium (HWE) expectations evaluated across SNP loci and populations. We used this test to evaluate potential non-random mating and family group biases within populations, or possible marker amplification problems (e.g. null alleles). Linkage disequilibrium was tested for all pairs of loci across populations using a simulated exact test in GENEPOP. Linkage analysis identifies non-random association of loci, where some loci may be physically linked in the genome; population specific linkage indicates likely population admixture. For all pairs of loci with significant non-random association (linkage) our protocol is to select the most informative of the two (e. g. highest F_{ST} and/or MAF), excluding the remaining locus in each pair from further downstream analyses. Statistical significance (α) was adjusted for the number of simultaneous tests (initial α = 0.05) for both HWE and linkage tests via the B-Y FDR method (Benjamini and Yekutieli 2001) as implemented by Narum (2006) to reduce false positive tests.

For the *O. nerka* baseline data, 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, and a 0.99 confidence interval for all SNP loci under an infinite alleles model. For the steelhead trout and Chinook salmon baseline datasets, outlier tests were implemented in ARLEQUIN version 3.5 (Excoffier *et al.* 2005). The method uses coalescent simulations to generate a null distribution under neutral expectations around observed F_{ST} values (with confidence intervals), assuming a finite island model. For test settings in ARLEQUIN we used 1000 simulated demes and 150,000 coalescent simulations; the hierarchical island model was not used due to high error rate (Narum

and Hess 2011). Results using both methods were plotted to represent the 1% and 99% confidence quantiles. Loci lying above or below these quantiles (outliers) may be under directional or balancing selection (respectively) in some populations. Evaluating adaptive diversity (selection) is confounded by the need to scrutinize the underlying demographic processes that also influence diversity. Many population genetic parameters (e.g., F_{ST} , Nm, Ne, gene flow) assume neutrality. Yet neutrality is difficult to determine. Relying solely on tests that detect “outliers” as a means of characterizing the selection candidacy of loci can often produce false positive results. This may occur for example, if loci are highly differentiated because of demography, substructure, or skewed patterns of isolation-by-distance (Akey 2009; Hermisson 2009; Narum and Hess 2011). In order to draw biological inferences from comparisons that reveal patterns of neutral genetic diversity it is important to account for, or eliminate adaptive influences contributed by non-neutral outlier loci (Luikart *et al.* 2003). This has been our practice for the steelhead trout baseline that is several years along in development. Since we have not yet begun to explore differences between adaptive and neutral divergence for *O. nerka* or our full suite of Chinook salmon SNPs, the baseline analyses presented in this report were conducted without excluding any candidate loci. Determining which candidate loci are under selection will require additional computationally demanding tests to show statistical associations with certain traits or landscape features. At this point in our analyses we have not determined the appropriate confidence level threshold for assuming positive selection among our *O. nerka* baseline populations. Some SNPs for Chinook salmon have been previously characterized (Matala et al. 2011).

Analyses for Chinook salmon and steelhead trout were conducted respective of lineage (e.g. coastal vs. inland *O. mykiss*). In addition to baseline screening, some SNPs in our steelhead trout panels have been evaluated in related studies, and identified as candidate loci. Specifically, two loci were found to be informative for differentiating anadromous and resident life history types: *Omy_ndk-152* and *Omy_LDHB-2_i6* (Narum et al. 2011), two loci are putatively associated with thermal stress-induced mortality: *Omy_hsp47-86*, and *Omy_OmyP9-180* (Narum et al. 2013), five SNP loci (*Omy_aldB-165*, *Omy_gdh-271*, *Omy_Ogo4-212*, *Omy_stat3-273*, and *Omy_tlr5-205*) have been previously identified as selection candidates associated with temperature variation in desert vs. montane environments (Narum et al. 2010a), and finally one locus (*Omy_hsf2-146*) is putatively associated with precipitation in the latter study. We refer to these ten loci as having “precedence” as loci under divergent selection (Appendix 2a). Chinook salmon candidate loci with similar selection precedence are described in detail in Matala et al. 2011 and Hess and Narum 2011.

The analysis program GENEPOP (Raymond and Rousset 1995) was used to calculate global F_{ST} (θ of Weir and Cockerham 1984) and matrices of pairwise F_{ST} among all pairs of collections respective of each species baseline data sets; F_{ST} indicates the proportion of total variation attributed to differences among collections. Pairwise matrices of Nei’s genetic distances (1972) and un-rooted neighbor-joining (NJ) phylograms were generated using PHYLIP version 3.68 (Felsenstein 1992). An NJ tree indicates similarities among baseline populations or groups of populations identified both by branch association (clustering) and by branch length in the genetic distance topology of the tree. Phylogenetic analyses were conducted respective of species lineage (e.g. coastal vs. inland *O. mykiss*). The SEQBOOT option was implemented to generate 1000 simulated data sets, and consensus topology with bootstrap support was generated using the CONSENSE option in PHYLIP. Multivariate principle coordinates analyses (PCA) were then

conducted in GenAlEx version 6.2 (Peakall and Smouse 2006) based on matrices of pairwise F_{ST} to quantify the amounts of variation observed among populations. Plots reveal major patterns of genetic similarity among populations. Significant variation that exists between distinct groups is revealed in the first few axes, where the first principal coordinate accounts for the greatest amount of variability in the data. Informativeness of each succeeding axis is diminished in order, while accounting for the greatest proportion of variability that remains.

Results

Steelhead trout

Among coastal lineage populations we observed a mean MAF of 0.246 across 188 SNPs (excluding hybrid detection loci), ranging from 0.001 at *Omy_impal-55* to 0.498 at *Omy_arp-630*. The mean MAF across inland lineage populations was 0.236, ranging from 0.024 at *Omy_nach-200* to 0.497 at *OMS00070*. Locus specific ranges varied widely (Appendix 2a). No populations within either lineage exhibited fixed allele frequencies for any of our 188 SNP loci. We observed 208 departures from expected genotypic proportions out of 26,190 possible tests (BY-FDR adjusted significance threshold; $\alpha=0.0046$). Overall, the deviations from Hardy-Weinburg Equilibrium (HWE) expectations spanned 100 discrete populations and 109 loci. Population specific departures were identified only in Abernathy Creek (Ref. #12) and in Canyon Creek (Ref. #9), each with 10 departures out of 188 loci. The only locus-specific HWE departure occurred at *OMS00087*, with 12 departures out of 145 populations; *OMS00087* was therefore omitted from all subsequent analyses. Tests for linkage disequilibrium revealed five pairs and one trio of loci that remained significantly out of equilibrium in at least 10% of populations after adjustment for multiple tests (P -value <0.0001). Linked SNP pairs were: *OMS00133* and *Omy_rapd-167*, *Omy_CRBF1-1* and *Omy_crb-106*, *Omy_Il-1b_028* and *Omy_Il1b-198*, *Omy_SECC22b-88* and *OMS00169*, *Omy_ndk-152* and *Omy_u09-52.284*, and the trio *Omy_GHSR-121*, *OMS00176* and *Omy_mapK3-10*. In each instance the locus with the largest F_{ST} (and MAF) was retained and the others were omitted prior to subsequent analyses, leaving 180 of the original 191 SNP loci in the data set (Appendix 2a).

The mean expected heterozygosity across loci and collections ranged from 0.269-0.362 for the coastal lineage, and 0.226-0.345 for the inland lineage. In outlier tests, plots of expected heterozygosity and genetic distance (F_{ST}) revealed eight candidate loci putatively under directional selection in the coastal lineage, and ten in the interior lineage (Appendix 2a). No influence of balancing selection was observed. Pairwise population F_{ST} matrices used for PCA analyses revealed nearly complete significance among all populations within each lineage, with only a few exceptions between closely adjacent populations. Divergence was greater within the inland lineage, where pairwise values were generally higher (range 0.0002 to 0.1889, mean 0.0421) compared to values within the coastal lineage (range 0.0001 to 0.1098, mean 0.0413). We observed among-group variation (F_{ST}) across loci that ranged from 0.0064-0.0941 for the coastal lineage (mean 0.0349), and 0.0031-0.1092 for the inland lineage (mean 0.0286). The overall mean pairwise genetic distance was 0.0692 across lineages. On average the pairwise genetic distances were lower in the coastal lineage although the average overall was higher than inland. We demonstrated genetic similarity among populations within each lineage through PCA cluster analyses to quantify variation and graphically display the relationship between collections (Figure 2), and based on phylogenetic relationships in the topology of an unrooted NJ phylogram (Figure 3a & 3b). The confidence or concordance ($>50\%$) of the NJ topology is indicated with

bootstrap values at the nodes. Results were complimentary and corroborate major distinctions previously identified (Behnke 2002; Currens et al. 2009; Blankenship et al. 2011), revealing defined clustering of the most genetically similar collections for steelhead, but populations between lineages were clearly divergent. In large part, the steelhead collections clustered accurately into geographic regions or major tributary, particularly in the Snake River for the inland lineage and Willamette River for the coastal lineage. Among the inland lineage the most recognizable distinctions occurred within major population groups or DPS, including the middle and south forks of both the Salmon River and Clearwater River. Similar to observations among Chinook salmon results, the Klickitat and Big White Salmon collections are among the most divergent overall, appearing intermediate between lineages.

Chinook salmon

The mean expected heterozygosity (allelic variability) across loci and collections ranged from 0.267-0.332 for the lower Columbia lineage, 0.276-0.320 for the ocean-type lineage, and 0.193-0.296 for the stream-type lineage. The SNP loci *Ots_zP3b-215* was fixed in both the ocean-type and stream-type lineages; locus *Ots_RAS1* was also fixed in the stream-type lineage. No loci were diagnostic for any particular lineage or life history type in the data set. Based on 12,710 tests for HWE deviations, the adjusted BY-FDR significance threshold was $\alpha=0.0050$. A total of 168 tests were significant, including 11 populations out of 145 at locus *Ots_EndoRB*, and 16 out of 188 loci for both the Entiat River stream-type and Little White Salmon River ocean-type collections. No loci or populations were dropped from analyses based on HWE tests. A plot of expected heterozygosity and genetic distance (F_{ST}) identified 20 SNP outlier loci, or candidate loci under directional selection across lineages, including 11 in both the stream-type and lower Columbia lineages and 3 in the ocean-type lineages (some outliers occurred in multiple lineages: Appendix 2b). Significant linkage disequilibrium was observed in four pair of loci, and for each pair one locus was dropped from further analyses (Appendix 2b): *Ots_OTALDBINT1-SNP1* and *Ots_aldb-177M*, *Ots_hsc71-3'-488* and *Ots_hsc71-5'-453*, *Ots_FGF6A* and *Ots_FGF6B_1*, and *Ots_OTSTF1-SNP1* and *Ots_Tnsf*.

For the 79 collections of Chinook salmon evaluated, we observed among-group variation (F_{ST}) across loci that ranged from 0.005 at locus *Ots_zP3b-215* to 0.346 at locus *Ots_MHC2* for the lower Columbia lineage (mean 0.046), 0.004 at locus *Ots_110689-218* to 0.386 at locus *Ots_Ots311-101x* for the ocean-type (mean 0.028), and 0.005 at locus *Ots_LWSop-638* to 0.130 at locus *Ots_TAPBP* for the stream-type (mean 0.043). The overall mean pairwise genetic distance (F_{ST}) was 0.050 for lower Columbia, 0.023 for ocean-type, and 0.036 for stream-type: the mean across lineages was 0.091. The least amount of among-group variation occurred within the ocean-type lineage, while the lower Columbia and stream-type lineages were comparable. We demonstrated genetic similarity among populations within each lineage through PCA cluster analyses to graphically display the relationship between collections (Figure 4), and based on phylogenetic relationships in the topology of an unrooted NJ phylogram (Figure 5a & 5b). The confidence or concordance (>50%) of the NJ topology is indicated with bootstrap values at the nodes. Results of the two analyses were complimentary, and revealed defined clustering of the most genetically similar collections for Chinook salmon, but collections between the three major lineages were clearly divergent. In large part, the Chinook salmon collections clustered accurately into geographic regions or major tributary (Figures 4 & 5). Note that nearly all tributary distinctions in the lower Columbia were represented with high confidence, where

spring-run and fall-run groups also appear to cluster together regardless of location. Ocean-type results were only slightly weaker, but three run types within that lineage generally also tend to cluster in close proximity. Relationships in the stream-type lineage are more variable, but the major divisions coincide well with major subbasins and regions within the Columbia River Basin. Such distinctions were likely highly influential in overall results (e.g., unusually high pairwise F_{ST} in the lower lineage), and those results may be reflective of stray influences within and/or among lineages (see Hess et al. 2011b). In a baseline companion project to evaluate population structure in the John Day River Basin over a 30 year period, we identified significant out-of-basin stray influences that appear to be occurring at an elevated rate in more recent years (Hess and Matala *in review*).

O. nerka

The mean expected heterozygosity across loci and collections ranged from 0.149 to 0.326. There were no fixed allele frequencies across loci and baseline collections, and no loci appear diagnostic for either life history type in the data set (kokanee or sockeye salmon). Among 1,842 total HWE tests across all collections we observed 23 departures from expected genotypic proportions using an BY-FDR adjusted significance threshold of $\alpha=0.0062$. We identified one locus specific deviation (*One_UCA-24*) which occurred in approximately 25% of collections (5 of 22), but there were no observed population specific deviations. A plot of expected heterozygosity and genetic distance (F_{ST}) revealed four SNP outlier loci, or candidate loci under directional selection across lineages: *One_HGFA-49*, *One_Prl2*, *One_srp09-127*, *One_U1216-230* (Appendix 2c). Further association testing and landscape genetic analysis will be required to confirm significant selection acting on these loci. Significant linkage disequilibrium was observed between *One_MHC2-190* and *One_MHC2-251*, and *One_ODC1-196* and *One_U508-533*. For the current evaluation, all loci were retained in analyses.

We observed among-group variation (F_{ST}) across loci that ranged from 0.028 at locus *One_U301-92*, to 0.606 at locus *One_Cytb_17* (mean 0.181). Interestingly, the latter is a maternally inherited locus located in the mitochondria. We demonstrated genetic similarity among populations within each region or major subbasin through phylogenetic relationships in the topology of an unrooted NJ phylogram (Figure 6). The confidence or concordance (>50%) of the NJ topology is indicated with bootstrap values at the nodes. Results revealed defined clustering of the most genetically similar collections for *O. nerka*. Collections within each region, particularly temporally stratified collections, exhibited substantial similarity while populations between regions (i.e., Wenatchee River, Deschutes River, Osoyos Lakes, and Snake River) were highly divergent (Figures 6 & 7). Results were complimentary to those from pairwise F_{ST} , indicating there is no significant difference between temporal Tumwater Dam samples, temporal Wells Dam samples, temporal Suttle Lake Samples, or temporal samples from Lake Billy Chinook and the Metolius River in the Deschutes River system. Based on these results, respective collections were combined by location across collection years, and the final number of collections that will be represented in the baseline for *O. nerka* is 13. In PCA cluster analyses to graphically display the relationship between collections (Figure 7), the thirteen collections generally cluster by region; however, Snake River populations are the most distinct (particularly Warm Lake in the South Fork Salmon River). Kokanee hatchery stocks from Meadow Creek and Whatcom Lake appear to cluster relatively tightly with populations which have likely experienced significant outplanting in the past (e.g., Whatcome Lake among lake

Suttle Lake and Petite Lake collections).

Discussion

We have compiled extensive data sets of SNP genotypes for Chinook salmon, steelhead trout, and *O. nerka* covering diverse regions in the Columbia River Basin (including the Snake River Basin). 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 to account for inter-annual variation. This strategy assures the greatest likelihood of discerning reproductively distinct aggregations for each species through time (Waples 1991), 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 2008b; Narum et al. 2010b) and steelhead trout (Blankenship et al. 2011; Matala et al. *in review*). Results further suggest SNPs are a class of markers that perform at least as well as μ SATs in terms of their potential for monitoring population distinctions and composition during fish migrations and fisheries harvests. In addition, we have demonstrated that SNPs offer an opportunity to characterize adaptive variation, which is beyond the scope of most μ SAT datasets that utilize neutral markers.

The expansion efforts reported here complement previously reported results. The continued expansion of SNP panels and updating of baseline collections will help us achieve a greater level of resolution (or statistical power to identify population distinctions), at least among the major tributaries and subbasins throughout the CRB. Such results will be most beneficial to the larger application of the baselines, namely GSI. Initial indications suggest an improved GSI quality with these updated data set (see sections 3 and 4 of this report) compared with previous years. Our steelhead baseline is robust and representative of the majority of watersheds within the Columbia/Snake River basins. With the updating effort that was completed this year (including contributions of a significant amount of data from IDFG) we now have over 145 separate collections, including spatially and temporally stratified coverage in many watersheds. On the basis of our SNP genotypes, steelhead appear to be a highly diverse species in the basin, with clear distinctions that corroborate previous studies and are consistent with biologically significant distinctions among life history types, lineages and ecotypes. A peer reviewed manuscript has been submitted for publication in (Canadian Journal of Fisheries and Aquatic Sciences) that examines demographic and adaptive divergence of steelhead using landscape analyses, which expands on some comprehensive published studies (e.g., Blankenship et al. 2011). In comparisons among species specific SNP panels, the panel for *O. nerka* is extremely powerful owing to the biological nature of the species and its distribution within the CRB. Our ability to differentiate among regions and in some cases within regions (the Deschutes River populations) exceeds what we have been able to ascertain with Chinook salmon and steelhead, as evidenced by comparisons of among-group variation. For example, in a related study, we have

shown that a mixed sample of sockeye salmon from Okanogan and Wenatchee River systems can be differentiated with 100% accuracy (data not shown).

We will begin to more closely examine those populations that display unique attributes or differences in contrast to, or in accordance with expectations based on published information (e.g., Big White Salmon River, Little White Salmon River, and Klickitat River). 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 (e.g., Matala et al. 2011). 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, and compare these results to some of our initial or preliminary findings as these baselines develop. This is particularly interesting for *O. nerka*, which have complex life history, but for which many anadromous populations have been extirpated; there is currently limited information available toward understanding the ability of populations to revert back to anadromy once stream corridors are made navigable. Our data will be implemented in current and ongoing application of PBT and GSI methods for each species. PBT can be used to validate assignment origins based on GSI. These additional and ongoing efforts will require further scrutiny of the genotypic data, as we have not yet identified significant selection candidate loci for some expanded data set.

Our future efforts include adding collections to the baseline to increase basin-wide coverage of all species, particularly those that account for stock transfer history of *O. nerka* throughout the basin, 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 for genetic applications, as evident by our updating effort in this report and with new RAD sequencing capabilities. A current Chinook RAD project is nearing the analysis phase, having genotyped a baseline encompassing approximately 50 discrete populations across the range of Chinook salmon. Using this technique we will potentially identify thousands of SNP loci. As we begin to construct a linkage map, the results will allow a more robust evaluation of which loci appear most influenced by selection as well as the corresponding traits underlying selection at those loci. Some HWE and linkage issues that point to problems with population admixture (e.g., straying) may persist and will require certain populations and/or loci be dropped from the baselines in the future.

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Figure Legends

Figure 1a. Map of study area and collections comprising the 2012 baselines. Chinook salmon collections are represented by red triangles, steelhead trout by yellow circles, and *O. nerka* by purple squares. Pink outline denotes Snake River, while green denotes relevant subbasins within the Columbia River Basin. One British Columbia collection is shown off scale. For reference, collection locations within each subbasin (latitude and longitude) are provided in appendices 1a-1c.

Figure 1b. Snake River subbasins and collections. BPA subbasins are: 1) Lower Snake, 2) Tucannon, 3) Asotin, 4) Snake Hells Canyon, 5) Clearwater, 6) Grande Ronde, 7) Imnaha, 8) Snake Lower Middle, and 9) Salmon.

Figure 1c. Columbia River subbasins and collections. BPA subbasins are: 10) Elochoman, 11) Cowlitz, 12) Kalama, 13) Lewis, 14) Willamette, 15) Sandy, 16) Hood, 17) Little White Salmon, 18) Big White Salmon, 19) Klickitat, 20) Fifteenmile, 21) Deschutes, 22) John Day, 23) Columbia Lower Middle, 24) Umatilla, 25) Walla Walla, 26) Yakima, 27) Crab, 28) Columbia Upper Middle, 29) Wenatchee, 30) Entiat, 31) Methow, and 32) Okanogan.

Figure 2. Steelhead trout principle coordinates analysis plots. Divergence in the steelhead trout coastal lineage (158 putative neutral loci; Matala et al. *in review*) is identified by axes 1&2 (a) and axes 1&3 (b), accounting for 66.8% of total variation. Divergence in the steelhead trout inland lineage (146 putative neutral loci) is identified by axes 1&2 (c) and axes 1&3 (d), accounting for 72.9% of total variation. Ellipses highlight clusters of populations by region or subbasin: 1) M. F. Salmon, 2) S. F. Salmon, 3) S. F. Clearwater, 4) M. F. Clearwater, 5) Yakima River, and 6) Klickitat River.

Figure 3a. Neighbor joining trees depicting distance between steelhead trout populations of the coastal lineage, represented by data for 158 loci; (*) indicates the run type is unknown, and circles identify known summer-run populations. Bootstrap support exceeding 50% appears in boxes at nodes.

Figure 3b. Neighbor joining trees based on SNPs classified as neutral from association tests (data not shown). Populations of the inland steelhead trout lineage are represented by data for 146 loci; Bootstrap support exceeding 50% appears at nodes.

Figure 4. Principal coordinates analysis plot showing distinct clustering of the three Chinook salmon lineages. The first two axes shown account for 93.13% of total variation. Note

potentially admixed collections from the Klickitat River (stream-type) and Entiat River (ocean-type), located off cluster for their respective lineages.

Figure 5. Neighbor joining trees depicting distance between Chinook salmon populations: a) stream-type, b) ocean-type, c) lower Columbia. Bootstrap support exceeding 50% appears at nodes. Numbered stream-type populations correspond with appendix 1b.

Figure 6. Principal coordinates analysis plot showing distinct clustering of 13 identified *O. nerka* populations (temporal collections have been combined). The first two axes account for 73.8% of total variation. The two plots show identical ordination of data points, from two different perspectives rotated on the three axes indicated.

Figure 7. *O. nerka* NJ-phylogram based on Neis distance (1972). Consensus in tree topology that has greater than 50% bootstrap support is shown in red text at nodes.

Figure 1a. Map of study area and collections comprising the 2012 baselines.

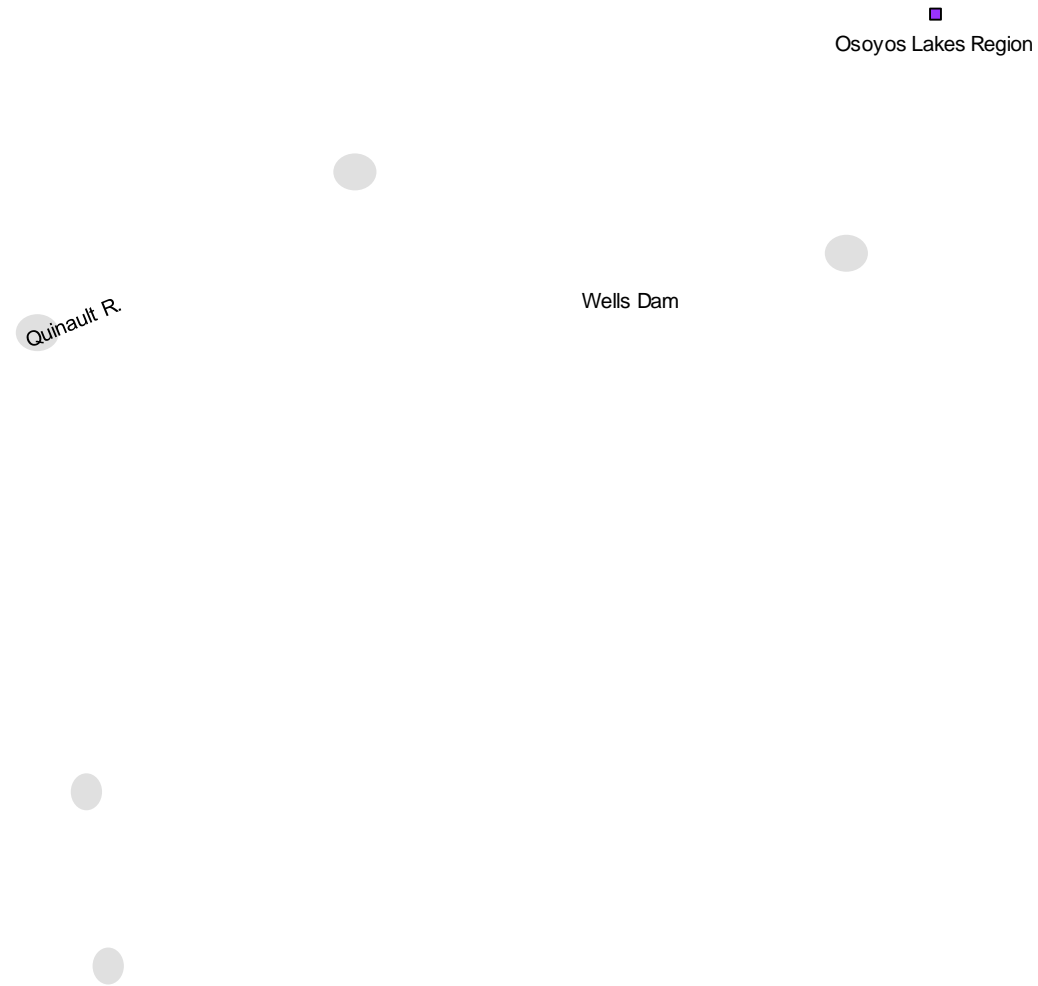


Figure 1b. Snake River subbasins and collections.

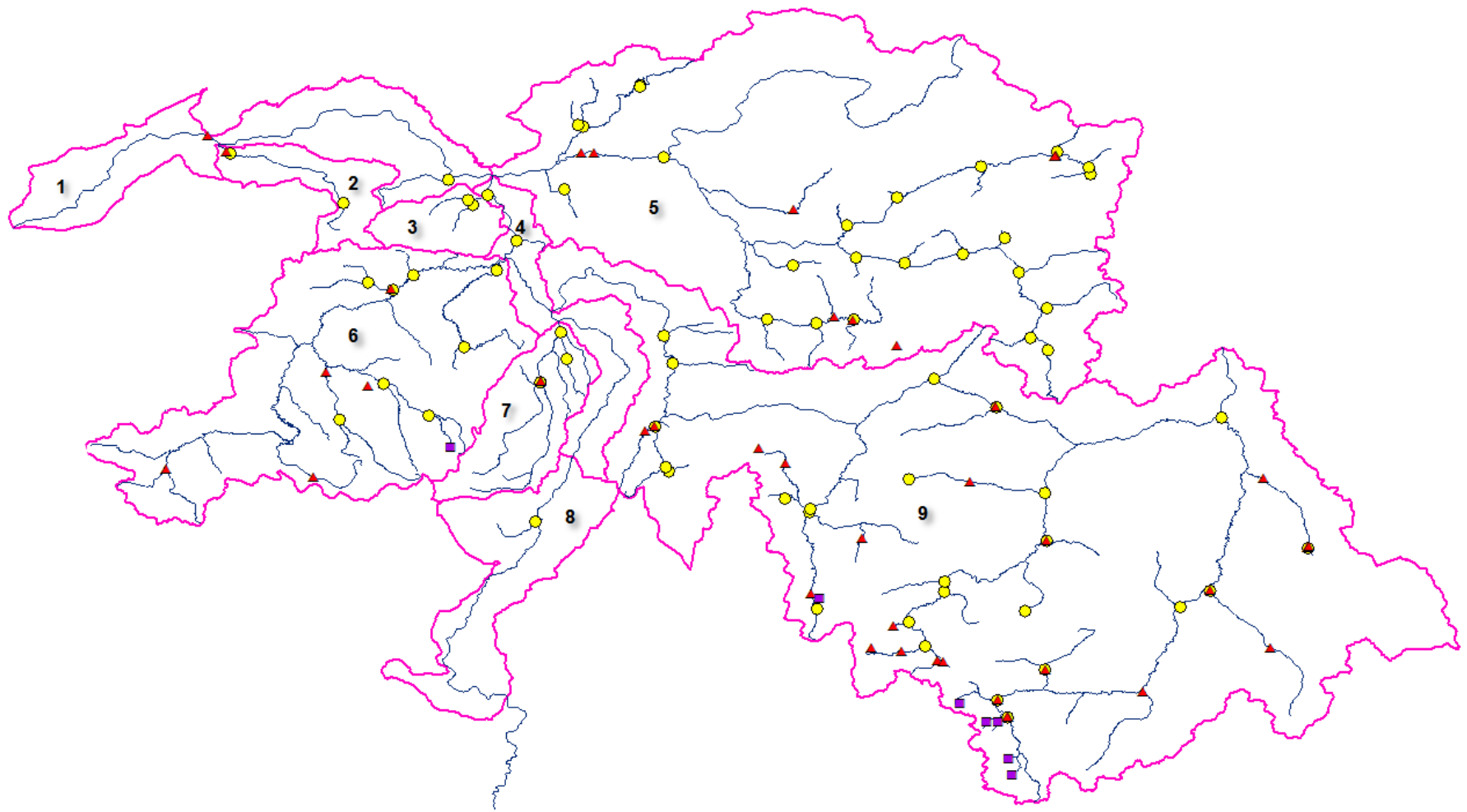


Figure 1c. Columbia River subbasins and collections.

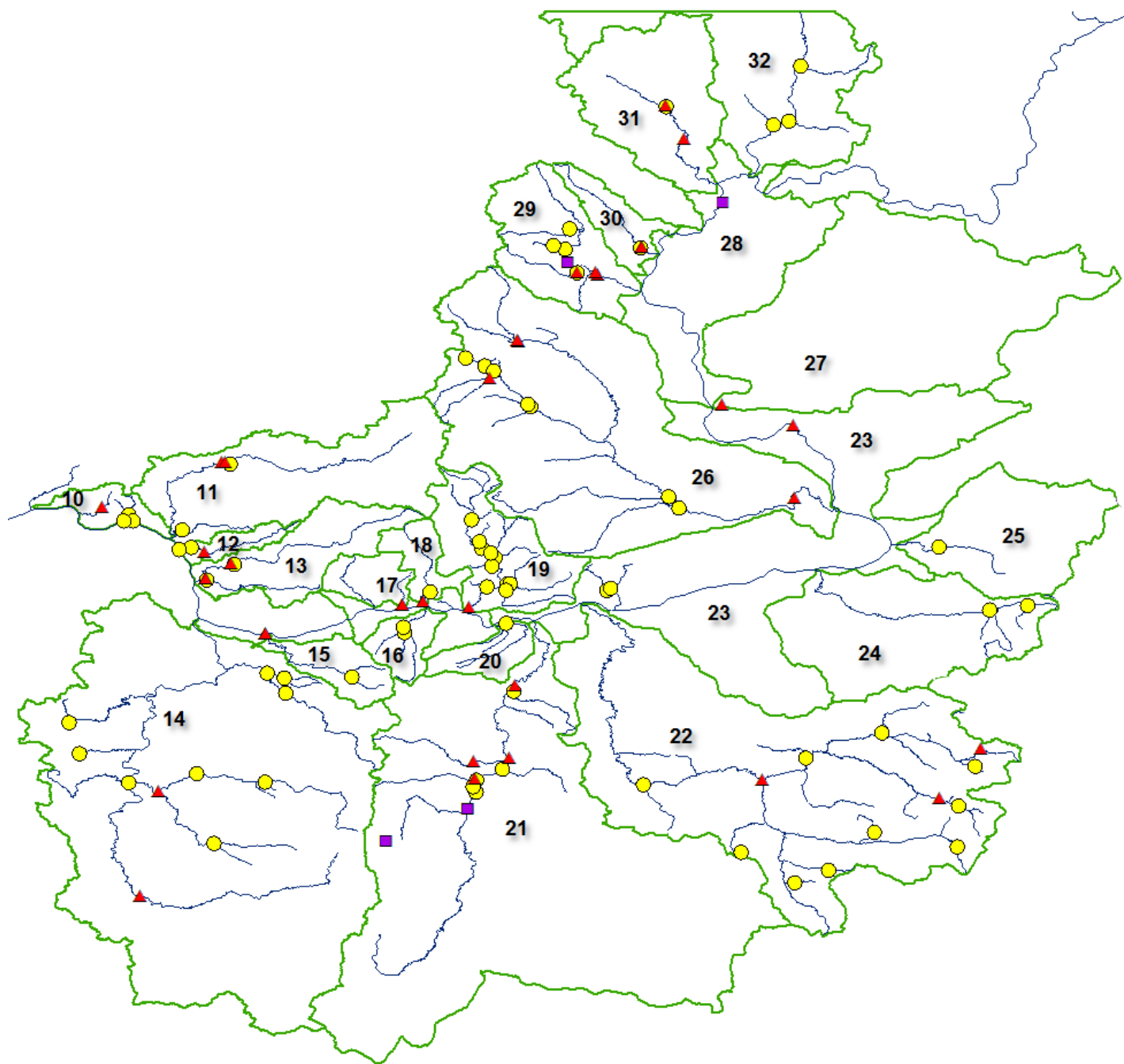
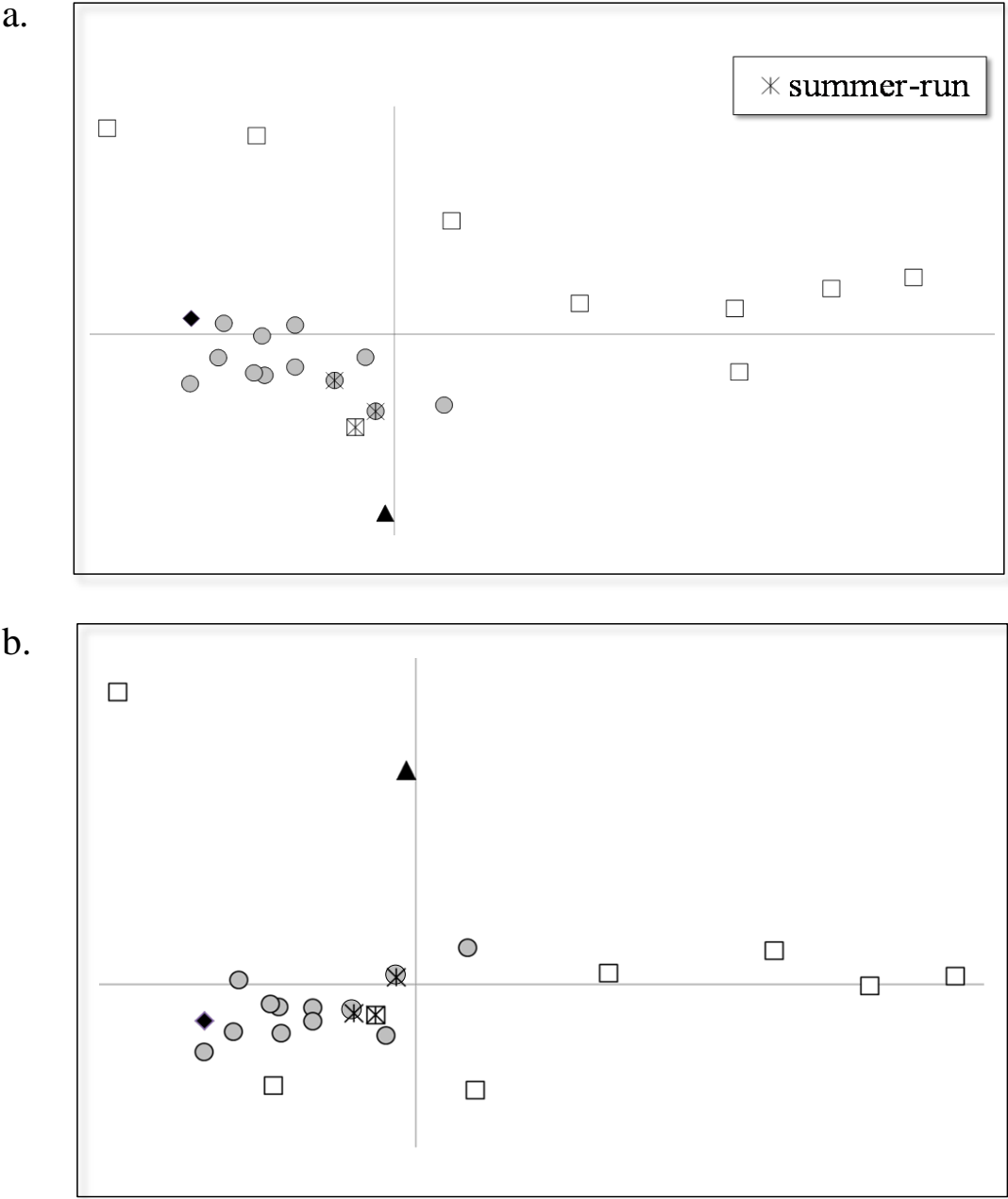
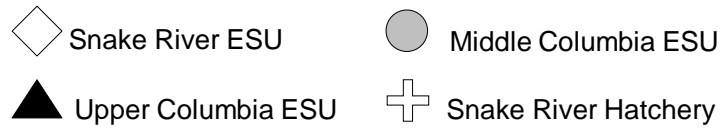


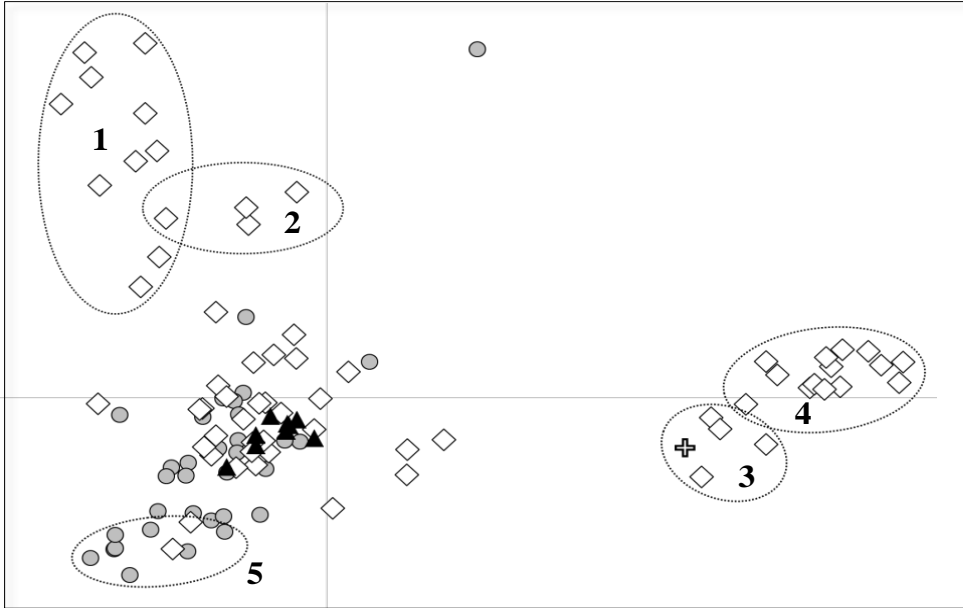
Figure 2. Steelhead trout principal coordinates analysis plots.

□ Willamette ● Lower Columbia ◆ Quinault ▲ Big White Salmon





c.



d.

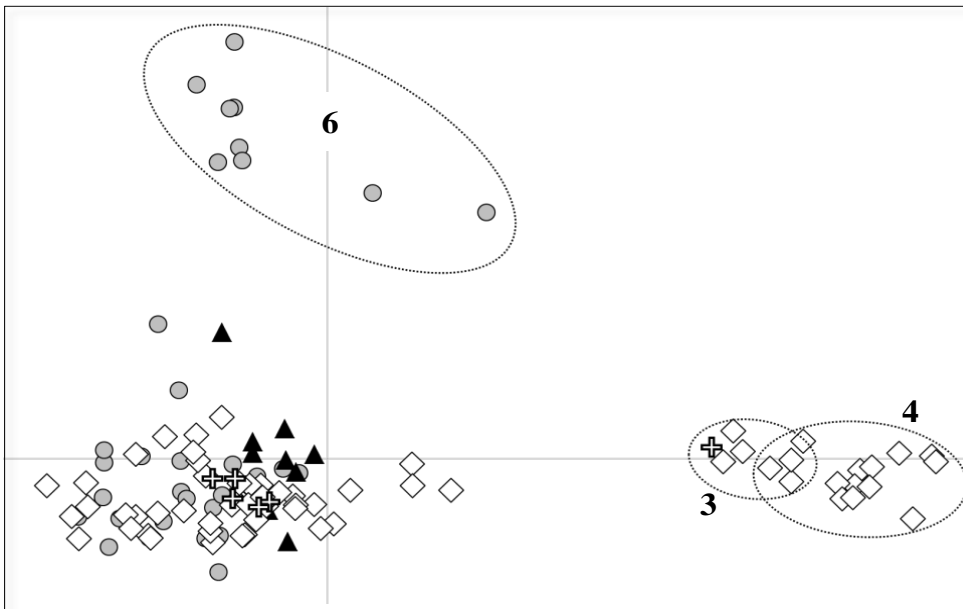


Figure 3a. Coastal lineage steelhead trout neighbor joining trees.

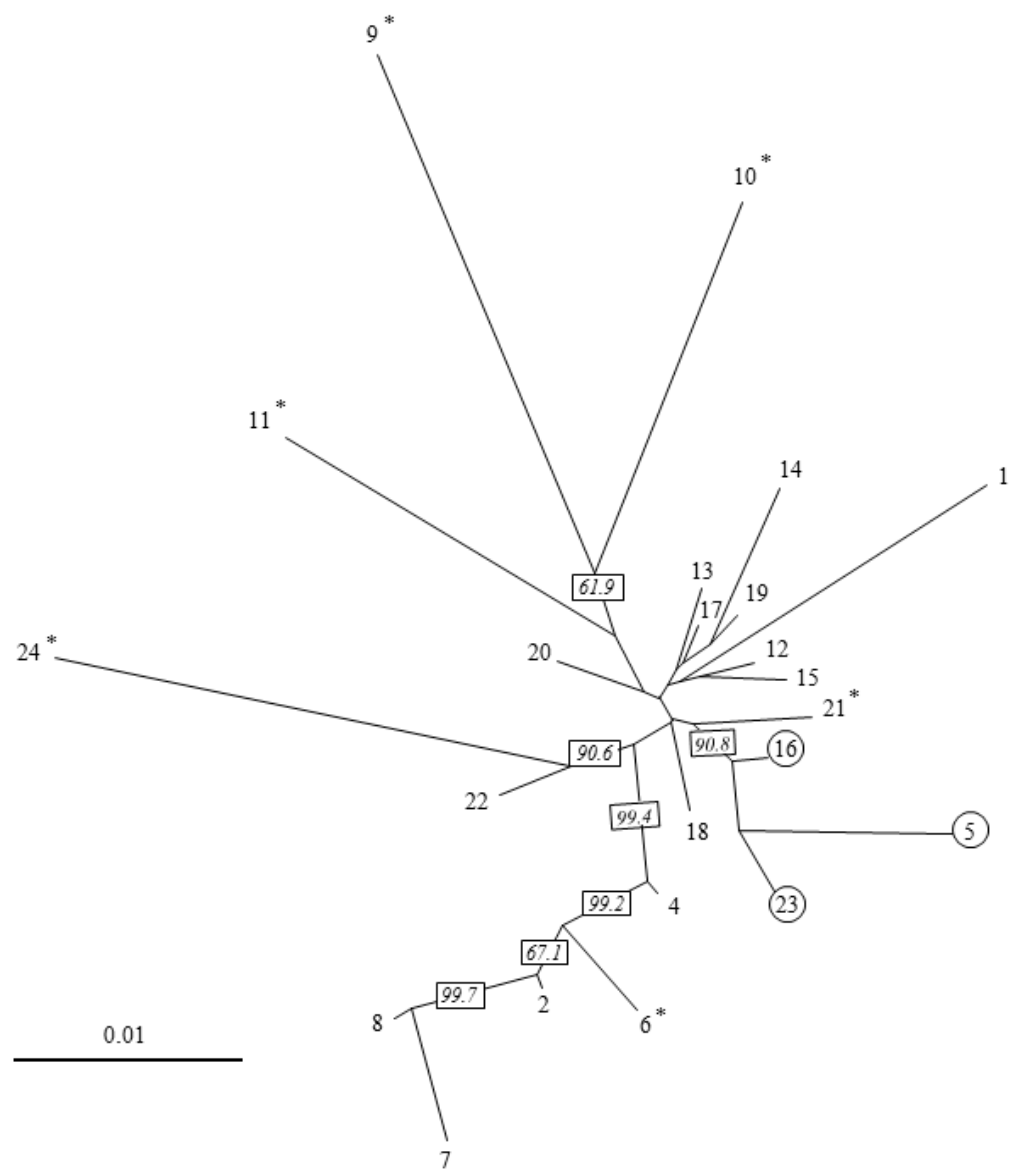


Figure 3b. Inland lineage steelhead trout neighbor joining trees.

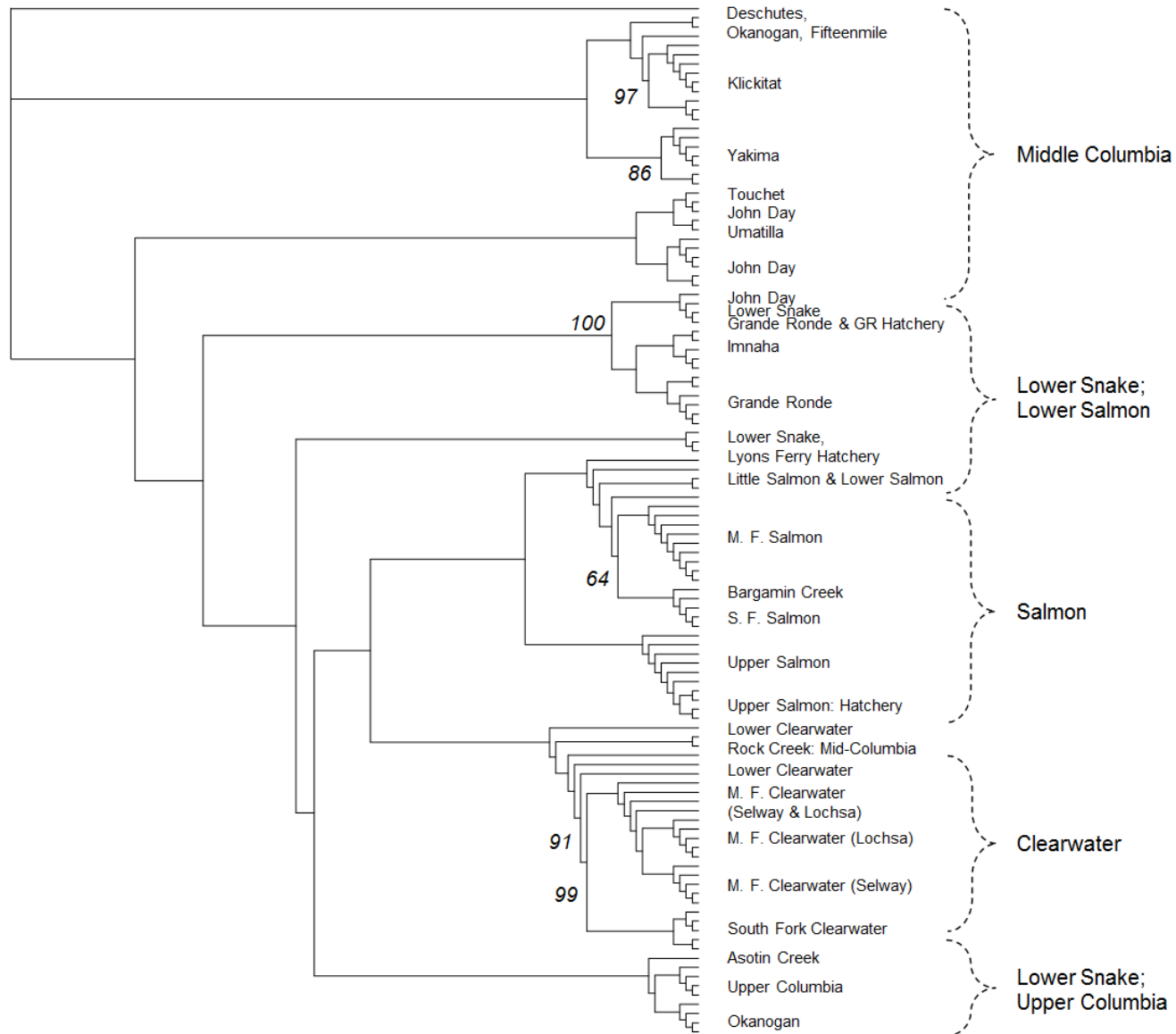


Figure 4. Chinook salmon principal coordinates analysis plot.

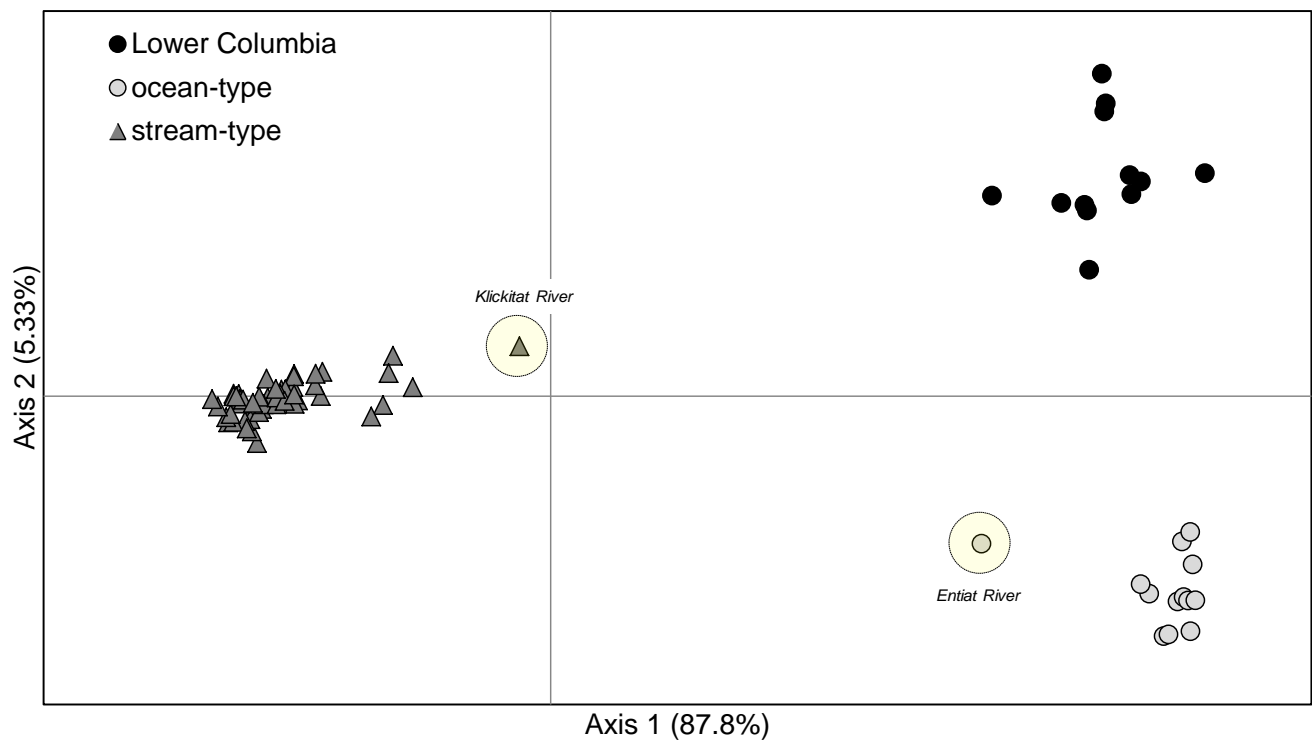
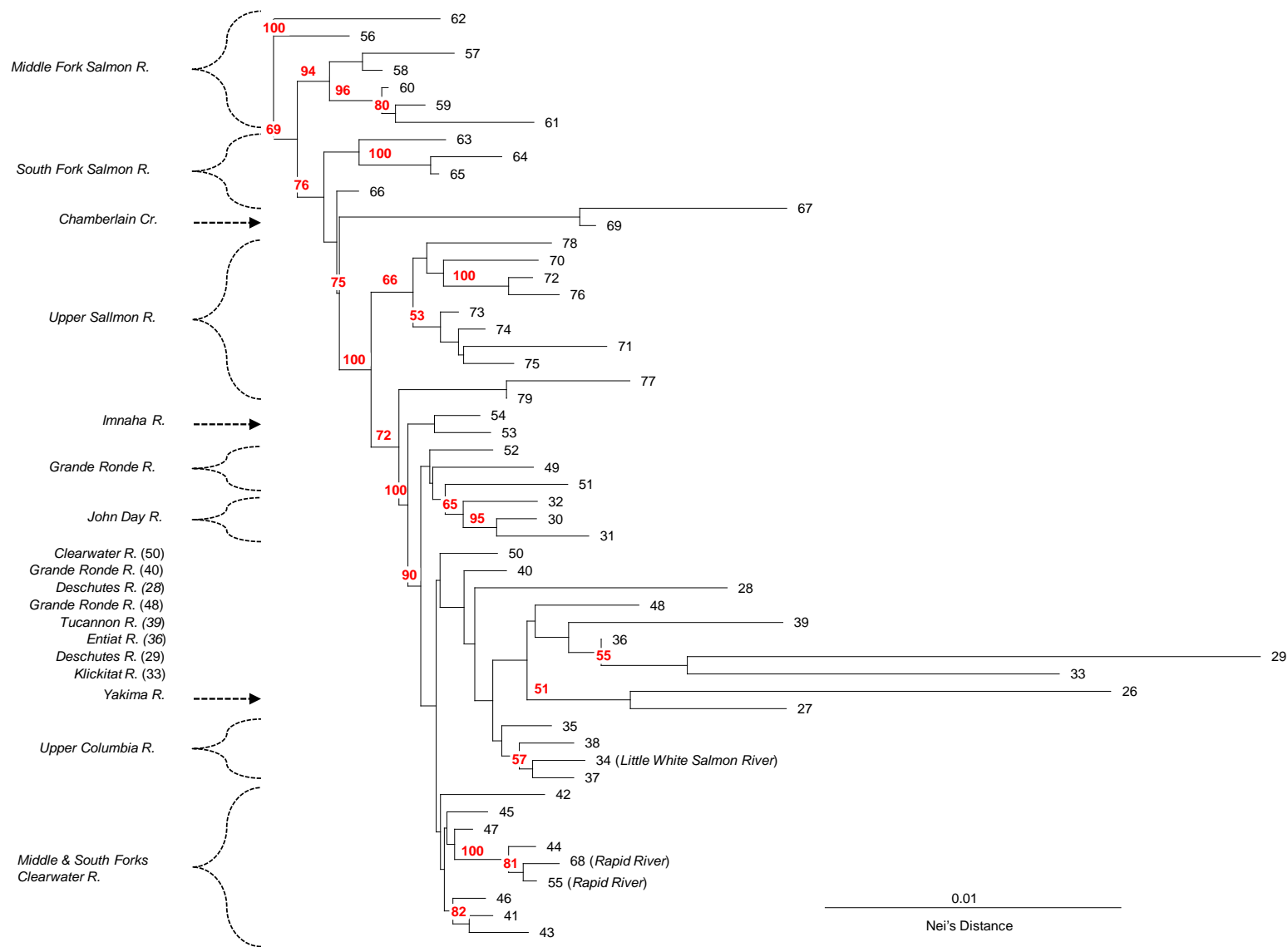
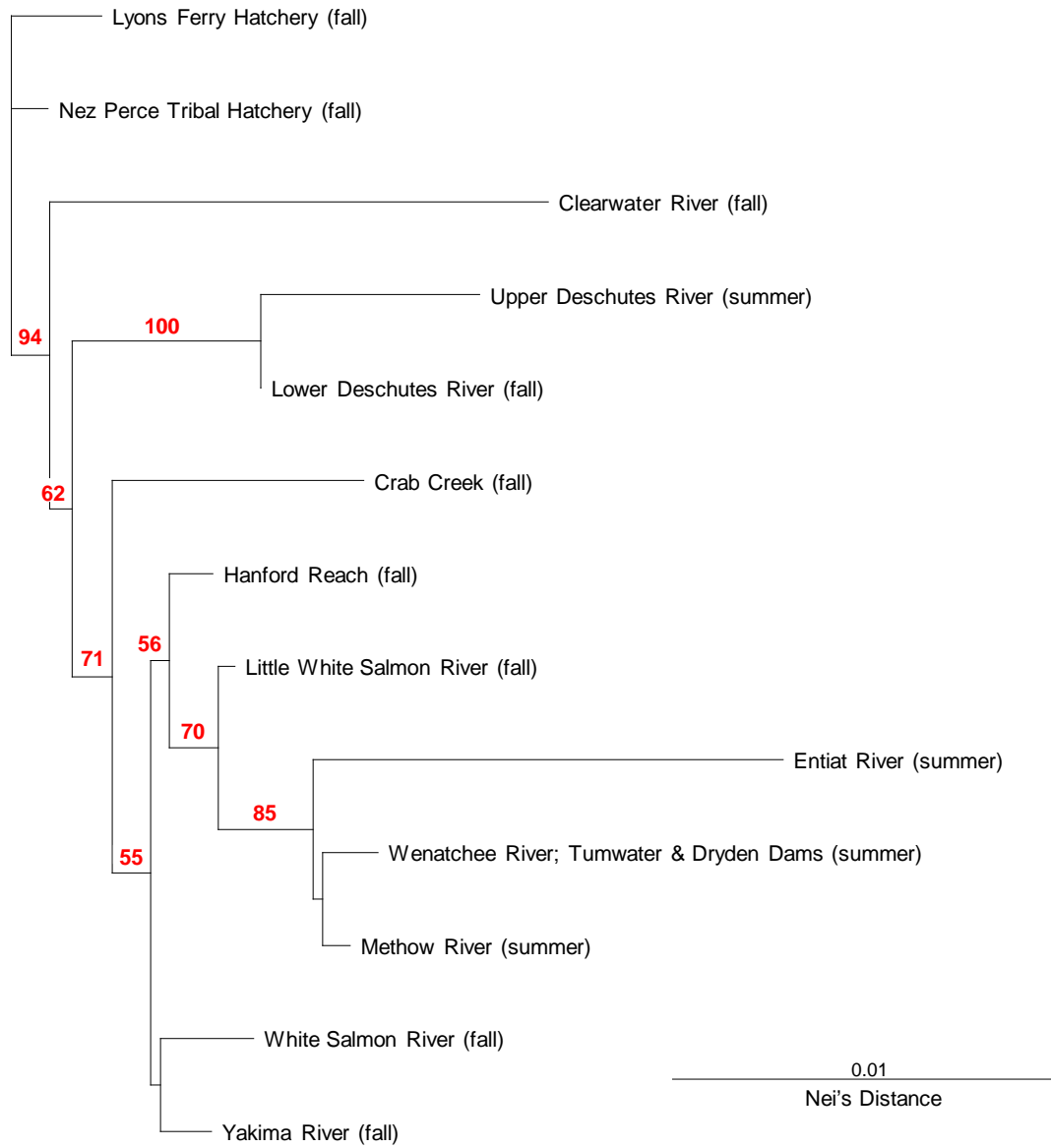


Figure 5. Chinook salmon neighbor joining trees.

a.)



b.)



c.)

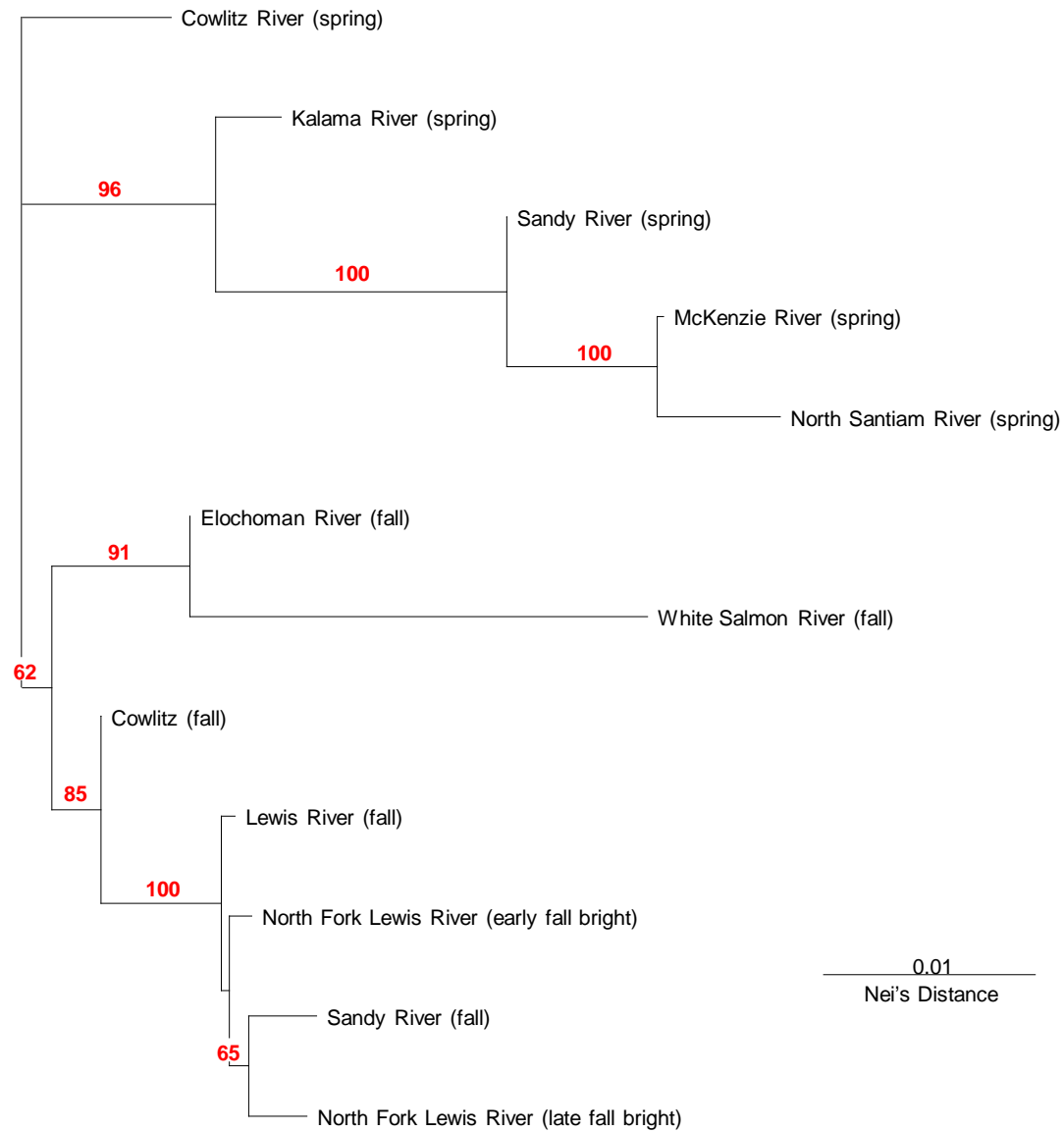
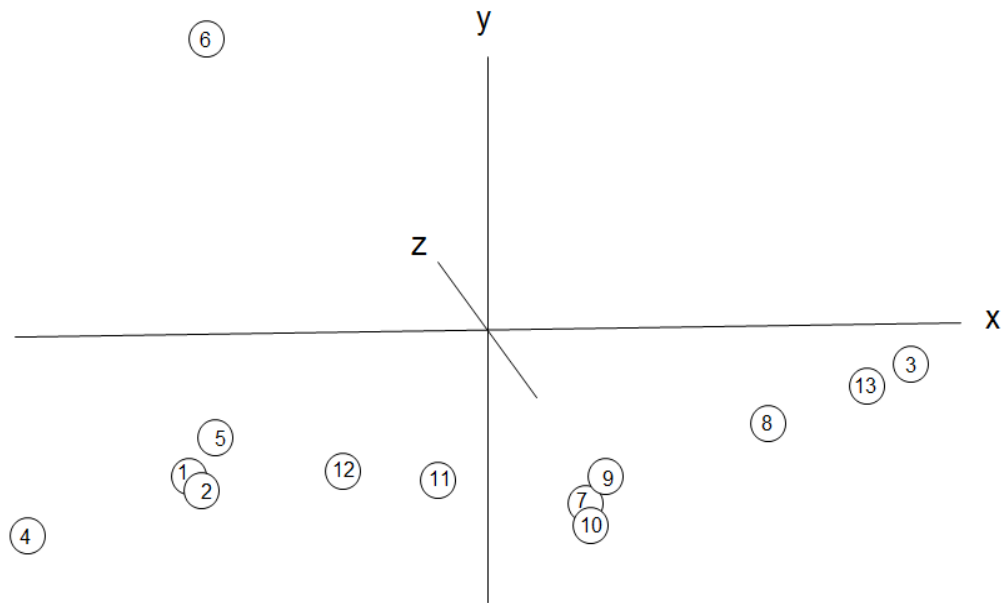


Figure 6. *O. nerka* principal coordinates analysis plot.



- | | |
|---------------------|--|
| 01.) Alturas Lake | 08.) Suttle Lake |
| 02.) Fishhook Creek | 09.) Lake Billy Chinook / Metolius River |
| 03.) Petite Lake | 10.) Meadow Cr. |
| 04.) Redfish Lake | 11.) Tumwater |
| 05.) Stanley Lake | 12.) Wells Dam |
| 06.) Warm Lake | 13.) Lake Whatcom |
| 07.) Wallowa River | |

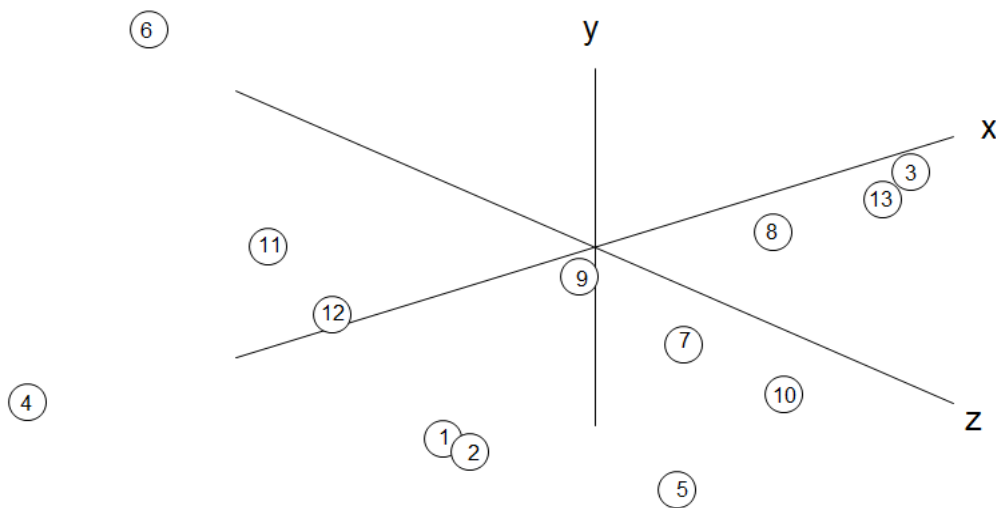
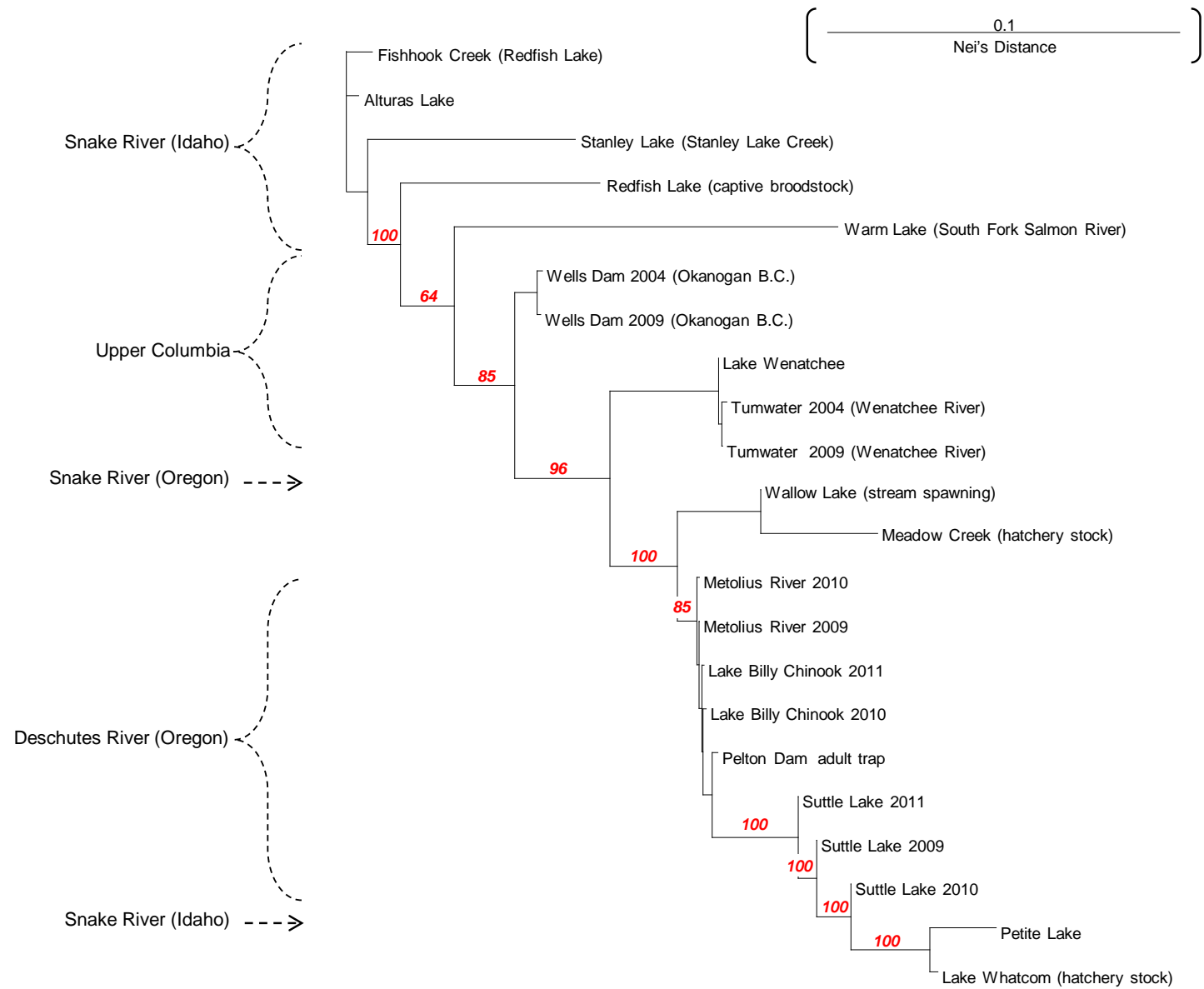


Figure 7. *O. nerka* NJ-phylogram based on Nei's distance (1972).



Appendix 1a. Description of 145 collections in the steelhead trout baseline. Origin is hatchery (Hat.) or natural origin (N). Run type is winter (W) or summer (S). Life stage is adult (A), juvenile unknown age (J), and smolts (S).

stream name (ref.#)	BPA subbasin	DPS	Lat.	Long.	(n)	origin	run	date	Life stage
<u>COASTAL LINEAGE</u>									
1	Quinault R.	na	47.3582	-123.9940	89	Hat.	WIN	2008	A
2	Clackamas R.	Willamette	45.2417	-122.2817	92	N	WIN	2000; 05	J/A
3	Eagle Cr.	Willamette	45.3514	-122.3840	47	N	WIN	2006	A
4	N. F. Eagle Cr.	Willamette	45.3254	-122.2885	43	N	WIN	2006	J/S
5	Skamania Stock	Willamette	45.2417	-122.2817	59	Hat.	S	2006	A
6	Little Rock/Mad Cr.	Willamette	44.7508	-122.3967	50	N	<u>U</u>	1996	J
7	N. F. Santiam/ Mad Cr.	Willamette	44.7970	-122.7730	39	N	WIN	2005	A
8	S. F. Santiam/ Wiley Cr.	Willamette	44.4136	-122.6772	93	N	WIN	1997; 05	J/A
9	Canyon Cr.	Willamette	44.9077	-123.4194	25	N	<u>U</u>	1997	J
10	Luckiamute Cr.	Willamette	44.7474	-123.1477	26	N	<u>U</u>	1997	J
11	Willamina Cr.	Willamette	45.0784	-123.4777	30	N	<u>U</u>	1997	J
12	Abernathy Cr.	Elochoman	46.2256	-123.1481	164	N	WIN	2007-08	A
13	Coweeman R.	Lower Columbia	46.1408	-122.8536	45	N	WIN	2006	A
14	Cowlitz R.	Cowlitz	46.5026	-122.5881	94	N	WIN	2005	A
15	Germany Cr.	Elochoman	46.1910	-123.1240	47	N	WIN	2005	S
16	Kalama R.	Kalama	46.0449	-122.8039	94	N	S	2005	A
17	Kalama R.	Kalama	46.0449	-122.8039	94	N	WIN	2005	A
18	E. F. Lewis R.	Lewis	45.8655	-122.7184	77	N	WIN	2005	A
19	N. F. Lewis R.	Lewis	45.9516	-122.5654	94	N	WIN	2006	A
20	Mill Cr.	Elochoman	46.1901	-123.1758	43	N	WIN	2005	S
21	Still Cr.	Sandy	45.3309	-121.9158	28	N	<u>U</u>	1998	J
22	East Fork Hood R.	Hood	45.5745	-121.6271	52	N	WIN	2007	S
23	West Frok Hood R.	Hood	45.6047	-121.6335	35	N	S	2007	S

24	Big White Salmon R.	Big White Salmon	LC	45.7993	-121.4846	78	N	<u>U</u>	1990; 05	J/A
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INLAND LINEAGE

25	Bowman Cr.	Klickitat	MC	45.8452	-121.0421	48	N	S	2005	J
26	Deadcanyon Cr.	Klickitat	MC	45.9420	-121.1439	34	N	S	2005	J
27	Lower Summit Cr.	Klickitat	MC	45.9876	-121.1255	45	N	S	2005	J
28	Lower Trout Cr.	Klickitat	MC	46.0378	-121.1994	48	N	S	2005	J
29	Lower White Cr.	Klickitat	MC	46.0133	-121.1500	33	N	S	2005	J
30	Snyder Cr.	Klickitat	MC	45.8281	-121.1724	47	N	S	2005	J
31	Surveyor Cr.	Klickitat	MC	46.1957	-121.2557	39	N	S	2005	J
32	Swale Cr.	Klickitat	MC	45.8091	-121.0652	48	N	S	2005	J
33	Upper Trout Cr.	Klickitat	MC	46.0774	-121.2122	46	N	S	2005	J
34	Lower Little Klickitat R.	Klickitat	MC	45.8434	-121.0605	46	N	S	2005	J
35	Buckhollow Cr.	Deschutes	MC	45.2507	-121.0230	63	N	S	2005-06	J
36	Mainstem Deschutes R.	Deschutes	MC	44.7260	-121.2478	61	N	S	2005-06	J
37	Pelton Dam Trap	Deschutes	MC	44.6942	-121.2312	45	N	S	1998	A
38	Shitike Cr.	Deschutes	MC	44.7615	-121.2288	31	N	S	2000	A
39	Trout Cr.	Deschutes	MC	44.8217	-121.0858	57	N	S	2007	J
40	Fifteenmile Cr.	Fifteenmile	MC	45.6251	-121.0656	91	N	S	2005	J
41	Baldy Cr.	John Day	MC	44.3629	-119.7700	25	N	S	2006	J
42	Beech Cr.	John Day	MC	44.4733	-119.0332	21	N	S	1996	J
43	Lower Mainstem J. D.	John Day	MC	44.7358	-120.3071	44	N	S	2006	J
44	Upper Mainstem J. D.	John Day	MC	44.3945	-118.5764	34	N	S	2000	J
45	Upper M. F. J. D.	John Day	MC	44.6195	-118.5679	107	N	S	1996; 05; 06	J
46	Big Wall Cr.	John Day	MC	44.8833	-119.4121	22	N	S	2006	J
47	Granite Cr.	John Day	MC	44.8383	-118.4770	18	N	S	2000	J
48	Middle N. F. J. D.	John Day	MC	45.0213	-118.9905	56	N	S	2005	J
49	Deer Cr.	John Day	MC	44.1956	-119.4716	18	N	S	2005	J
50	Murderers Cr.	John Day	MC	44.2646	-119.2857	18	N	S	2005	J
51	Rock Cr.	Columbia Lower Mid	MC	45.8066	-120.5104	126	N	S	2009	J

52	Squaw Cr.	Columbia Lower Mid	MC	45.8191	-120.4890	138	N	S	2009	J
53	Iskuulpa Cr.	Umatilla	MC	45.6997	-118.3971	148	N	S	2010	A/J
54	Umatilla R.	Umatilla	MC	45.7240	-118.1879	34	N	S	2005	J
55	Touchet R.	Walla Walla	MC	46.0470	-118.6770	86	N	S	1995	U
56	N. F. Little Naches R.	Yakima	MC	47.0897	-121.2881	21	N	S	2008	J
57	Nile/ Naches R.	Yakima	MC	46.8338	-120.9451	59	N	S	2005	J
58	Pileup/ Naches R.	Yakima	MC	47.0448	-121.1829	26	N	S	2005	J
59	Quartz/ Naches R.	Yakima	MC	47.0178	-121.1338	26	N	S	2005	J
60	Rattlesnake/ Naches R.	Yakima	MC	46.8201	-120.9307	36	N	S	2005	J
61	Satus Cr.	Yakima	MC	46.2621	-120.1124	46	N	S	2009	J
62	Toppenish Cr.	Yakima	MC	46.3240	-120.1697	44	N	S	2009	J
63	Chiwaukum R.	Wenatchee	UC	47.6881	-120.7407	54	N	S	2007	J
64	Icicle Cr./ Wells Stock	Wenatchee	UC	47.5591	-120.6742	23	Hat.	S	2007	J
65	Leavenworth-NFH	Wenatchee	UC	47.5591	-120.6742	19	Hat.	S	2007	A
66	Nason Cr.	Wenatchee	UC	47.8018	-120.7146	21	N	S	2006	J
67	Peshastin R.	Wenatchee	UC	47.4923	-120.6378	99	N	S	2005	J
68	Upper Chiwaukum R.	Wenatchee	UC	47.7107	-120.8051	29	N	S	2007	J
69	Entiat R.	Entiat	UC	47.6964	-120.3227	94	U	S	2006	J
70	Methow R.	Methow	UC	48.4756	-120.1819	90	N	S	2007	S
71	Bonaparte Cr.	Okanogan	UC	48.6998	-119.4399	99	U	S	2010	J
72	Omak Cr.	Okanogan	UC	48.3957	-119.5043	94	N/HAT	S	2005	A
73	Salmon Cr.	Okanogan	UC	48.3747	-119.5911	98	N/HAT	S	2010	A
74	Tucannon R.	Tucannon	Snake	46.3097	-117.6572	105	N	S	2005; 2010	A
75	Alpowa Cr.	Asotin	Snake	46.4076	-117.2198	98	N	S	2010	A
76	Asotin Cr.	Asotin	Snake	46.3228	-117.1368	98	N	S	2008; 2010	A
77	Asotin Cr.	Asotin	Snake	46.3442	-117.0551	49	N	S	2000	J
78	Captain John Cr.	Snake Hells Canyon	Snake	46.1515	-116.9340	56	N	S	2000	J
79	George Cr.	Asotin	Snake	46.3029	-117.1168	95	N	S	2010	A
80	Mission Cr.	Clearwater	Snake	46.3672	-116.7360	49	N	S	2000	J
81	Big Bear Cr.	Clearwater	Snake	46.6306	-116.6562	98	N	S	2007-08; 2010-11	A
82	E. F. Potlatch R.	Clearwater	Snake	46.7984	-116.4194	156	N	S	2008; 2010-11	A

83	Little Bear Cr.	Clearwater	Snake	46.6372	-116.6780	151	N	S	2007-08; 2010-11	A
84	W. F. Potlatch R.	Clearwater	Snake	46.8054	-116.4182	85	N	S	2009-10	A
85	Bear/ Selway R.	Clearwater	Snake	46.0191	-114.8378	35	N	S	2000	J
86	Gedney/ Selway R.	Clearwater	Snake	46.0583	-115.3141	45	N	S	2000	J
87	Little Clearwater/ Selway R.	Clearwater	Snake	45.7441	-114.7895	59	N	S	2008	J
88	Mainstem Selway R.	Clearwater	Snake	45.6921	-114.7175	76	N	S	2008	J
89	N. F. Moose/ Selway R.	Clearwater	Snake	46.1634	-114.9006	92	N	S	2000, 2004	J
90	OHara/ Selway R.	Clearwater	Snake	46.0809	-115.5179	47	N	S	2000	J
91	Three Links/ Selway R.	Clearwater	Snake	46.0981	-115.0728	47	N	S	2000	J
92	Whitecap/ Selway R.	Clearwater	Snake	45.8689	-114.7205	76	N	S	2008	J
93	Canyon/ Lochsa R.	Clearwater	Snake	46.2161	-115.5559	46	N	S	2011	J
94	Colt/ Lochsa R.	Clearwater	Snake	46.4311	-114.5395	38	N	S	2000	J
95	Crooked Fork/Lochsa R.	Clearwater	Snake	46.5251	-114.6786	44	N	S	2000	J
96	Fish/ Lochsa R.	Clearwater	Snake	46.3336	-115.3471	99	N	S	2010	A
97	Lake/ Lochsa R.	Clearwater	Snake	46.4632	-114.9965	47	N	S	2000	J
98	Storm/ Lochsa R.	Clearwater	Snake	46.4607	-114.5467	38	N	S	2000	J
99	Clear Cr.	Clearwater	Snake	46.0486	-115.7814	45	N	S	2000	J
100	Crooked R.	Clearwater	Snake	45.8211	-115.5272	104	N	S	2007-08	A
101	Johns Cr.	Clearwater	Snake	45.8224	-115.8887	36	N	S	2000	J
102	Tenmile Cr.	Clearwater	Snake	45.8057	-115.6833	46	N	S	2000	J
103	Slate Cr.	Salmon	Snake	45.6380	-116.2828	46	N	S	2000	J
104	Whitebird Cr.	Salmon	Snake	45.7523	-116.3198	59	N	S	2000-01	J
105	Crooked Cr.	Grande Ronde	Snake	45.9770	-117.5550	95	N	S	2001	J
106	Elk Cr.	Grande Ronde	Snake	45.7053	-117.1529	45	N	S	2000	J
107	Joseph Cr.	Grande Ronde	Snake	46.0278	-117.0177	45	N	S	2011	A
108	Little Minam R.	Grande Ronde	Snake	45.4004	-117.6722	48	N	S	2000	J
109	Lostine R.	Grande Ronde	Snake	45.5521	-117.4898	45	N	S	2000	J
110	Menatchee R.	Grande Ronde	Snake	46.0075	-117.3651	68	N	S	1999	J
111	Wenaha R.	Grande Ronde	Snake	45.9453	-117.4513	93	N	S	2001	J
112	Big Sheep Cr.	Imnaha	Snake	45.5574	-116.8345	61	N	S	2001	J
113	Camp Cr.	Imnaha	Snake	45.5572	-116.8352	23	N	S	2001	J

114	Cow Cr.	Imnaha	Snake	45.7681	-116.7496	44	N	S	2000	J
115	Lightning Cr.	Imnaha	Snake	45.6554	-116.7265	38	N	S	2000	J
116	Boulder Cr.	Salmon	Snake	45.2019	-116.3114	47	N	S	2000	J
117	Hazard Cr.	Salmon	Snake	45.1836	-116.2995	43	N	S	2000	J
118	Rapid R.	Salmon	Snake	45.3737	-116.3569	99	N	S	2003; 09	A
119	East Fork S. F. Salmon R.	Salmon	Snake	45.0127	-115.7129	45	N	S	2000	J
120	Lick Cr.	Salmon	Snake	45.0692	-115.8140	39	N	S	2010	J
121	Secesh R.	Salmon	Snake	45.0268	-115.7082	45	N	S	2000	J
122	Stolle Meadows	Salmon	Snake	44.6070	-115.6810	45	N	S	2000	J
123	Chamberlain Cr.	Salmon	Snake	45.4523	-114.9310	46	N	S	2000	J
124	Bargamin Cr.	Salmon	Snake	45.5716	-115.1919	46	N	S	2000	J
125	Camas Cr.	Salmon	Snake	44.8918	-114.7222	56	N	S	2000	J
126	Loon Cr.	Salmon	Snake	44.5976	-114.8123	84	N	S	1999-00	J
127	Lower Big Cr.	Salmon	Snake	45.0941	-114.7343	46	N	S	2000	J
128	Marsh Cr.	Salmon	Snake	44.4493	-115.2301	59	N	S	2000	J
129	Pistol Cr.	Salmon	Snake	44.7217	-115.1488	23	N	S	2000	J
130	Rapid R.	Salmon	Snake	44.6790	-115.1490	31	N	S	2000	J
131	Sulphur Cr.	Salmon	Snake	44.5526	-115.2974	42	N	S	2000	J
132	Upper Big Cr.	Salmon	Snake	45.1523	-115.2975	45	N	S	2000	J
133	Hayden Cr.	Salmon	Snake	44.8616	-113.6319	84	N	S	2009-10	J
134	Morgan Cr.	Salmon	Snake	44.6135	-114.1641	37	N	S	2000	J
135	N. F. Salmon R.	Salmon	Snake	45.4094	-113.9918	99	N	S	2010	A
136	Pahsimeroi Weir	Salmon	Snake	44.6844	-114.0403	96	N	S	2006; 10	A
137	Sawtooth Weir	Salmon	Snake	44.1506	-114.8851	105	N	S	2005; 10	A
138	Valley Cr.	Salmon	Snake	44.2231	-114.9272	44	N	S	2005	J
139	W. F. Yankee Fork R.	Salmon	Snake	44.3514	-114.7297	117	N	S	2004; 08	J
140	Dworshak Hatchery	Clearwater	Snake	na	na	114	Hat.	S	2008-09	A
141	Oxbow Hatchery	Snake Lower Middle	Snake	na	na	90	Hat.	S	2008-09	A
142	Pahsimeroi Hatchery	Salmon	Snake	na	na	146	Hat.	S	2008-09	A
143	Sawtooth Hatchery	Salmon	Snake	na	na	93	Hat.	S	2008-09	A
144	Tucannon/Lyons Ferry Hatchery	Tucannon	Snake	na	na	89	Hat.	S	2009	A

145	Wallowa Hatchery	Grande Ronde	Snake	na	na	93	Hat.	S	2009	A
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Appendix 1b. Description of 79 collections in the Chinook salmon baseline. Lineage is LC – Lower Columbia, OT – ocean type, and ST – stream type. Run type is fall (F), late fall bright (LFB), early fall bright (EFB), spring (SP) or summer (SU). Origin is hatchery (Hat.) or natural origin (N). Life stage is adult (A), carcass (C), juvenile unknown age (J), and smolts (S). Unknown status is indicated by “U”.

	Collection	BPA Subbasin	Region	Lineage	(n)	Lat	Long	Run	Origin	Age
1	Cowlitz R.	Cowlitz	Low. Columbia	LC	89	46.5130	-122.6350	SP	Hat.	A
2	Cowlitz R.	Cowlitz	Low. Columbia	LC	94	46.5100	-122.6150	F	Hat.	A
3	Elochoman R.	Elochoman	Low. Columbia	LC	81	46.2610	-123.2980	F	N	A
4	Kalama R.	Kalama	Low. Columbia	LC	85	46.0170	-122.7330	SP	Hat.	A
5	Lewis R.	Lewis	Low. Columbia	LC	93	45.9530	-122.5840	F	N	A
6	North Fork Lewis R.	Lewis	Low. Columbia	LC	81	45.8670	-122.7240	*(LFB)	N	A
7	North Fork Lewis R.	Lewis	Low. Columbia	LC	94	45.8670	-122.7240	*(EFB)	N	A
8	Sandy R.	Sandy	Low. Columbia	LC	46	45.5630	-122.3950	SP	N	A
9	Sandy R.	Sandy	Low. Columbia	LC	82	45.5630	-122.3950	F	N	A
10	McKenzie R.	Willamette	Low. Columbia	LC	75	44.1170	-123.0860	SP	Hat.	A
11	North Santiam R.	Willamette	Low. Columbia	LC	75	44.6970	-122.9830	SP	Hat.	A

12	White Salmon R.	Big White Salmon	Mid. Columbia	LC	77	45.7440	-121.5250	SP/F	N	J
13	White Salmon R.	Big White Salmon	Mid. Columbia	OT	90	45.7440	-121.5250	F	N	J
14	Lower Deschutes R.	Deschutes	Mid. Columbia	OT	90	45.2800	-121.0200	F	N	A
15	Hanford Reach	Columbia Lower Mid.	Mid. Columbia	OT	90	46.7130	-119.4810	F	N	A
16	Lower Crab Creek	Crab	Mid. Columbia	OT	94	46.8281	-119.8740	F	N	U
17	Upper Deschutes R.	Deschutes	Mid. Columbia	OT	90	44.8780	-121.0480	SU	N	J
18	Little White Salmon R.	Little White Salmon	Mid. Columbia	OT	99	45.7220	-121.6410	F	Hat.	J
19	Lower Yakima R.	Yakima	Mid. Columbia	OT	60	46.3120	-119.4730	F	N	A
20	Entiat R.	Entiat	Up. Columbia	OT	51	47.6960	-120.3210	SU	N	A
21	Methow R.	Methow	Up. Columbia	OT	94	48.2960	-120.0840	SU	N	J
22	Tumwater & Dryden	Wenatchee	Up. Columbia	OT	93	47.5420	-120.5590	SU	N	A
23	Nez Perce Tribal Hat.	Clearwater	Low. Clearwater	OT	85	46.5190	-116.6650	F	Hat.	A
24	Clearwater R.	Clearwater	Low. Clearwater	OT	152	46.5200	-116.6100	F	Hat.	A
25	Lyons Ferry Hatchery	Tucannon	Low. Snake	OT	90	46.5890	-118.2200	F	Hat.	A
26	American R.	Yakima	Mid. Columbia	ST	66	46.9760	-121.1580	SP	N	A
27	Cle Elum R.	Yakima	Mid. Columbia	ST	86	47.1780	-120.9990	SP	Hat.	U
28	Shitike Cr.	Deschutes	Mid. Columbia	ST	93	44.7640	-121.2380	SP	N	J
29	Warm Springs R.	Deschutes	Mid. Columbia	ST	88	44.8610	-121.2440	SP	Hat.	A
30	John Day R.	John Day	Mid. Columbia	ST	76	44.7600	-119.6500	SP	N	J/A
31	Middle Fork John Day r.	John Day	Mid. Columbia	ST	47	44.6593	-118.6720	SP	N	A
32	North Fork John Day r.	John Day	Mid. Columbia	ST	42	44.9319	-118.4470	SP	N	A
33	Klickitat R.	Klickitat	Mid. Columbia	ST	84	45.7100	-121.2700	SP	Hat.	A
34	Little White Salmon R.	Little White Salmon	Mid. Columbia	ST	93	45.7220	-121.6410	SP	Hat.	J
35	Winthrop NFH	Methow	Up. Columbia	ST	94	48.4760	-120.1870	SP	Hat.	A
36	Entiat R.	Entiat	Up. Columbia	ST	92	47.6960	-120.3210	SP	N	J
37	Leavenworth-NFH	Wenatchee	Up. Columbia	ST	88	47.5590	-120.6720	SP	Hat.	A
38	Peshastin Cr.	Wenatchee	Up. Columbia	ST	86	47.5580	-120.5750	SP	N	J
39	Tucannon R.	Tucannon	Low. Snake	ST	82	46.5260	-118.1420	SP	N	A
40	Dworshak Hatchery	Clearwater	Low. Clearwater	ST	92	47.1800	-121.0020	SP	U	U

41	Lochsa R. (Powell)	Clearwater	M. F. Clearwater	ST	94	46.5080	-114.6810	SP	Hat.	A
42	Crooked F Lochsa R	Clearwater	M. F. Clearwater	ST	29	46.5060	-114.6810	SP	N	C
43	Powell Weir	Clearwater	M. F. Clearwater	ST	32	46.5060	-114.6870	SP	N	A
44	Newsome Cr.	Clearwater	S. F. Clearwater	ST	82	45.8310	-115.6080	SP	N	A
45	Red R.	Clearwater	S. F. Clearwater	ST	73	45.7100	-115.3440	SP	N	A, C
46	Crooked R. Weir	Clearwater	S. F. Clearwater	ST	67	45.8170	-115.5270	SP	N	A
47	Lolo Cr	Clearwater	S. F. Clearwater	ST	89	46.2790	-115.7750	SP	N	J
48	Wenaha R.	Grande Ronde	Grande Ronde	ST	48	45.9460	-117.4550	SP	N	J
49	Upper Grande Ronde R.	Grande Ronde	Grande Ronde	ST	46	45.1930	-118.3950	SP	N	J
50	Catherine Cr	Grande Ronde	Grande Ronde	ST	94	45.1580	-117.7790	SP	N	A
51	Lostine R	Grande Ronde	Grande Ronde	ST	177	45.5420	-117.5550	SP	N	J
52	Minam R	Grande Ronde	Grande Ronde	ST	81	45.6000	-117.7290	SP	N	J
53	Imnaha R	Imnaha	Imnaha	ST	46	45.5610	-116.8340	SP	N	J
54	Imnaha R.	Imnaha	Imnaha	ST	91	45.5610	-116.8340	SP	N	A
55	Rapid R.	Salmon	Little Salmon	ST	91	45.3720	-116.3560	SP	N	A
56	Big Cr.	Salmon	M. F. Salmon	ST	89	45.1380	-115.0380	SP	N	A
57	Capehorn Cr.	Salmon	M. F. Salmon	ST	113	44.3880	-115.1740	SP	N	C, J
58	Marsh Cr.	Salmon	M. F. Salmon	ST	67	44.3810	-115.1530	SP	N	C
59	Elk Cr.	Salmon	M. F. Salmon	ST	91	44.4420	-115.4540	SP	N	C, J
60	Bear Valley Cr.	Salmon	M. F. Salmon	ST	85	44.4270	-115.3280	SP	N	C
61	Sulphur Cr.	Salmon	M. F. Salmon	ST	37	44.5340	-115.3580	SP	N	C, J
62	Camas Cr.	Salmon	M. F. Salmon	ST	61	44.8920	-114.7210	SP	N	J
63	Johnson Cr.	Salmon	S. F. Salmon	ST	92	44.8990	-115.4920	SP	N	A
64	Lake Cr, Summit Cr.	Salmon	S. F. Salmon	ST	78	45.2790	-115.9220	SP	N	C
65	Secesh R.	Salmon	S. F. Salmon	ST	134	45.2170	-115.8080	SP	N	C, J
66	SF Salmon R.	Salmon	S. F. Salmon	ST	143	44.6670	-115.7030	SP	N	A, C
67	Chamberlain Cr.	Salmon	Salmon	ST	45	45.4540	-114.9330	SP	N	J
68	Rapid R.	Salmon	Salmon	ST	93	45.3530	-116.3940	SP	Hat.	A
69	Chamberlain Cr.	Salmon	Salmon	ST	70	45.4540	-114.9330	SP	N	C, J
70	East Fork Salmon R.	Salmon	Up. Salmon	ST	94	44.2590	-114.3170	SP	N	A
71	W. F. Yankee Fork R.	Salmon	Up. Salmon	ST	75	44.3490	-114.7270	SP	N	J

72	Pahsimeroi R.	Salmon	Up. Salmon	ST	92	44.4410	-113.7870	SP	U	U
73	Sawtooth Hatchery	Salmon	Up. Salmon	ST	94	44.1520	-114.8810	SP	Hat.	A
74	Sawtooth Weir	Salmon	Up. Salmon	ST	92	44.1510	-114.8850	SP	N	A
75	Valley Cr.	Salmon	Up. Salmon	ST	59	44.2230	-114.9270	SP	N	C
76	Pahsimeroi R.	Salmon	Up. Salmon	ST	97	44.6820	-114.0390	SP	N	A, C
77	Hayden Cr.	Salmon	Up. Salmon	ST	80	44.8620	-113.6320	SP	N	C, J
78	Lemhi R. (upper)	Salmon	Up. Salmon	ST	96	44.8690	-113.6250	SP	N	C, J
79	Lemhi R. (lower)	Salmon	Up. Salmon	ST	90	45.1530	-113.8140	SP	N	J

Appendix 1c. Sockeye salmon and Kokanee collections included in the *O. nerka* SNP baseline. Collections are either kokanee (K), sockeye salmon (sock), or unknown origin (U). Origin is known hatchery stock (Hat), natural origin (N) or unknown (U).

	Collection	BPA Subbasin	Lake/River	Year	(n)	Lat	Long	life history	Origin
01	Alturas Lake	Salmon	Alturas Lake	2008	59	43.9091	-114.8645	K	U
02	Fishhook Creek	Salmon	Redfish Lake	2007	96	44.1283	-114.9708	K	U
03	Pettit Lake Creek	Salmon	Pettit Lake	2003	70	43.9781	-114.8824	K	U
04	Captive Broodstock	Salmon	Redfish Lake	2009	86	44.1310	-114.9267	Sock	Hat.
05	Stanley Lake Creek	Salmon	Stanley Lake	2011	53	44.2089	-115.0837	K	U
06	Warm Lake	Salmon	South Fork Salmon River	2003	68	44.6482	-115.6706	K	N
07	*Wallowa Lake	Grande Ronde	Wallowa River	2011	43	45.2812	-117.2092	**K	N
08	Unknown	Wenatchee	Wenatchee	2011	92	Unknown	Unknown	Sock	N
09	Lake Creek outlet	Deschutes	Suttle Lake	2010	92	44.4268	-121.7265	**K	U
10	Lake Creek outlet	Deschutes	Suttle Lake	2011	100	44.4268	-121.7265	**K	U
11	Lake Creek outlet	Deschutes	Suttle Lake	2009	99	44.4268	-121.7265	**K	U
12	Pelton Fish Trap	Deschutes	Deschutes R.	2009	23	44.6027	-121.2801	Sock	U
13	SWW Facility	Deschutes	Lake Billy Chinook	2011	98	44.6027	-121.2801	K	U
14	SWW Facility	Deschutes	Lake Billy Chinook	2011	100	44.6027	-121.2801	K	U
15	Metolius River Inlet	Deschutes	Lake Billy Chinook	2010	98	44.6027	-121.2801	K	U

16	Metolius River Inlet	Deschutes	Lake Billy Chinook	2010	94	44.6027	-121.2801	K	U
17	Meadow Creek	na: B.C. Okanogan	Meadow Creek	2005	46	50.2167	-116.9833	K	Hat.
18	Tumwater Dam	Wenatchee	Wenatchee	2004	97	47.6165	-120.7229	sock	N
19	Tumwater Dam	Wenatchee	Wenatchee	2009	155	47.6165	-120.7229	sock	N
20	‡Wells Dam	Upper Middle Columbia	Upper Middle Columbia	2004	91	47.9454	-119.8660	sock	N
21	‡Wells Dam	Upper Middle Columbia	Upper Middle Columbia	2009	121	47.9454	-119.8660	sock	N
22	Lake Whatcom	na: Puget Sound	Lake Whatcom	2005	46	48.6732	-122.2775	K	Hat.

* *sample represents the stream spawning type: this location supports both lake spawning and stream spawning types.*

‡ *fish captured at this site are presumed to be individuals from the Osoyoos Lake population in the trans-border Okanogan region (U.S. & Canada).*

** *collections are presumed kokanee; however, anadromous sockeye were indigenous to these locations in Oregon, but have been extirpated.*

Appendix 2a. Steelhead trout SNP descriptive statistics by locus. Numbers (HWE) are Hardy-Weinberg deviations (HWE) per locus. Outliers occurred in the coastal lineage (CO) or inland lineage (IN), and some loci have selection precedence in landscape and association tests for adaptive divergence. Linked loci are matched by letter, and the locus with lowest F_{ST} and minor allele frequency (MAF) is identified (*).

SNP locus	HWE	outlier	linked	Overall F_{ST}	<u>Coastal MAF</u>		<u>Inland MAF</u>	
					mean	range	mean	range
M09AAC.055	0	---	---	0.057	0.033	0.096	0.085	0.322
M09AAD.076	1	---	---	0.119	0.122	0.404	0.474	0.522
M09AAE.082	2	---	---	0.091	0.394	0.433	0.252	0.656
M09AAJ.163	1	---	---	0.059	0.435	0.262	0.303	0.547
OMGH1PROM1-SNP1	1	---	---	0.073	0.099	0.375	0.114	0.375
OMS00002	1	---	---	0.046	0.341	0.520	0.325	0.486
OMS00003	1	---	---	0.062	0.316	0.378	0.157	0.402
OMS00006	0	---	---	0.043	0.438	0.397	0.490	0.645
OMS00008	0	---	---	0.086	0.032	0.128	0.179	0.456
OMS00013	0	---	---	0.244	0.479	0.388	0.099	0.406
OMS00014	0	---	---	0.048	0.035	0.234	0.036	0.186
OMS00015	0	---	---	0.069	0.145	0.316	0.070	0.405
OMS00017	2	---	---	0.066	0.315	0.452	0.296	0.667
OMS00018	0	---	---	0.068	0.185	0.503	0.121	0.345
OMS00024	5	---	---	0.065	0.265	0.478	0.359	0.686
OMS00030	1	---	---	0.035	0.094	0.269	0.098	0.198
OMS00039	2	---	---	0.050	0.369	0.438	0.475	0.691
OMS00048	1	---	---	0.120	0.432	0.324	0.136	0.364
OMS00052	2	---	---	0.035	0.170	0.287	0.192	0.353
OMS00053	1	---	---	0.078	0.171	0.338	0.440	0.596
OMS00056	0	---	---	0.041	0.291	0.415	0.217	0.409
OMS00057	2	---	---	0.051	0.388	0.384	0.357	0.606
OMS00058	2	---	---	0.063	0.323	0.283	0.419	0.598
OMS00061	0	---	---	0.109	0.289	0.305	0.081	0.235
OMS00062	1	---	---	0.032	0.280	0.240	0.256	0.426
OMS00064	1	CO	---	0.078	0.468	0.594	0.394	0.607
OMS00068	3	---	---	0.063	0.195	0.514	0.331	0.529
OMS00070	3	---	---	0.063	0.395	0.376	0.497	0.672
OMS00071	0	---	---	0.069	0.346	0.280	0.431	0.590
OMS00072	3	---	---	0.039	0.493	0.346	0.445	0.553
OMS00074	1	---	---	0.082	0.396	0.453	0.490	0.755
OMS00077	1	---	---	0.086	0.333	0.429	0.406	0.748
OMS00078	0	---	---	0.041	0.187	0.331	0.282	0.499
OMS00079	0	---	---	0.050	0.429	0.310	0.483	0.710
OMS00087	12	---	*HWE	dropped	---	---	---	---
OMS00089	1	---	---	0.059	0.265	0.423	0.285	0.571
OMS00090	0	---	---	0.079	0.334	0.462	0.460	0.607
OMS00092	1	---	---	0.077	0.230	0.397	0.156	0.493
OMS00095	2	---	---	0.051	0.002	0.029	0.064	0.251
OMS00096	1	CO	---	0.219	0.268	0.614	0.218	0.461

OMS00101	2	---	---	0.055	0.450	0.392	0.417	0.639
OMS00105	1	---	---	0.090	0.323	0.327	0.397	0.580
OMS00106	0	---	---	0.069	0.359	0.564	0.250	0.546
OMS00111	0	---	---	0.061	0.118	0.213	0.188	0.508
OMS00112	1	---	---	0.054	0.109	0.343	0.183	0.444
OMS00114	2	---	---	0.042	0.016	0.110	0.082	0.212
OMS00118	2	CO	---	0.162	0.197	0.521	0.398	0.552
OMS00119	0	---	---	0.062	0.230	0.446	0.144	0.398
OMS00120	1	---	---	0.095	0.412	0.365	0.192	0.475
OMS00121	3	---	---	0.048	0.400	0.377	0.472	0.604
OMS00129	6	---	---	0.068	0.067	0.385	0.188	0.421
OMS00132	0	---	---	0.041	0.419	0.499	0.413	0.608
OMS00133	1	---	(A)	0.173	0.301	0.430	0.056	0.198
OMS00138	1	---	---	0.056	0.059	0.214	0.117	0.380
OMS00143	0	---	---	0.057	0.031	0.103	0.098	0.299
OMS00149	0	---	---	0.067	0.122	0.376	0.064	0.319
OMS00151	0	---	---	0.045	0.102	0.270	0.183	0.391
OMS00154	1	---	---	0.120	0.442	0.282	0.224	0.426
OMS00169	0	---	*(E)	---	---	---	---	---
OMS00173	1	---	---	0.050	0.045	0.138	0.128	0.302
OMS00174	0	CO	---	0.043	0.049	0.237	0.048	0.167
OMS00175	1	---	---	0.075	0.388	0.307	0.418	0.560
OMS00176	1	---	*(B)	---	---	---	---	---
OMS00179	1	---	---	0.066	0.497	0.529	0.307	0.477
OMS00180	0	---	---	0.141	0.244	0.264	0.345	0.559
Omy_101832-195	0	---	---	0.065	0.434	0.633	0.445	0.772
Omy_101993-189	2	---	---	0.072	0.163	0.287	0.256	0.567
Omy_102505-102	0	---	---	0.048	0.320	0.530	0.372	0.567
Omy_103705-558	2	---	---	0.063	0.286	0.294	0.119	0.271
Omy_104519-624	2	---	---	0.058	0.299	0.489	0.311	0.525
Omy_105075-162	1	---	---	0.091	0.302	0.387	0.104	0.364
Omy_105105-448	3	---	---	0.057	0.314	0.374	0.435	0.555
Omy_105385-406	0	---	---	0.070	0.408	0.482	0.440	0.615
Omy_105714-265	0	---	---	0.062	0.314	0.609	0.320	0.559
Omy_107031-704	0	---	---	0.307	0.182	0.305	0.197	0.586
Omy_107285-69	0	---	---	0.045	0.147	0.236	0.173	0.414
Omy_107806-34	0	---	---	0.093	0.494	0.493	0.339	0.839
Omy_108007-193	1	---	---	0.171	0.136	0.370	0.418	0.643
Omy_109243-222	0	---	---	0.058	0.320	0.501	0.184	0.355
Omy_109894-185	4	---	---	0.054	0.265	0.470	0.332	0.524
Omy_110064-419	1	---	---	0.095	0.121	0.320	0.367	0.672
Omy_110201-359	0	---	---	0.164	0.457	0.580	0.126	0.372
Omy_111383-51	1	---	---	0.061	0.428	0.453	0.437	0.733
Omy_113490-159	1	---	---	0.104	0.370	0.573	0.386	0.691
Omy_114315-438	2	---	---	0.107	0.179	0.269	0.439	0.657
Omy_114587-480	1	---	---	0.080	0.132	0.244	0.314	0.508
Omy_116733-349	2	---	---	0.058	0.150	0.306	0.274	0.570
Omy_128923-433	0	---	---	0.114	0.183	0.340	0.495	0.686
Omy_128996-481	4	---	---	0.166	0.418	0.321	0.113	0.640
Omy_129870-756	1	---	---	0.036	0.135	0.424	0.168	0.328

Omy_130524-160	2	---	---	0.035	0.423	0.300	0.390	0.546
Omy_97077-73	0	---	---	0.053	0.087	0.298	0.037	0.145
Omy_97660-230	0	---	---	0.069	0.176	0.422	0.362	0.641
Omy_97865-196	1	---	---	0.037	0.022	0.090	0.054	0.171
Omy_97954-618	1	CO	---	0.080	0.069	0.483	0.181	0.509
Omy_99300-202	0	---	---	0.042	0.173	0.257	0.207	0.444
Omy_ada10-71	2	---	---	0.062	0.340	0.599	0.209	0.427
Omy_aldB-165	0	2	---	0.077	0.417	0.311	0.321	0.542
Omy_anp-17	1	IN	---	0.093	0.267	0.341	0.354	0.735
Omy_aromat-280	1	---	---	0.044	0.265	0.324	0.193	0.549
Omy_arp-630	0	---	---	0.057	0.498	0.495	0.435	0.694
Omy_aspAT-123	0	---	---	0.070	0.102	0.252	0.273	0.681
Omy_b1-266	1	---	---	0.077	0.466	0.595	0.286	0.363
Omy_b9-164	4	IN	---	0.110	0.036	0.128	0.106	0.449
Omy_BAC-B4-324	0	---	---	0.166	0.064	0.192	0.460	0.509
Omy_BAC-F5.284	0	---	---	0.053	0.103	0.324	0.067	0.245
Omy_BAMBI2.312	3	---	---	0.072	0.102	0.280	0.119	0.632
Omy_bcAKala-380rd	2	CO	---	0.149	0.267	0.561	0.341	0.618
Omy_ca050-64	1	---	---	0.056	0.153	0.212	0.344	0.428
Omy_carban1-264	0	---	---	0.075	0.004	0.058	0.108	0.349
Omy_cd28-130	0	---	---	0.252	0.363	0.445	0.045	0.308
Omy_cd59-206	0	CO	---	0.064	0.340	0.600	0.282	0.553
Omy_cd59b-112	1	---	---	0.050	0.147	0.224	0.131	0.387
Omy_cin-172	0	---	---	0.044	0.233	0.301	0.212	0.421
Omy_colla1-525	0	---	---	0.040	0.203	0.377	0.305	0.412
Omy_cox1-221	0	---	---	0.064	0.457	0.553	0.367	0.579
Omy_cox2-335	0	---	---	0.048	0.090	0.254	0.164	0.354
Omy_crb-106	6	---	*(C)	---	---	---	---	---
Omy_CRBF1-1	2	---	(C)	0.091	0.241	0.410	0.083	0.297
Omy_e1-147	1	---	---	0.087	0.204	0.470	0.076	0.264
Omy_g1-103	0	---	---	0.058	0.024	0.222	0.082	0.245
Omy_g12-82	0	---	---	0.053	0.443	0.427	0.468	0.645
Omy_G3PD_2-371	1	---	---	0.039	0.163	0.281	0.178	0.483
Omy_gadd45-332	0	---	---	0.100	0.002	0.023	0.107	0.467
Omy_gdh-271	0	2	---	0.055	0.173	0.313	0.104	0.390
Omy_gh-475	2	---	---	0.042	0.087	0.273	0.123	0.273
Omy_GHSR-121	0	---	(B)	0.075	0.142	0.462	0.081	0.317
Omy_gluR-79	1	---	---	0.044	0.488	0.347	0.482	0.599
Omy_hsc715-80	1	---	---	0.059	0.386	0.309	0.413	0.517
Omy_hsf1b-241	2	---	---	0.067	0.195	0.410	0.099	0.487
Omy_hsf2-146	1	4	---	0.127	0.028	0.096	0.310	0.610
Omy_hsp47-86	0	1	---	0.042	0.366	0.342	0.234	0.364
Omy_hsp70aPro-329	0	---	---	0.077	0.098	0.250	0.064	0.443
Omy_hus1-52	4	---	---	0.158	0.370	0.411	0.098	0.290
Omy_IL17-185	4	---	---	0.065	0.369	0.495	0.462	0.645
Omy_IL1b-.028	0	---	(D)	0.077	0.378	0.458	0.184	0.389
Omy_IL1b-163	0	IN	---	0.455	0.197	0.539	0.143	0.601
Omy_IL1b-198	0	---	*(D)	---	---	---	---	---
Omy_IL6-320	1	---	---	0.042	0.201	0.323	0.229	0.465
Omy_imp1-55	0	---	---	0.064	0.001	0.011	0.093	0.305

Omy_inos-97	0	---	---	0.056	0.030	0.130	0.064	0.267
Omy_LDHB-1_i2	2	---	---	0.040	0.067	0.400	0.092	0.193
Omy_LDHB-2_e5	0	---	---	0.064	0.332	0.400	0.169	0.478
Omy_LDHB-2_i6	1	3	---	0.068	0.092	0.214	0.032	0.223
Omy_lpl-220	0	---	---	0.034	0.198	0.359	0.163	0.370
Omy_mapK3-103	0	---	*(B)	---	---	---	---	---
Omy_mcsf-268	0	---	---	0.071	0.121	0.188	0.045	0.224
Omy_metA-161	0	---	---	0.076	0.487	0.399	0.281	0.535
Omy_metB-138	2	CO	---	0.058	0.098	0.468	0.170	0.462
Omy_myoD-178	1	---	---	0.055	0.045	0.101	0.122	0.353
Omy_nach-200	1	---	---	0.051	0.032	0.263	0.024	0.124
Omy_NaKATPa3-50	1	---	---	0.046	0.234	0.255	0.312	0.480
Omy_ndk-152	1	IN; 3	(F)	0.562	0.229	0.509	0.077	0.547
Omy_nips-299	3	---	---	0.039	0.051	0.233	0.064	0.213
Omy_nkef-241	0	---	---	0.073	0.328	0.311	0.455	0.614
Omy_ntl-27	0	---	---	0.083	0.253	0.458	0.393	0.731
Omy_nxt2-273	5	IN	---	0.098	0.127	0.285	0.096	0.581
Omy_Ogp4-212	5	2	---	0.066	0.319	0.641	0.442	0.639
Omy_OmyP9-180	6	1	---	0.092	0.311	0.312	0.105	0.281
Omy_Ots249-227	1	---	---	0.096	0.379	0.642	0.313	0.561
Omy_oxct-85	0	---	---	0.076	0.273	0.456	0.115	0.329
Omy_p53-262	0	---	---	0.067	0.067	0.308	0.190	0.495
Omy_pad-196	1	---	---	0.042	0.001	0.029	0.049	0.199
Omy_ppie-232	2	---	---	0.043	0.110	0.269	0.140	0.479
Omy_rapd-167	1	---	*(A)	---	---	---	---	---
Omy_rbm4b-203	0	---	---	0.095	0.425	0.505	0.196	0.489
Omy_redd1-410	0	---	---	0.036	0.222	0.247	0.210	0.525
Omy_sast-264	1	---	---	0.080	0.389	0.687	0.178	0.403
Omy_SECC22b-88	1	---	(E)	0.079	0.051	0.301	0.045	0.204
Omy_srp09-37	1	---	---	0.064	0.134	0.206	0.321	0.476
Omy_sSOD-1	0	---	---	0.221	0.318	0.481	0.044	0.330
Omy_star-206	0	IN	---	0.275	0.467	0.475	0.097	0.601
Omy_stat3-273	0	2	---	0.045	0.368	0.288	0.236	0.434
Omy_sys1-188	1	---	---	0.061	0.077	0.207	0.113	0.385
Omy_tlr3-377	0	---	---	0.045	0.120	0.290	0.102	0.289
Omy_tlr5-205	1	2	---	0.081	0.236	0.460	0.085	0.265
Omy_txnlp-343	1	---	---	0.097	0.491	0.609	0.238	0.523
Omy_u07-79-166	1	---	---	0.209	0.480	0.353	0.119	0.391
Omy_u09-52.284	2	---	*(F)	---	---	---	---	---
Omy_u09-53.469	2	IN	---	0.098	0.251	0.275	0.372	0.771
Omy_u09-54-311	2	---	---	0.063	0.363	0.309	0.274	0.561
Omy_u09-56.119	1	IN	---	0.222	0.475	0.431	0.149	0.654
Omy_U11_2b-154	1	---	---	0.055	0.321	0.555	0.247	0.512
Omy_UT16_2-173	0	IN	---	0.094	0.177	0.270	0.085	0.625
Omy_vamp5-303	5	---	---	0.139	0.428	0.572	0.255	0.606
Omy_vatf-406	0	IN	---	0.112	0.352	0.343	0.401	0.841
Omy_zg57-91	0	---	---	0.065	0.020	0.096	0.113	0.418
OMY1011SNP	0	---	---	0.066	0.465	0.332	0.293	0.626
Ocl_gshpx-357	---	---	hybrid	---	---	---	---	---
Omy_myclarp404-111	---	---	hybrid	---	---	---	---	---

Omy_Omyclmk438-96 --- --- hybrid --- --- --- ---

"precedence" references: 1. *Thermal stress association (Narum et al. in review)*; 2. *Temperature association (Narum et al. 2010b)*;
3. *Anadromy association (Narum et al. 2011)*; 4. *Precipitation association (Narum et al 2010b)*.

Appendix 2b. Chinook Salmon SNP descriptive statistics by locus. Numbers (HWE) are Hardy-Weinberg deviations (HWE) per locus. Outliers occurred in the stream-type lineage (ST), ocean-type lineage (OT), or lower Columbia (LC). Linked loci are matched by letter, and the locus with lowest F_{ST} and MAF is identified (*). Overall for MAF and F_{ST} are results calculated across lineage. “Allele 1” and “Allele 2” identify the mean allele frequencies by lineage.

Locus	HWE	outlier	linked	Overall		mean LC		mean OT		mean ST	
				F_{ST}	MAF	allele 1	allele 2	allele 1	allele 2	allele 1	allele 2
Ots_OTALDBINT1-SNP1	1	---	(A)	0.217	0.209	0.295	0.705	0.579	0.421	0.101	0.899
Ots_100884-287	0	---	---	0.048	0.195	0.196	0.804	0.109	0.891	0.216	0.784
Ots_110201-363	1	---	---	0.029	0.324	0.273	0.727	0.325	0.675	0.335	0.665
Ots_115987-325	0	---	---	0.248	0.419	0.423	0.577	0.116	0.884	0.727	0.273
Ots_CirpA	2	---	---	0.443	0.320	0.307	0.693	0.171	0.829	0.885	0.115
Ots_ntl-255	1	---	---	0.096	0.449	0.404	0.596	0.312	0.688	0.641	0.359
Ots_ppie-245	2	ST	---	0.482	0.439	0.098	0.902	0.070	0.930	0.782	0.218
Ots_101554-407	0	---	---	0.192	0.422	0.139	0.861	0.118	0.882	0.559	0.441
Ots_105132-200	0	---	---	0.071	0.163	0.026	0.974	0.062	0.938	0.218	0.782
Ots_110495-380	1	ST	---	0.331	0.293	0.315	0.685	0.390	0.610	0.870	0.130
Ots_117432-409	0	---	---	0.143	0.415	0.386	0.614	0.331	0.669	0.690	0.310
Ots_HMGB1-73	1	---	---	0.298	0.279	0.309	0.691	0.790	0.210	0.149	0.851
Ots_OTDESMIN19-SNP1	1	---	---	0.179	0.451	0.123	0.877	0.226	0.774	0.577	0.423
Ots_101704-143	0	---	---	0.197	0.247	0.396	0.604	0.575	0.425	0.135	0.865
Ots_105385-421	1	---	---	0.146	0.336	0.157	0.843	0.039	0.961	0.447	0.553
Ots_110551-64	1	---	---	0.029	0.209	0.220	0.780	0.224	0.776	0.203	0.797
Ots_118205-61	1	---	---	0.026	0.190	0.193	0.807	0.192	0.808	0.188	0.812
Ots_Thio	0	---	---	0.156	0.376	0.274	0.726	0.482	0.518	0.737	0.263
Ots_105407-117	1	---	---	0.077	0.386	0.233	0.767	0.502	0.498	0.391	0.609
Ots_110689-218	1	---	---	0.033	0.215	0.138	0.862	0.227	0.773	0.229	0.771
Ots_118938-325	0	---	---	0.082	0.320	0.457	0.543	0.424	0.576	0.264	0.736
Ots_Est740	0	---	---	0.050	0.485	0.410	0.590	0.403	0.597	0.565	0.435
Ots_OTSTF1-SNP1	1	---	(B)	0.188	0.475	0.449	0.551	0.125	0.875	0.638	0.362

Ots_101119-381	0	---	---	0.093	0.026	0.059	0.941	0.090	0.910	0.003	0.997
Ots_redd1-187	1	---	---	0.080	0.210	0.103	0.897	0.046	0.954	0.273	0.727
Ots_102213-210	0	---	---	0.087	0.030	0.031	0.969	0.114	0.886	0.010	0.990
Ots_u1002-75	1	---	---	0.099	0.296	0.439	0.561	0.798	0.202	0.740	0.260
Ots_102457-132	0	---	---	0.393	0.200	0.382	0.618	0.514	0.486	0.962	0.038
Ots_102801-308	1	---	---	0.028	0.192	0.186	0.814	0.113	0.887	0.212	0.788
Ots_102867-609	0	---	---	0.107	0.065	0.165	0.835	0.172	0.828	0.016	0.984
Ots_112301-43	0	---	---	0.094	0.189	0.437	0.563	0.156	0.844	0.142	0.858
Ots_104569-86	0	---	---	0.196	0.243	0.473	0.527	0.510	0.490	0.879	0.121
Ots_123921-111	0	---	---	0.035	0.157	0.118	0.882	0.212	0.788	0.153	0.847
Ots_106499-70	0	---	---	0.074	0.353	0.469	0.531	0.520	0.480	0.288	0.712
Ots_NFYB-147	1	---	---	0.084	0.129	0.003	0.997	0.013	0.987	0.186	0.814
Ots_107074-284	0	---	---	0.333	0.173	0.496	0.504	0.552	0.448	0.967	0.033
Ots_107285-93	0	---	---	0.113	0.062	0.237	0.763	0.067	0.933	0.022	0.978
Ots_107806-821	2	---	---	0.050	0.358	0.235	0.765	0.293	0.707	0.401	0.599
Ots_108820-336	0	---	---	0.204	0.426	0.217	0.783	0.066	0.934	0.558	0.442
Ots_108007-208	1	ST	---	0.327	0.204	0.434	0.566	0.512	0.488	0.944	0.056
Ots_112419-131	0	---	---	0.191	0.237	0.494	0.506	0.485	0.515	0.120	0.880
Ots_108390-329	0	---	---	0.200	0.076	0.300	0.700	0.170	0.830	0.004	0.996
Ots_124774-477	0	---	---	0.229	0.209	0.335	0.665	0.584	0.416	0.091	0.909
Ots_108735-302	3	---	---	0.156	0.205	0.358	0.642	0.453	0.547	0.111	0.889
Ots_109693-392	1	---	---	0.142	0.093	0.303	0.697	0.155	0.845	0.032	0.968
Ots_parp3-286	1	---	---	0.039	0.147	0.118	0.882	0.047	0.953	0.178	0.822
Ots_111681-657	0	---	---	0.111	0.148	0.373	0.627	0.194	0.806	0.087	0.913
Ots_txnlp-321	1	---	---	0.082	0.099	0.029	0.971	0.004	0.996	0.137	0.863
Ots_112208-722	1	---	---	0.353	0.218	0.359	0.641	0.508	0.492	0.942	0.058
Ots_103122-180	2	LC	---	0.393	0.328	0.411	0.589	0.156	0.844	0.854	0.146
Ots_117242-136	1	---	---	0.205	0.181	0.329	0.671	0.491	0.509	0.073	0.927
Ots_109525-816	1	---	---	0.050	0.166	0.037	0.963	0.171	0.829	0.194	0.806
Ots_117259-271	2	---	---	0.642	0.267	0.189	0.811	0.246	0.754	0.971	0.029
Ots_112820-284	0	---	---	0.068	0.197	0.259	0.741	0.349	0.651	0.147	0.853

Ots_118175-479	0	---	---	0.137	0.090	0.296	0.704	0.155	0.845	0.029	0.971
Ots_brp16-64	0	---	---	0.127	0.228	0.321	0.679	0.499	0.501	0.143	0.857
Ots_122414-56	0	---	---	0.237	0.108	0.342	0.658	0.274	0.726	0.016	0.984
Ots_127236-62	0	LC	---	0.442	0.197	0.464	0.536	0.412	0.588	0.973	0.027
Ots_pigh-105	1	---	---	0.093	0.368	0.154	0.846	0.220	0.780	0.450	0.550
Ots_128302-57	2	---	---	0.610	0.290	0.163	0.837	0.229	0.771	0.948	0.052
Ots_128693-461	0	---	---	0.161	0.134	0.310	0.690	0.321	0.679	0.050	0.950
Ots_104415-88	0	---	---	0.172	0.333	0.021	0.979	0.120	0.880	0.454	0.546
Ots_129144-472	0	---	---	0.216	0.090	0.153	0.847	0.349	0.651	0.013	0.987
Ots_112876-371	1	---	---	0.193	0.304	0.474	0.526	0.404	0.596	0.816	0.184
Ots_130720-99	3	---	---	0.411	0.232	0.357	0.643	0.404	0.596	0.948	0.052
Ots_129458-451	0	---	---	0.144	0.211	0.340	0.660	0.454	0.546	0.124	0.876
Ots_131460-584	1	LC	---	0.409	0.215	0.447	0.553	0.405	0.595	0.952	0.048
Ots_pop5-96	1	---	---	0.092	0.189	0.078	0.922	0.027	0.973	0.253	0.747
Ots_131906-141	0	---	---	0.129	0.109	0.331	0.669	0.150	0.850	0.050	0.950
Ots_99550-204	0	---	---	0.112	0.072	0.246	0.754	0.103	0.897	0.026	0.974
Ots_DDX5-171	1	LC; ST	---	0.211	0.218	0.421	0.579	0.470	0.530	0.113	0.887
Ots_Est1363	4	LC; ST	---	0.722	0.323	0.182	0.818	0.045	0.955	0.939	0.061
Ots_HFABP-34	0	---	---	0.196	0.109	0.282	0.718	0.284	0.716	0.028	0.972
Ots_hnRNPL-533	1	---	---	0.175	0.405	0.206	0.794	0.063	0.937	0.531	0.469
Ots_Hsp90a	1	LC	---	0.476	0.190	0.486	0.514	0.603	0.397	0.025	0.975
Ots_il13Ra2B-37	1	---	---	0.094	0.276	0.071	0.929	0.133	0.867	0.356	0.644
Ots_GCSH	3	---	---	0.570	0.343	0.246	0.754	0.051	0.949	0.894	0.106
Ots_il-1racp-166	0	---	---	0.126	0.486	0.346	0.654	0.188	0.812	0.590	0.410
Ots_nelfd-163	1	---	---	0.533	0.216	0.344	0.656	0.363	0.637	0.983	0.017
Ots_tpx2-125	0	---	---	0.058	0.077	0.007	0.993	0.027	0.973	0.104	0.896
Ots_OTSM-TA-SNP1	0	---	---	0.112	0.060	0.179	0.821	0.156	0.844	0.011	0.989
Ots_vatf-251	2	---	---	0.331	0.250	0.414	0.586	0.738	0.262	0.096	0.904
Ots_P450-288	2	---	---	0.208	0.460	0.272	0.728	0.084	0.916	0.593	0.407
Ots_stk6-516	0	---	---	0.094	0.013	0.011	0.989	0.063	0.937	0.001	0.999
Ots_TCTA-58	0	ST	---	0.223	0.151	0.441	0.559	0.308	0.692	0.049	0.951

Ots_u1007-124	0	---	---	0.133	0.062	0.139	0.861	0.199	0.801	0.011	0.989
Ots_U2362-227	1	---	---	0.145	0.090	0.302	0.698	0.140	0.860	0.032	0.968
Ots_U2362-330	0	---	---	0.247	0.373	0.056	0.944	0.048	0.952	0.522	0.478
Ots_U2446-123	2	---	---	0.065	0.323	0.162	0.838	0.290	0.710	0.366	0.634
Ots_unk1104-38	1	---	---	0.137	0.431	0.291	0.709	0.101	0.899	0.541	0.459
Ots_unk1832-39	1	LC	---	0.097	0.390	0.265	0.735	0.194	0.806	0.464	0.536
Ots_unk3513-49	2	---	---	0.043	0.241	0.190	0.810	0.275	0.725	0.245	0.755
Ots_unk7936-50	0	---	---	0.093	0.134	0.318	0.682	0.179	0.821	0.082	0.918
Ots_unk8200-45	0	---	---	0.052	0.007	0.030	0.970	0.003	0.997	0.003	0.997
Ots_unk9480-51	1	---	---	0.421	0.360	0.227	0.773	0.164	0.836	0.846	0.154
Ots_zn593-346	0	---	---	0.038	0.015	0.001	0.999	0.005	0.995	0.020	0.980
Ots_102414-395	1	---	---	0.062	0.493	0.465	0.535	0.668	0.332	0.457	0.543
Ots_105105-613	2	---	---	0.198	0.492	0.273	0.727	0.163	0.837	0.643	0.357
Ots_106747-239	1	---	---	0.064	0.403	0.463	0.537	0.274	0.726	0.421	0.579
Ots_110064-383	0	---	---	0.149	0.461	0.352	0.648	0.241	0.759	0.653	0.347
Ots_113242-216	0	---	---	0.189	0.235	0.418	0.582	0.540	0.460	0.120	0.880
Ots_113457-40R	0	---	---	0.376	0.277	0.249	0.751	0.427	0.573	0.900	0.100
Ots_123048-521	0	---	---	0.092	0.048	0.121	0.879	0.145	0.855	0.008	0.992
Ots_128757-61R	0	---	---	0.171	0.196	0.332	0.668	0.488	0.512	0.096	0.904
Ots_94857-232R	0	---	---	0.058	0.482	0.352	0.648	0.466	0.534	0.515	0.485
Ots_94903-99R	1	---	---	0.067	0.495	0.360	0.640	0.431	0.569	0.541	0.459
Ots_96222-525	0	---	---	0.247	0.171	0.448	0.552	0.418	0.582	0.049	0.951
Ots_96500-180	2	---	---	0.126	0.476	0.221	0.779	0.271	0.729	0.581	0.419
Ots_96899-357R	0	---	---	0.062	0.089	0.002	0.998	0.018	0.982	0.125	0.875
Ots_97077-179R	1	---	---	0.213	0.107	0.150	0.850	0.384	0.616	0.030	0.970
Ots_AldB1-122	0	---	---	0.052	0.105	0.105	0.895	0.137	0.863	0.097	0.903
Ots_aldb-177M	2	---	*(A)	0.185	0.128	0.208	0.792	0.302	0.621	0.069	0.931
Ots_ARNT	2	LC	---	0.505	0.441	0.092	0.908	0.029	0.971	0.791	0.209
Ots_arp-436	0	---	---	0.358	0.121	0.245	0.755	0.438	0.485	0.018	0.982
Ots_AsnRS-60	0	---	---	0.034	0.177	0.089	0.911	0.158	0.842	0.201	0.799
Ots_aspat-196	0	---	---	0.182	0.059	0.210	0.790	0.147	0.853	0.004	0.996

Ots_C3N3	55	---	---	0.459	0.210	0.307	0.693	0.535	0.465	0.958	0.042
Ots_Cath_D141	0	---	---	0.042	0.020	0.013	0.987	0.064	0.936	0.010	0.990
Ots_CCR7	0	---	---	0.128	0.018	0.114	0.886	0.005	0.995	0.000	1.000
Ots_CD59-2	2	---	---	0.028	0.382	0.351	0.649	0.335	0.665	0.400	0.600
Ots_CD63	0	---	---	0.283	0.203	0.416	0.584	0.586	0.414	0.932	0.068
Ots_cox1-241	1	---	---	0.440	0.375	0.239	0.761	0.105	0.895	0.836	0.164
Ots_CRB211	1	---	---	0.068	0.020	0.018	0.982	0.085	0.915	0.004	0.996
Ots_E2-275	0	---	---	0.224	0.457	0.405	0.595	0.101	0.899	0.680	0.320
Ots_EndoRB1-486	11	---	---	0.147	0.114	0.268	0.732	0.270	0.730	0.041	0.959
Ots_EP-529	0	---	---	0.048	0.045	0.043	0.957	0.122	0.878	0.027	0.973
Ots_ETIF1A	1	---	---	0.233	0.461	0.234	0.766	0.177	0.823	0.694	0.306
Ots_FARSLA-220	4	---	---	0.748	0.314	0.177	0.823	0.041	0.959	0.955	0.045
Ots_FGF6A	1	---	*(C)	0.101	0.344	0.498	0.502	0.906	0.094	0.630	0.370
Ots_FGF6B_1	1	---	(C)	0.122	0.490	0.145	0.855	0.541	0.459	0.555	0.445
Ots_GDH-81x	0	---	---	0.085	0.314	0.383	0.617	0.517	0.483	0.249	0.751
Ots_GH2_1	0	---	---	0.036	0.027	0.002	0.998	0.004	0.996	0.039	0.961
Ots_GnRH-271	0	---	---	0.038	0.018	0.000	1.000	0.002	0.998	0.026	0.974
Ots_GPDH-338	0	---	---	0.046	0.017	0.021	0.979	0.057	0.943	0.006	0.994
Ots_GPH-318	0	---	---	0.079	0.155	0.143	0.857	0.027	0.973	0.189	0.811
Ots_GST-207	0	---	---	0.101	0.044	0.105	0.895	0.149	0.851	0.005	0.995
Ots_GST-375	0	---	---	0.042	0.006	0.031	0.969	0.006	0.994	0.000	1.000
Ots_GTH2B-550	3	---	---	0.212	0.403	0.161	0.839	0.036	0.964	0.546	0.454
Ots_hsc71-3'-488	2	---	(D)	0.423	0.382	0.190	0.810	0.146	0.854	0.827	0.173
Ots_hsc71-5'-453	0	---	*(D)	0.279	0.171	0.459	0.541	0.662	0.338	0.952	0.048
Ots_hsp27b-150	1	---	---	0.200	0.138	0.193	0.807	0.451	0.549	0.050	0.950
Ots_HSP90B-100	1	---	---	0.416	0.391	0.185	0.815	0.134	0.866	0.818	0.182
Ots_IGF-I.1-76	2	---	---	0.100	0.129	0.001	0.999	0.003	0.997	0.188	0.812
Ots_Ikaros-250	5	OT	---	0.157	0.082	0.044	0.956	0.090	0.910	0.089	0.911
Ots_IL11	2	OT; ST	---	0.618	0.312	0.199	0.801	0.178	0.822	0.919	0.081
Ots_IL8R_C8	1	---	---	0.291	0.410	0.023	0.977	0.050	0.950	0.582	0.418
Ots_mapK-3'-309	2	---	---	0.087	0.447	0.470	0.530	0.328	0.672	0.626	0.374

Ots_mapKpr-151	0	---	---	0.080	0.212	0.073	0.927	0.093	0.907	0.271	0.729
Ots_MHC1	3	---	---	0.502	0.270	0.333	0.667	0.232	0.768	0.939	0.061
Ots_MHC2	0	LC; ST	---	0.139	0.345	0.267	0.733	0.379	0.621	0.354	0.646
Ots_mybp-85	1	---	---	0.264	0.248	0.459	0.541	0.421	0.579	0.897	0.103
Ots_Myc-366	0	---	---	0.017	0.004	0.005	0.995	0.012	0.988	0.001	0.999
Ots_myo1a-384	0	---	---	0.131	0.091	0.284	0.716	0.109	0.891	0.044	0.956
Ots_myoD-364	0	---	---	0.386	0.253	0.345	0.655	0.375	0.625	0.925	0.075
Ots_nkef-192	3	---	---	0.303	0.396	0.014	0.986	0.023	0.977	0.571	0.429
Ots_NOD1	3	---	---	0.351	0.487	0.131	0.869	0.055	0.945	0.708	0.292
Ots_nramp-321Redesign	5	---	---	0.797	0.294	0.202	0.798	0.041	0.959	0.978	0.022
Ots_LWSop-638	0	---	---	0.109	0.018	0.115	0.885	0.005	0.995	0.000	1.000
Ots_Ots311-101x	0	---	---	0.319	0.086	0.361	0.639	0.125	0.798	0.015	0.985
Ots_P450	4	ST	---	0.714	0.296	0.193	0.807	0.102	0.898	0.963	0.037
Ots_P53	2	---	---	0.121	0.312	0.367	0.633	0.688	0.312	0.760	0.240
Ots_PGK-54	2	---	---	0.514	0.222	0.263	0.737	0.437	0.563	0.975	0.025
Ots_Prl2	0	---	---	0.141	0.375	0.416	0.584	0.394	0.606	0.727	0.273
Ots_RAG3	0	---	---	0.378	0.326	0.328	0.672	0.213	0.787	0.862	0.138
Ots_RAS1	0	---	---	0.026	0.003	0.021	0.979	0.000	1.000	0.000	1.000
Ots_RFC2-558	1	---	---	0.361	0.154	0.496	0.504	0.568	0.432	0.991	0.009
Ots_S7-1	1	---	---	0.055	0.389	0.492	0.508	0.617	0.383	0.637	0.363
Ots_SCIkF2R2-135	0	---	---	0.047	0.367	0.287	0.713	0.316	0.684	0.397	0.603
Ots_SL	2	---	---	0.703	0.281	0.213	0.787	0.131	0.869	0.973	0.027
Ots_SWS1op-182	1	---	---	0.162	0.430	0.247	0.753	0.365	0.635	0.691	0.309
Ots_TAPBP	2	OT; ST	---	0.304	0.412	0.058	0.942	0.660	0.340	0.688	0.312
Ots_TGFB	0	---	---	0.086	0.110	0.278	0.722	0.175	0.825	0.056	0.944
Ots_TLR3	0	---	---	0.271	0.454	0.207	0.793	0.182	0.818	0.709	0.291
Ots_TNF	0	---	---	0.043	0.011	0.044	0.956	0.023	0.977	0.000	1.000
Ots_Tnsf	2	---	*(B)	0.135	0.267	0.104	0.896	0.071	0.929	0.350	0.650
Ots_u07-07.161	1	LC	---	0.091	0.461	0.425	0.575	0.383	0.617	0.603	0.397
Ots_u07-17.135	0	---	---	0.051	0.092	0.024	0.976	0.028	0.972	0.123	0.877
Ots_u07-18.378	0	---	---	0.206	0.230	0.404	0.596	0.559	0.441	0.113	0.887

Ots_u07-20.332	0	---	---	0.090	0.020	0.091	0.909	0.039	0.961	0.000	1.000
Ots_u07-25.325	0	---	---	0.231	0.329	0.012	0.988	0.044	0.956	0.469	0.531
Ots_u07-49.290	1	---	---	0.091	0.400	0.463	0.537	0.647	0.353	0.327	0.673
Ots_u07-53.133	1	LC	---	0.314	0.230	0.438	0.562	0.468	0.532	0.917	0.083
Ots_u07-57.120	2	---	---	0.716	0.327	0.138	0.862	0.065	0.935	0.939	0.061
Ots_u07-64.221	0	---	---	0.025	0.004	0.001	0.999	0.019	0.981	0.001	0.999
Ots_u202-161	1	---	---	0.457	0.251	0.256	0.744	0.409	0.591	0.941	0.059
Ots_u211-85	1	---	---	0.303	0.417	0.043	0.957	0.026	0.974	0.594	0.406
Ots_u4-92	0	---	---	0.058	0.055	0.139	0.861	0.065	0.935	0.034	0.966
Ots_u6-75	0	---	---	0.044	0.094	0.059	0.941	0.029	0.971	0.117	0.883
Ots_unk526	1	---	---	0.046	0.142	0.111	0.889	0.156	0.844	0.145	0.855
Ots_zP3b-215	0	---	---	0.005	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Ots_ZR-575	5	ST	---	0.579	0.322	0.184	0.816	0.157	0.843	0.914	0.086

Appendix 2c. *O. nerka* SNP descriptive statistics by locus. Numbers (HWE) are Hardy-Weinberg deviations (HWE) per locus. Selection outlier loci are identified by “X”. Linked loci are matched by letter, and F_{ST} and allele frequencies are the mean across collections.

Locus	HWE	outlier	linked	Overall (mean)		
				Fst	allele 1	allele 2
One_ACBP-79	0	---	---	0.300	0.663	0.337
One_agt-132	1	---	---	0.382	0.504	0.496
One_aldB-152	0	---	---	0.354	0.496	0.504
One_apoe-83	0	---	---	0.079	0.925	0.075
One_c3-98	4	---	---	0.121	0.072	0.928
One_CD9-269	0	---	---	0.110	0.792	0.208
One_cetn1-167	0	---	---	0.102	0.193	0.807
One_CFP1	0	---	---	0.321	0.412	0.588
One_cin-177	0	---	---	0.310	0.683	0.317
One_Cytb_17	0	X	---	0.759	0.223	0.777
One_dds-529	0	---	---	0.251	0.694	0.306
One_DDX5-86	0	---	---	0.267	0.698	0.302
One_E2-65	0	---	---	0.052	0.985	0.015
One_gdh-212	1	---	---	0.246	0.262	0.738
One_GHII-2165	0	---	---	0.239	0.503	0.497
One_ghsR-66	0	---	---	0.142	0.978	0.022
One_GPDH-201	0	---	---	0.124	0.451	0.549
One_GTHa	0	---	---	0.186	0.729	0.271
One_HGFA-49	1	X	---	0.442	0.434	0.566
One_Hpal-71	0	---	---	0.224	0.506	0.494
One_Hpal-99	0	---	---	0.150	0.251	0.749
One_hsc71-220	0	---	---	0.372	0.367	0.633
One_Hsp47	1	---	---	0.264	0.322	0.678
One_IL8r-362	1	---	---	0.094	0.953	0.047
One_ins-107	0	---	---	0.081	0.545	0.455
One_KCT1-453	0	---	---	0.184	0.254	0.746
One_KPNA-422	0	---	---	0.259	0.653	0.347
One_LEI-87	0	---	---	0.178	0.767	0.233
One_lpp1-44	0	---	---	0.164	0.909	0.091
One_MARCKS-241	0	---	---	0.064	0.072	0.928
One_metA-253	0	---	---	0.254	0.735	0.265
One_MHC2-190	0	---	(A)	0.326	0.438	0.562
One_MHC2-251	0	---	(A)	0.511	0.302	0.621
One_Mkpro-129	0	---	---	0.362	0.847	0.153
One_ODC1-196	0	---	(B)	0.217	0.233	0.767
One_Ots208-234	0	---	---	0.236	0.611	0.389
One_Ots213-181	0	---	---	0.296	0.401	0.599
One_p53-534	0	---	---	0.192	0.152	0.848
One_pax7-248	0	---	---	0.142	0.070	0.930
One_PIP_3	0	---	---	0.275	0.511	0.489
One_Prl2	0	X	---	0.325	0.399	0.601
One_psme2-354	0	---	---	0.043	0.030	0.970
One_rab1a-76	0	---	---	0.225	0.851	0.149
One_RAG3-93	0	---	---	0.304	0.698	0.302
One_redd1-414rd	0	---	---	0.254	0.330	0.670
One_RFC2-102	0	---	---	0.235	0.686	0.314
One_RFC2-285	0	---	---	0.284	0.880	0.120
One_RH2op-395	0	---	---	0.047	0.941	0.059
One_rpo2j-261	0	---	---	0.396	0.794	0.206

One_sast-211	0	---	---	0.089	0.864	0.136
One_spf30-207	0	---	---	0.042	0.947	0.053
One_srp09-127	0	X	---	0.478	0.228	0.772
One_ssrd-135	0	---	---	0.138	0.476	0.524
One_STC-410	0	---	---	0.066	0.044	0.956
One_STR07	0	---	---	0.297	0.290	0.710
One_SUMO1-6	1	---	---	0.095	0.130	0.870
One_sys1-230	1	---	---	0.128	0.319	0.681
One_taf12-248	0	---	---	0.037	0.971	0.029
One_Tf_ex11-750	0	---	---	0.106	0.126	0.874
One_Tf_in3-182	0	---	---	0.066	0.968	0.032
One_txnip-401	0	---	---	0.106	0.848	0.152
One_U1003-75	0	---	---	0.310	0.216	0.784
One_U1004-183	0	---	---	0.216	0.449	0.551
One_U1009-91	0	---	---	0.228	0.221	0.779
One_U1010-81	0	---	---	0.216	0.183	0.817
One_U1012-68	0	---	---	0.278	0.545	0.455
One_U1013-108	0	---	---	0.194	0.072	0.928
One_U1014-74	2	---	---	0.194	0.402	0.598
One_U1024-197	0	---	---	0.176	0.258	0.742
One_U1101	0	---	---	0.262	0.562	0.438
One_U1105	1	---	---	0.355	0.438	0.562
One_U1201-492	0	---	---	0.077	0.648	0.352
One_U1202-1052	0	---	---	0.063	0.967	0.033
One_U1203-175	1	---	---	0.324	0.706	0.294
One_U1204-53	0	---	---	0.174	0.852	0.148
One_U1205-57	0	---	---	0.125	0.060	0.940
One_U1206-108	0	---	---	0.268	0.287	0.713
One_U1207-231	0	---	---	0.218	0.546	0.454
One_U1208-67	1	---	---	0.110	0.744	0.256
One_U1209-111	0	---	---	0.059	0.049	0.951
One_U1212-106	0	---	---	0.302	0.536	0.464
One_U1214-107	1	---	---	0.258	0.172	0.828
One_U1215-82	0	---	---	0.169	0.633	0.367
One_U1216-230	0	X	---	0.517	0.355	0.645
One_U301-92	0	---	---	0.024	0.035	0.965
One_U401-224	0	---	---	0.233	0.336	0.664
One_U502-167	0	---	---	0.262	0.816	0.184
One_U503-170	0	---	---	0.167	0.259	0.741
One_U504-141	0	---	---	0.138	0.428	0.572
One_U508-533	0	---	(B)	0.516	0.913	0.087
One_UCA-24	5	---	---	0.535	0.373	0.550
One_vamp5-255	0	---	---	0.096	0.937	0.063
One_vatf-214	0	---	---	0.247	0.276	0.724
One_VIM-569	0	---	---	0.165	0.148	0.852
One_ZNF-61	0	---	---	0.204	0.514	0.486
One_Zp3b-49	1	---	---	0.329	0.264	0.736

Section 3: Genetic Stock Identification of Chinook Salmon and Steelhead Harvest 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 mixed stock applications of salmonids (Shaklee et al. 1999, Winans et al. 2004, Beacham et al. 2006, and Beacham et al. 2011). These methods have been demonstrated to be useful even at relatively fine geographic scales within the Columbia River Basin (CRB) (Hess et al. 2011, Hess and Narum 2011). Within the CRB, Chinook salmon consist of three major genetic lineages and steelhead consist of two major genetic lineages, and lineages of both species can be further broken into populations that are genetically structured on a finer spatial scale (e.g., Waples et al. 2004; Narum et al. 2010; Blankenship et al. 2011). In this study we use 192 SNP markers to discriminate seventeen reporting groups for Chinook salmon and fourteen reporting groups for steelhead.

Despite continuous improvements of the power of our Chinook salmon and steelhead baselines in GSI applications (Hess et al. 2012), we have determined that further improvement in the detail of data and accuracy of stock assignments could be made by utilizing a recently developed genetic technology, parentage based tagging (PBT), in combination with GSI, in a tiered approach for stock identification. PBT is an efficient approach for mass tagging of fish. The method is carried out by first genotyping a set of potential parents which then provides the opportunity to assign a set of genotyped offspring to their true parent pair. PBT is currently being utilized on a broad scale in the Columbia River Basin to tag all Chinook salmon and steelhead Snake River hatchery broodstock (Steele et al. 2011). This application has effectively tagged all Snake River hatchery Chinook salmon and steelhead starting with the 2008 brood years. When parent pairs of a Snake River hatchery fish are identified with PBT, we can provide accurate information including age of the fish and the source hatchery in which its parents were spawned. We use PBT in this Chinook salmon and steelhead harvest study to identify all Snake River hatchery-origin fish, and then we use GSI to estimate stock-of-origin of all other hatchery fish that were not assigned with PBT (i.e. non-Snake River hatchery-origin) and all wild fish. PBT can assign the 3- and 4-year old Snake River hatchery-origin spring Chinook salmon (i.e. brood years 2008-2009) from the 2012 spring Chinook salmon harvest and the 1-, 2-, and 3-ocean age Snake River hatchery-origin steelhead (i.e. brood years 2008-2012) from the 2012 steelhead harvest. Eventually the PBT baseline will be expanded beyond the Snake River, and this year we have been able to add to our analyses the first middle Columbia River hatchery, Klickitat spring Chinook salmon hatchery.

Fisheries conducted in the mainstem of the lower and middle Columbia River provide an ideal and important application of genetic stock analyses because the fish harvested consist of mixtures of stocks from a large extent of the CRB. Further, Chinook salmon 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, PBT and GSI can be used to address two primary questions: 1) how are Chinook salmon and steelhead 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 has the potential to be used by fisheries managers to shape the various fisheries in a sustainable way that would protect less abundant stocks, while targeting the stocks of adequate abundance.

Thus, our study had three primary objectives: 1) utilize a combination of PBT and GSI analyses to estimate stock composition of Chinook salmon passing Bonneville Dam; 2) determine stock composition of Chinook salmon harvested in sport, commercial, and tribal fisheries in the mainstem Columbia River; and 3) utilize a combination of PBT and GSI to estimate stock composition of steelhead harvested above Bonneville Dam in the zone 6 fishery. Increasingly, we are tailoring our analyses to address specific questions that fisheries managers have presented to us. For example, in 2012 managers proposed extending the geographic boundary of one of the mark selective spring-run Chinook salmon sport fisheries above Bonneville Dam that occurs at the mouth of the Wind River. This extension created a larger “bubble” boundary at the mouth of the Wind River and was intended to increase Columbia River mainstem fishing access while maintaining targeted focus on Wind River spring-run Chinook salmon. We tested how this extended boundary affected stock composition of the Wind River sport harvest by comparing stock proportions among the various samples from other fisheries and Bonneville Dam that were analyzed this year. For steelhead, we sampled a group of fish that were non-adipose clipped and captured in the tribal fishery harvest above Bonneville Dam in zone 6. This latter project was in collaboration with Alan Byrne (Idaho Department of Fish and Game) and Stuart Ellis (Columbia River Inter-Tribal Fish Commission) and is currently being written into a TAC report. A high-seas fishing vessel fishing in Alaskan waters also provided samples of steelhead by-catch for examination.

Methods

Tissue collection of Chinook salmon and steelhead

Tissues were sampled from Chinook salmon in 2012 from a total of five different mixture sources: 1) Bonneville Dam (results discussed in section 4), and the spring-run seasons of the following fisheries: 2) lower river commercial, 3) lower river sport, and 4) Wind R. sport, 5) tribal ceremonial. In addition, we selected a group of fall-run harvest samples that had coded wire tags in order to test the accuracy of GSI fall-run reporting groups. The tribal ceremonial harvest was conducted in the spring and sampled by Bubba Holliday as part of the Warm Springs fishery program. These other harvest tissues were collected in coordination with existing monitoring programs led by Washington Department of Fish and Wildlife (WDFW) and Oregon Department of Fish and Wildlife (ODFW). The spring-run fisheries were sampled below Bonneville Dam in the sport and commercial (regions A and B), and sampled above Bonneville Dam in region 01 as part of the Wind River sport fishery and Warm Springs tribal ceremonial fishery (Figure 1, Table 1). The fall-run fisheries were sampled below Bonneville Dam (regions A and B via the sport and commercial fisheries). Stock proportions were calculated for some groupings within each fishery source, such that stock proportions could be compared across geographic regions as well as adipose-clipped versus non-adipose-clipped categories for particular fisheries. We use the following four main geographic regions (Figure 1): Region A corresponds to our grouping of pre-existing Oregon and Washington state sport fishing zones 1-4 (or commercial zones 4-5), Region B corresponds to our grouping of sport zones 5-10 (or commercial zones 1-3), and 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.

Table 1. Characteristics of Chinook salmon harvest samples by fishery source, geographic region, and weekly strata.

Fishery source	Region	Statistical week																																											Total
		Spring												Fall																															
		10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	33	34	35	36	37	38	39	40	41	42	43																	
Lower_River_commercial(HOR)	B						228	216																									444												
	A						48	88																																					
Lower_River_sport(HOR)	B	3	14	1	10	28	43	148	353					14	1		2																617												
	A	14	26	24	63	32	17	59	225					18																															
Bonneville Dam(HOR)	-									94	171	239	146	73	57	73	51																	904											
Bonneville Dam(NOR)	-									26	50	111	89	55	73	39	36																	479											
Warm_Springs_Ceremonial(HOR)	01								24	175	174																							373											
Warm_Springs_Ceremonial(NOR)	01								6	33	23																							62											
Wind_River_sport(HOR)	01								9	45	157	83	51	2	2																			349											
Fall_Commercial(HOR/CWT)	A/B																	21	15	37	34			2	9	1	3	1					123												
Fall_Sport(HOR/CWT)	A/B																	1	8	7	12	10	9	3	1								51												
Total		17	40	25	73	60	336	511	608	337	463	507	318	211	133	114	89	22	23	44	46	10	9	5	10	1	3	1					4016												

Note: Statistical week 10 equals 2/27/2012-3/04/2012, week 25 equals 6/11/2012-6/17/2012, week 33 equals 8/6/2012-8/12/2012 and week 43 equals 10/15/2012-10/21/2012. Regions are shown in Figure 1. Hatchery-origin “HOR” and natural-origin “NOR” were determined from the absence or presence of adipose fins, respectively. In the case of the fall fisheries, only fish with coded wire tags “CWT” were selected for genotyping.

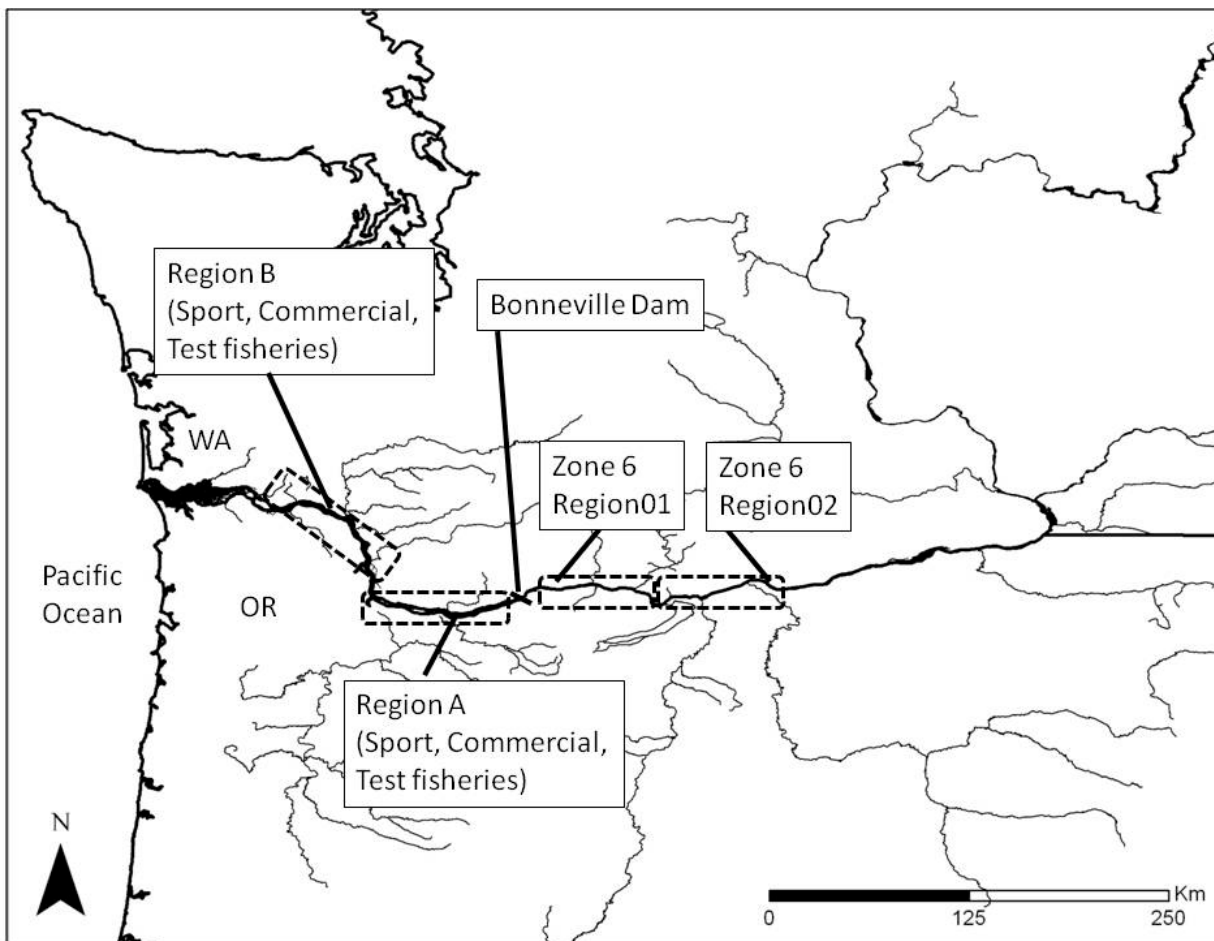


Figure 1. Map of sources of Chinook salmon and steelhead mixtures.

Non-tribal fisheries on spring-run Chinook salmon are mark-selective based on absence or presence of the adipose fin to distinguish hatchery fish from natural origin fish, 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 from Bonneville Dam and the tribal ceremonial harvest above Bonneville Dam in zone 6.

Tissues were sampled from steelhead in 2012 from three fishery mixture sources: 1) Bonneville Dam (results discussed in section 4), the tribal fishery in zone 6 (Figure 1), and fishery by-catch from a high seas Japanese fishing vessel in Alaskan waters. The tribal fishery was conducted during the regular summer steelhead management period (July 1st-October 31st) and was sampled as part of the Yakama Nation fishery monitoring program. This fishery is not mark-selective, and we conducted genetic stock analyses on the subset of samples that were not adipose-clipped (putatively natural-origin). However, there are a few steelhead hatcheries in the Snake River that release unmarked smolts and so a proportion of the samples in our dataset were expected to be unmarked hatchery-origin.

Molecular data

A total of 192 SNP loci were used for genotyping Chinook salmon (Section 2, Appendix 2b). However, as explained in Section 2 (this report), the following six loci were excluded:

Ots_SEXY1 (a marker for sex determination), *Ots_aldb-177M* (found to be linked to another

marker, *Ots_OTALDBINT1-SNP1*, which was retained), *Ots_hsc71-5'-453* (found to be linked to another marker, *Ots_hsc71-3'-488*, which was retained), *Ots_FGF6A* (found to be linked to another marker, *Ots_FGF6B_1*, which was retained), *Ots_Tnsf* (found to be linked to another marker, *Ots_OTSTF1-SNP1*, which was retained), and *Ots_zP3b-215* found to be monomorphic in the Columbia River Chinook salmon baseline. Therefore, we used 186 total SNP markers for GSI. Information on SNP baseline development and details of laboratory protocols involved in using these DNA markers for genotyping fish is available in Section 2. For PBT analyses, we used 95 of the 192 SNP loci (Hess et al. 2012; Section 1, Appendix 1), which have previously been demonstrated to provide accurate parent assignments (Steele et al. 2011).

There were a total of 192 SNP loci used for genotyping steelhead (Section 2, Appendix 2a). Basic QAQC (Section 2) resulted in the exclusion of seven loci found to be linked and one locus that exhibited significant Hardy-Weinberg deviation. In addition, there are 3 diagnostic markers for distinguishing cutthroat from steelhead/rainbow trout and a sex-determining marker which also had to be excluded for GSI applications. Therefore, we used 180 total SNP markers for GSI of steelhead mixtures.

GSI baselines for Chinook salmon and steelhead

Chinook salmon GSI analyses were performed using the updated baseline referred to as “Ots186SNP_Feb26_2013v1ONCOR”. This baseline includes the combined expansion data from both CRITFC and IDFG described in Section 2. Seventy-seven collections were delineated into the following 17 reporting groups: W_Cascade_sp, W_Cascade_fa, Willamette_sp, Spring_Cr_Group_Tule, Klickitat_sp, Deschutes_R_sp, John_Day_sp, Yakima_sp, Upper_Columbia_R_sp, Lower_Snake_sp, RapidR_Clearwater_sp, SF_Salmon_sp, MF_Salmon_sp, Chamberlain_Cr_sp, Upper_Salmon_sp, Interior_Columbia_R_su/fa, and Columbia_Rogue (Table 2). These reporting groups were primarily determined by the relative genetic similarity among populations according to a phylogenetic analysis. STRUCTURE v2.3.2 (Pritchard et al. 2000) was used to identify strays (>80% assignment to a different lineage) in each collection by setting a K value of 3 or higher; 30 strays were swapped into a more appropriate collection (e.g. in Entiat R., interior ocean-type and stream-type lineages co-occur and have slightly overlapping spring and summer run-timing and so must be genetically identified to make a pure collection) and 30 strays were removed entirely from the dataset (results not shown). Genetic distances were computed from allele frequencies based on Nei’s (1972) genetic distance, with the PHYLIP v 3.69 (Felsenstein 1989) and 1000 bootstrap replicates were performed. Distances were clustered using the Neighbor – Joining method (Saitou and Nei, 1987), and a consensus tree was constructed (<http://evolution.genetics.washington.edu/phylip/>).

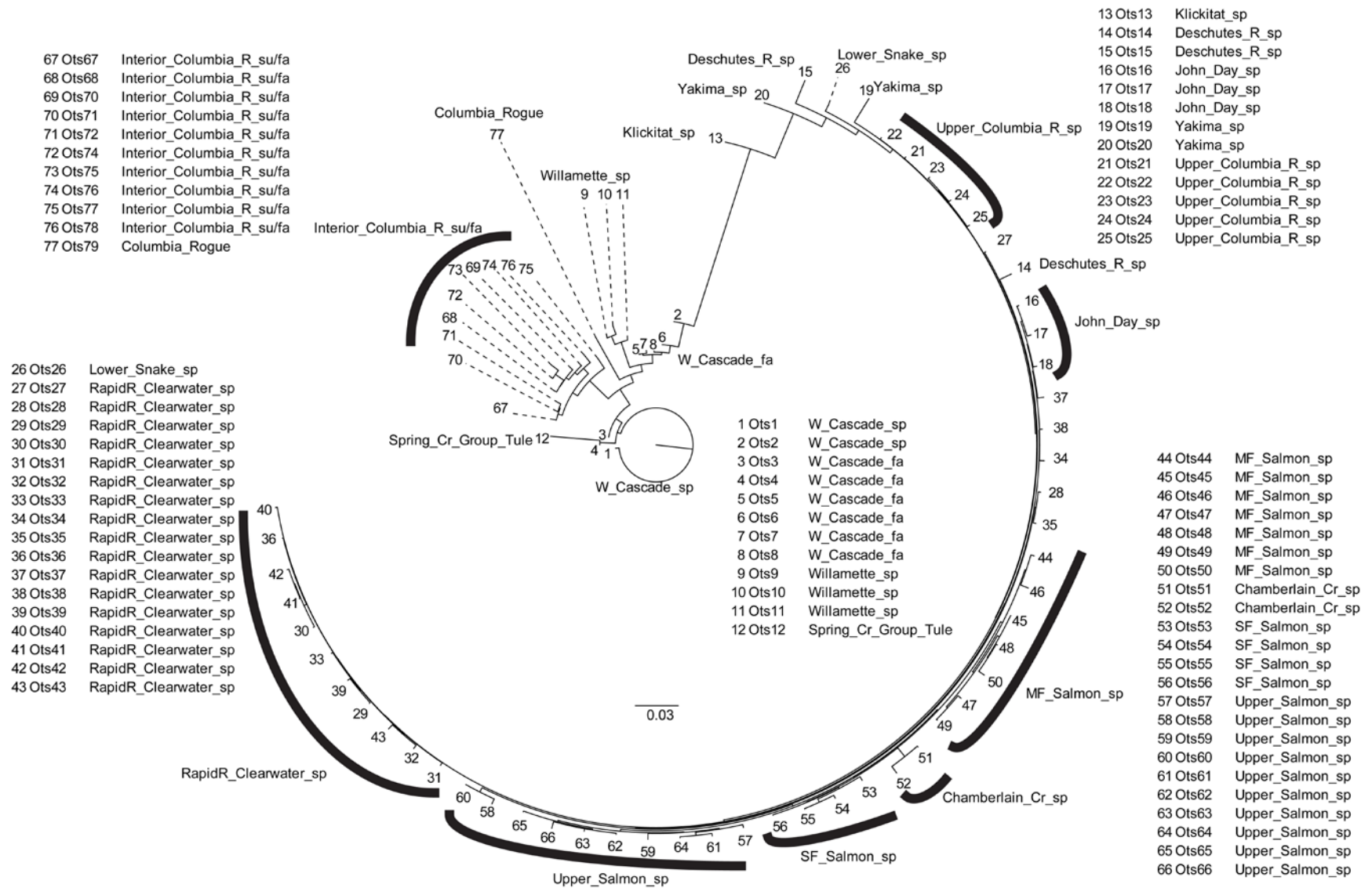


Figure 2. Neighbor-joining tree of Chinook salmon baseline populations using Nei's 1972 genetic distance of 186 SNP loci. The

clusters are labeled with names of reporting groups used to aggregate the collections based on a combination of factors including genetic similarity, life history, and geographic proximity. We used a final set of 17 reporting groups for all GSI analyses (Table 2).

The Upper_Columbia_R_sp reporting group includes the following Bonneville pool hatchery stocks: Carson (Ots21), and Little White Salmon R. (Ots24) because they are genetically indistinguishable from Upper Columbia R. spring Chinook salmon. This composite group is notable because inclusion of these Bonneville pool stocks explains why a large proportion of fish from the Wind R. sport fishery should assign to this Upper_Columbia_R_sp reporting group. The Columbia_Rogue reporting group represents an out-of-basin genetic stock (originating from the Rogue R., OR) that is reared within the Columbia R. at Youngs Bay. Basic QAQC was performed to remove duplicate individuals and strays from the reference populations in the baseline. The baseline and reporting groups file is available on request.

Table 2. Sample sizes and reporting groups of Chinook salmon baseline populations.

#	Collection	N	Lineage	Reporting Group
Ots1	Cowlitz R.	90	LC	W_Cascade_sp
Ots2	Kalama R.	83	LC	W_Cascade_sp
Ots3	Cowlitz	82	LC	W_Cascade_fa
Ots4	Elochoman R.	86	LC	W_Cascade_fa
Ots5	Lewis R.	93	LC	W_Cascade_fa
Ots6	North Fork Lewis R. late	84	LC	W_Cascade_fa
Ots7	North Fork Lewis R. early	94	LC	W_Cascade_fa
Ots8	Sandy R.	83	LC	W_Cascade_fa
Ots9	McKenzie R.	79	LC	Willamette_sp
Ots10	North Santiam R.	79	LC	Willamette_sp
Ots11	Sandy R.	48	LC	Willamette_sp
Ots12	White Salmon R.	77	LC	Spring_Cr_Group_Tule
Ots13	Klickitat R.	85	ST	Klickitat_sp
Ots14	Shitike Cr.	93	ST	Deschutes_R_sp
Ots15	Warm Springs R.	90	ST	Deschutes_R_sp
Ots16	John Day R.	78	ST	John_Day_sp
Ots17	Middle Fork John Day	47	ST	John_Day_sp
Ots18	North Fork John Day	42	ST	John_Day_sp
Ots19	American R.	76	ST	Yakima_sp
Ots20	Cle Elum R.	88	ST	Yakima_sp
Ots21	Carson_stock_(WNFH)	82	ST	Upper_Columbia_R_sp
Ots22	Entiat R.	98	ST	Upper_Columbia_R_sp
Ots23	Leavenworth-NFH	90	ST	Upper_Columbia_R_sp
Ots24	Little White Salmon R.	93	ST	Upper_Columbia_R_sp
Ots25	Peshastin Cr.	87	ST	Upper_Columbia_R_sp
Ots26	Tucannon R.	85	ST	Lower_Snake_sp
Ots27	Dworshak	88	ST	RapidR_Clearwater_sp
Ots28	Imnaha R.	92	ST	RapidR_Clearwater_sp
Ots29	Lochsa-Powell	77	ST	RapidR_Clearwater_sp
Ots30	Newsome Cr.	82	ST	RapidR_Clearwater_sp

Ots31	Catherine Cr	93	ST	RapidR_Clearwater_sp
Ots32	Crooked F Lochsa R	27	ST	RapidR_Clearwater_sp
Ots33	Crooked R Weir	67	ST	RapidR_Clearwater_sp
Ots34	Upper Grande Ronde	43	ST	RapidR_Clearwater_sp
Ots35	Imnaha R	43	ST	RapidR_Clearwater_sp
Ots36	Lolo Cr	89	ST	RapidR_Clearwater_sp
Ots37	Lostine R	176	ST	RapidR_Clearwater_sp
Ots38	Minam R	80	ST	RapidR_Clearwater_sp
Ots39	Powell Weir	31	ST	RapidR_Clearwater_sp
Ots40	Red R	72	ST	RapidR_Clearwater_sp
Ots41	Rapid R	91	ST	RapidR_Clearwater_sp
Ots42	Rapid R.	93	ST	RapidR_Clearwater_sp
Ots43	Wenaha R.	45	ST	RapidR_Clearwater_sp
Ots44	Big Cr.	89	ST	MF_Salmon_sp
Ots45	Bear Valley Cr	80	ST	MF_Salmon_sp
Ots46	Camas Cr	58	ST	MF_Salmon_sp
Ots47	Capehorn Cr	112	ST	MF_Salmon_sp
Ots48	Elk Cr	84	ST	MF_Salmon_sp
Ots49	Marsh Cr	66	ST	MF_Salmon_sp
Ots50	Sulphur Cr	35	ST	MF_Salmon_sp
Ots51	Chamberlain Cr (pre-2008)	70	ST	Chamberlain_Cr_sp
Ots52	Chamberlain Cr (post-2008)	55	ST	Chamberlain_Cr_sp
Ots53	Johnson Cr.	92	ST	SF_Salmon_sp
Ots54	Lake Cr, Summit Cr	74	ST	SF_Salmon_sp
Ots55	Secesh R	131	ST	SF_Salmon_sp
Ots56	SF Salmon R	140	ST	SF_Salmon_sp
Ots57	EF Salmon R	187	ST	Upper_Salmon_sp
Ots58	Hayden Cr	79	ST	Upper_Salmon_sp
Ots59	Lemhi (upper)	95	ST	Upper_Salmon_sp
Ots60	Lemhi (lower)	90	ST	Upper_Salmon_sp
Ots61	Pahsimeroi R	92	ST	Upper_Salmon_sp
Ots62	Sawtooth Weir	91	ST	Upper_Salmon_sp
Ots63	Valley Cr	57	ST	Upper_Salmon_sp
Ots64	Pahsimeroi	92	ST	Upper_Salmon_sp
Ots65	Sawtooth-Upper Salmon	90	ST	Upper_Salmon_sp
Ots66	W. F. Yankee Fork	75	ST	Upper_Salmon_sp
Ots67	Entiat R.	52	OT	Interior_Columbia_R_su/fa
Ots68	Hanford Reach	93	OT	Interior_Columbia_R_su/fa
Ots70	Lower Yakima R.	62	OT	Interior_Columbia_R_su/fa
Ots71	Methow	87	OT	Interior_Columbia_R_su/fa
Ots72	Tumwater & Dryden	92	OT	Interior_Columbia_R_su/fa
Ots74	Lower Deschutes R.	90	OT	Interior_Columbia_R_su/fa
Ots75	Upper Deschutes R.	89	OT	Interior_Columbia_R_su/fa

Ots76	Clearwater-NPTH	85	OT	Interior_Columbia_R_su/fa
Ots77	Clearwater	143	OT	Interior_Columbia_R_su/fa
Ots78	Lyons Ferry	90	OT	Interior_Columbia_R_su/fa
Ots79	Youngs Bay	91	Coast	Columbia_Rogue
Total		6409		

Note: Chinook salmon baseline collections (n=6409). Lineages are: ST- stream type, OT – ocean type, and LC – Lower Columbia. Refer to Appendix 1b, Section 2 for details on these collections based on similar names. “Sp”, “su”, and “fa” notation designate spring-, summer-, and fall-run-timing, respectively.

Steelhead GSI analyses utilized the updated baseline referred to as “OmySNPbase180JEHonor.txt”. This baseline includes the combined expansion data from both CRITFC and IDFG described in the study by Matala et al. (in review). The 131 baseline collections were delineated into the following 13 Columbia River Basin reporting groups: LOWCOL, SKAMAN, WILLAM, BWSALM, KICKR, MGILCS, YAKIMA, UPPCOL, SFCLWR, UPCLWR, SFSALM, MFSALM, and UPSALM (Table 3). The MGILCS reporting group includes the following Snake River stocks: George Cr, Asotin Cr, Alpowa Cr, and Tucannon R as well as the reporting groups from the Grande Ronde, Imnaha, Lower Clearwater and Lower Salmon R.; because they are genetically indistinguishable from other middle Columbia River stocks. The QAQC process relied heavily on the analyses in Section 2, and we excluded most hatchery populations (Skamania stocks were the exception, and remain in the baseline as a reference), and populations that had HWE deviations. We created a map of the geographic distribution of the baseline collections and reporting groups (Figure 3).

Table 3. Sample numbers of steelhead baseline collections and reporting group accuracy.

Ref#	Collection	N	RpGrv2.2	Leave-one-out		100% simulations		
				% Correct	RpGr Avg.	100% Sims	95% C.I.	RpGr Avg.
1	Quinault	89	WCOAST	100.0%	100.0%	100.0%	(0.9951, 1.0000)	100.0%
2	Mill	43	LOWCOL	89.3%	87.8%	99.3%	(0.9772, 1.0000)	97.3%
15	Germany	47	LOWCOL	86.4%		99.5%	(0.9798, 1.0000)	
13	Coweeman	45	LOWCOL	95.2%		99.2%	(0.9746, 1.0000)	
14	Cowlitz	94	LOWCOL	100.0%		100.0%	(0.9957, 1.0000)	
17	Kalama	94	LOWCOL	96.0%		96.0%	(0.9136, 0.9971)	
18	E. F. Lewis	77	LOWCOL	70.5%		98.5%	(0.9488, 1.0000)	
19	N. F. Lewis	94	LOWCOL	94.1%		99.8%	(0.9894, 1.0000)	
10	Luckiamute	26	LOWCOL	100.0%		100.0%	(0.9963, 1.0000)	
11	Willamina	30	LOWCOL	100.0%		99.7%	(0.9851, 1.0000)	
21	Still	28	LOWCOL	69.2%		82.4%	(0.7656, 0.8965)	
22	East Fork Hood	52	LOWCOL	65.0%		95.8%	(0.9297, 0.9888)	
16	Kalama	94	SKAMAN	71.4%	80.5%	94.5%	(0.8835, 0.9816)	97.2%
5	Skamania Stock	59	SKAMAN	89.5%		99.9%	(0.9894, 1.0000)	

2	Clackamas	92	WILLAM	88.6%	86.4%	99.9%	(0.9935, 1.0000)	99.4%
4	N. F. Eagle	43	WILLAM	75.0%		98.4%	(0.9549, 0.9999)	
3	Eagle	47	WILLAM	89.5%		98.1%	(0.9545, 1.0000)	
6	Little Rock/Mad	50	WILLAM	87.5%		99.8%	(0.9896, 1.0000)	
7	N. F. Santiam/ Mad	39	WILLAM	94.4%		100.0%	(0.9994, 1.0000)	
8	S. F. Santiam/ Wiley	93	WILLAM	83.3%		100.0%	(0.9953, 1.0000)	
24	Big White Salmon	78	BWSALM	90.5%	90.5%	100.0%	(0.9954, 1.0000)	100.0%
33	Upper Trout	46	KLICKR	96.3%	77.2%	100.0%	(1.0000, 1.0000)	99.3%
31	Surveyor	39	KLICKR	100.0%		100.0%	(0.9995, 1.0000)	
30	Snyder	47	KLICKR	89.5%		100.0%	(1.0000, 1.0000)	
27	Lower Summit	45	KLICKR	78.9%		99.0%	(0.9719, 1.0000)	
28	Lower Trout	48	KLICKR	61.9%		99.2%	(0.9753, 1.0000)	
29	Lower White	33	KLICKR	85.7%		99.0%	(0.9715, 1.0000)	
34	Lower Little Klickitat	46	KLICKR	66.7%		98.9%	(0.9696, 1.0000)	
26	Deadcanyon	34	KLICKR	58.3%		98.6%	(0.9598, 1.0000)	
25	Bowman	48	KLICKR	50.0%		98.4%	(0.9592, 1.0000)	
32	Swale	48	KLICKR	84.4%		99.6%	(0.9849, 1.0000)	
40	Fifteen	91	MGILCS	89.2%	85.4%	98.5%	(0.9592, 1.0000)	96.5%

37	Pelton	45	MGILCS	72.7%	81.1%	(0.7069, 0.8947)
38	Shitike	31	MGILCS	66.7%	94.3%	(0.8914, 0.9846)
35	Buckhollow	63	MGILCS	81.8%	96.3%	(0.9182, 0.9920)
39	Trout	57	MGILCS	75.0%	96.5%	(0.9327, 0.9906)
36	Mainstem Deschutes	61	MGILCS	100.0%	99.9%	(0.9950, 1.0000)
42	Beech	21	MGILCS	85.7%	98.7%	(0.9634, 1.0000)
44	Upper Mainstem J. D.	34	MGILCS	100.0%	99.9%	(0.9941, 1.0000)
41	Baldy	25	MGILCS	100.0%	99.7%	(0.9874, 1.0000)
43	Lower Mainstem J. D.	44	MGILCS	100.0%	97.4%	(0.9376, 0.9978)
45	Upper M. F. J. D.	107	MGILCS	96.0%	99.5%	(0.9811, 1.0000)
47	Granite	18	MGILCS	100.0%	99.9%	(0.9950, 1.0000)
48	Middle N. F. J. D.	56	MGILCS	87.5%	98.7%	(0.9620, 1.0000)
46	Big Wall	22	MGILCS	85.7%	99.0%	(0.9660, 1.0000)
49	Deer	18	MGILCS	100.0%	99.5%	(0.9848, 1.0000)
50	Murderers	18	MGILCS	100.0%	99.0%	(0.9704, 1.0000)
51	Rock	126	MGILCS	76.5%	98.7%	(0.9656, 1.0000)
52	Squaw	138	MGILCS	78.9%	97.3%	(0.9414, 0.9974)
53	Iskuulpa	148	MGILCS	87.6%	98.4%	(0.9617, 0.9981)

54	Umatilla	34	MGILCS	88.9%	99.5%	(0.9832, 1.0000)
55	Touchet	86	MGILCS	92.2%	99.6%	(0.9837, 1.0000)
79	George Cr	95	MGILCS	69.6%	88.5%	(0.7870, 0.9522)
76	Asotin Cr	98	MGILCS	74.6%	88.9%	(0.8055, 0.9551)
75	Alpowa Cr	98	MGILCS	63.1%	92.0%	(0.8338, 0.9677)
74	Tucannon R	105	MGILCS	74.2%	97.4%	(0.9423, 0.9949)
108	Little Minam R	48	MGILCS	95.7%	99.5%	(0.9861, 1.0000)
109	Lostine R	45	MGILCS	100.0%	98.8%	(0.9680, 1.0000)
106	Elk Cr	45	MGILCS	90.3%	99.8%	(0.9903, 1.0000)
107	Joseph Cr	45	MGILCS	83.3%	96.7%	(0.9201, 0.9955)
100	Crooked Cr	95	MGILCS	84.9%	98.1%	(0.9432, 0.9990)
110	Menatchee Cr	68	MGILCS	88.2%	97.1%	(0.9349, 0.9942)
111	Wenaha R	93	MGILCS	87.0%	97.2%	(0.9385, 0.9952)
78	Captain John Cr	56	MGILCS	88.2%	98.9%	(0.9536, 1.0000)
112	Big Sheep Cr	61	MGILCS	84.2%	97.4%	(0.9442, 0.9949)
113	Camp Cr	23	MGILCS	83.3%	95.2%	(0.9115, 0.9919)
114	Cow Cr	44	MGILCS	85.7%	91.8%	(0.8616, 0.9738)
115	Lightning Cr	38	MGILCS	77.8%	96.3%	(0.9260, 0.9919)

84	WF Potlatch R	85	MGILCS	91.4%		98.8%	(0.9737, 1.0000)	
82	EF Potlatch R	156	MGILCS	85.4%		99.2%	(0.9708, 1.0000)	
81	Big Bear Cr	98	MGILCS	81.7%		97.9%	(0.9491, 0.9997)	
83	Little Bear Cr	151	MGILCS	86.6%		98.9%	(0.9742, 1.0000)	
116	Boulder Cr	47	MGILCS	72.4%		92.4%	(0.8421, 0.9684)	
118	Rapid R	99	MGILCS	75.9%		98.3%	(0.9611, 1.0000)	
103	Slate Cr	46	MGILCS	77.4%		80.1%	(0.7229, 0.8758)	
104	Whitebird Cr	59	MGILCS	78.8%		92.8%	(0.8725, 0.9723)	
60	Rattlesnake/ Naches	36	YAKIMA	78.6%	80.4%	95.2%	(0.9142, 0.9847)	97.6%
57	Nile/ Naches	59	YAKIMA	80.0%		97.8%	(0.9509, 0.9999)	
58	Pileup/ Naches	26	YAKIMA	87.5%		96.6%	(0.9382, 0.9905)	
59	Quartz/ Naches	26	YAKIMA	81.8%		98.2%	(0.9571, 0.9995)	
56	N. F. Little Naches	21	YAKIMA	60.0%		98.4%	(0.9640, 0.9997)	
61	Satus	46	YAKIMA	80.0%		97.3%	(0.9471, 0.9929)	
62	Toppenish	44	YAKIMA	95.0%		99.8%	(0.9908, 1.0000)	
67	Peshastin	99	UPPCOL	55.2%	62.9%	87.8%	(0.8145, 0.9290)	91.1%
63	Chiwaukum	54	UPPCOL	81.3%		95.3%	(0.9075, 0.9889)	
68	Upper Chiwaukum	29	UPPCOL	50.0%		83.6%	(0.7651, 0.9035)	

66	Nason	21	UPPCOL	72.7%		84.5%	(0.7545, 0.9362)	
69	Entiat	94	UPPCOL	53.1%		90.3%	(0.8340, 0.9514)	
70	Methow	90	UPPCOL	52.1%		87.0%	(0.7832, 0.9451)	
71	Bonaparte	99	UPPCOL	62.0%		94.5%	(0.8921, 0.9828)	
72	Omak	94	UPPCOL	79.5%		97.6%	(0.9408, 0.9965)	
73	Salmon	98	UPPCOL	60.5%		99.3%	(0.9785, 1.0000)	
100	Crooked R	104	SFCLWR	87.8%	83.0%	99.3%	(0.9745, 1.0000)	95.7%
102	Tenmile Cr	46	SFCLWR	77.4%		99.6%	(0.9864, 1.0000)	
101	John's Cr	36	SFCLWR	88.2%		86.8%	(0.7897, 0.9188)	
99	Clear Cr	45	SFCLWR	78.6%		97.0%	(0.9420, 0.9932)	
94	Colt Cr	38	UPCLWR	100.0%	93.9%	99.9%	(0.9908, 1.0000)	99.1%
98	Storm Cr	38	UPCLWR	100.0%		99.9%	(0.9947, 1.0000)	
95	Crooked F Lochsa R	44	UPCLWR	87.0%		99.4%	(0.9739, 1.0000)	
97	Lake Cr	47	UPCLWR	100.0%		99.9%	(0.9950, 1.0000)	
96	Fish Cr	99	UPCLWR	93.7%		99.9%	(0.9901, 1.0000)	
93	Canyon Cr	46	UPCLWR	75.0%		98.0%	(0.9535, 0.9972)	
88	Selway R	76	UPCLWR	97.9%		99.9%	(0.9948, 1.0000)	
87	Little Clearwater R	59	UPCLWR	97.6%		99.8%	(0.9920, 1.0000)	

92	Whitecap Cr	76	UPCLWR	100.0%		99.9%	(0.9946, 1.0000)	
85	Bear Cr	35	UPCLWR	100.0%		99.9%	(0.9930, 1.0000)	
89	NF Moose Cr	92	UPCLWR	98.1%		99.9%	(0.9923, 1.0000)	
91	Three Links Cr	47	UPCLWR	96.7%		100.0%	(0.9953, 1.0000)	
86	Gedney Cr	45	UPCLWR	96.0%		98.7%	(0.9643, 1.0000)	
90	O'Hara Cr	47	UPCLWR	72.7%		92.0%	(0.8656, 0.9664)	
119	EF SF Salmon R	45	SFSALM	82.6%	89.5%	99.5%	(0.9838, 1.0000)	99.1%
122	Stolle Meadows	45	SFSALM	100.0%		99.3%	(0.9751, 1.0000)	
121	Secesh R	45	SFSALM	93.5%		98.9%	(0.9764, 1.0000)	
120	Lick Cr	39	SFSALM	81.8%		98.6%	(0.9642, 1.0000)	
128	Marsh Cr	59	MFSALM	100.0%	91.0%	99.9%	(0.9953, 1.0000)	97.6%
131	Sulphur Cr	42	MFSALM	95.7%		99.9%	(0.9925, 1.0000)	
130	Rapid R (MF)	31	MFSALM	100.0%		99.8%	(0.9902, 1.0000)	
129	Pistol Cr	23	MFSALM	100.0%		99.9%	(0.9951, 1.0000)	
126	Loon Cr	84	MFSALM	92.2%		99.7%	(0.9884, 1.0000)	
125	Camas Cr	56	MFSALM	96.9%		99.6%	(0.9860, 1.0000)	
132	Big Cr (upper)	45	MFSALM	90.9%		99.9%	(0.9952, 1.0000)	
127	Big Cr (lower)	46	MFSALM	89.5%		99.4%	(0.9818, 1.0000)	

123	Chamberlain Cr	46	MFSALM	76.5%		90.2%	(0.8462, 0.9488)	
124	Bargamin Cr	46	MFSALM	68.4%		87.8%	(0.8341, 0.9207)	
137	Sawtooth Weir	105	UPSALM	66.1%	61.0%	96.4%	(0.9269, 0.9893)	93.7%
138	Valley Cr	44	UPSALM	52.0%		93.7%	(0.8842, 0.9742)	
139	WF Yankee F Salmon	117	UPSALM	73.3%		97.1%	(0.9395, 0.9938)	
134	Morgan Cr	37	UPSALM	68.2%		95.6%	(0.9105, 0.9898)	
136	Pahsimeroi Weir	96	UPSALM	65.5%		94.9%	(0.9115, 0.9820)	
133	Hayden Cr	84	UPSALM	55.6%		94.3%	(0.8977, 0.9750)	
135	NF Salmon R	99	UPSALM	46.6%		83.9%	(0.7468, 0.8948)	

Note: Averaged values for each reporting group are shaded dark green, light green, orange, yellow, and red to indicate ranges of >90%, 80-90%, 70-80%, 60-70%, and below 60%, respectively.

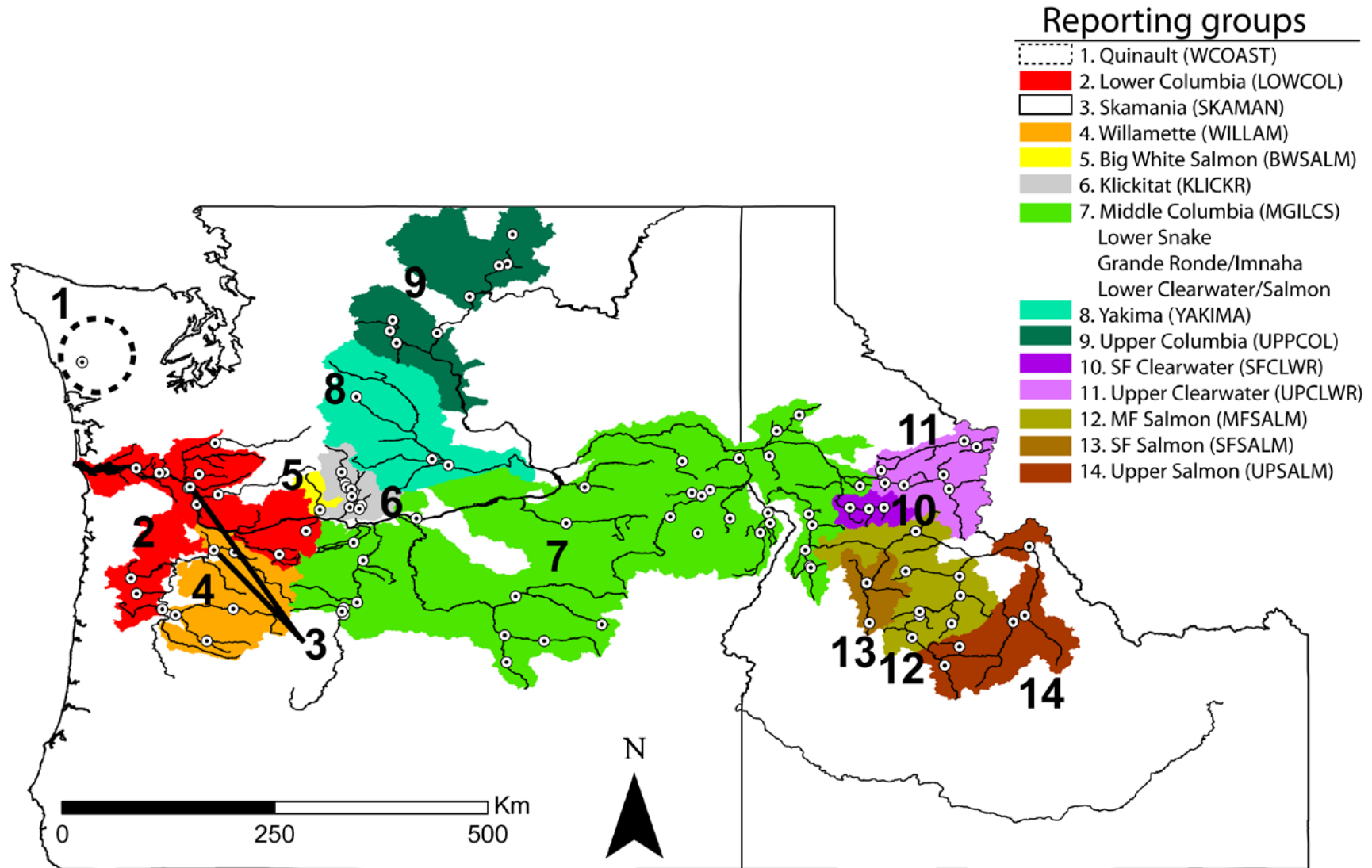


Figure 3. Map of steelhead baseline collections and reporting groups.

Baseline power analyses

The accuracy of the Chinook salmon and steelhead baselines was characterized using mixture simulations performed by the program ONCOR v1.0 (available at <http://www.montana.edu/kalinowski>). This program has a feature, “100% simulations” which can evaluate the power of the baseline to analyze mixture samples at the reporting group level (Anderson et al. 2008). It has been recommended that reporting groups meet a threshold of 90% proportions in these 100% mixture simulation analyses to be useful for fishery management applications (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. ONCOR was also used to assign individuals from the baseline in a “leave-one-out” analysis to estimate correct individual assignment to reporting groups. To examine how much greater power 188 SNPs can generate compared to our previous 92-SNP baseline, we excluded all SNPs that weren’t part of the 92 SNP marker set and ran all these analyses.

In addition to simulations, we examined how well GSI assignments corresponded to known origin fish based on CWT data. In total, 174 CWT were selected for genotyping and had been sampled during the Chinook salmon fall-run season (Table 1). The hatchery-of-origin information was fit into our reporting group categories to make it compatible for comparing to GSI results.

Combined application of PBT and GSI

We combined PBT and GSI results together by first accepting all confident PBT assignments to hatchery broodstock for the Chinook salmon and steelhead individuals and then for the remaining individuals, we used the best estimate GSI assignments to provide likely population of origin. Current PBT baselines allowed us to identify the source hatcheries of 3- and 4-year old Snake River and Klickitat River spring-run Chinook salmon (2008-2009 broodyears) and the 1-, 2-, and 3-ocean age steelhead from the Snake River (2008-2010 broodyears). Genotypes of parents and offspring were analyzed with SNPPITv1.0, a software program developed for large PBT datasets based on SNP markers (Anderson 2010). Analyses with SNPPIT were performed on the Klickitat R. and Snake River Chinook salmon SY2008 and SY2009 parental data (“cleaned” baseline data file from Sep. 19, 2012). This dataset represents a ‘cleaned’ version that was processed via an R script (i.e. no duplicates, no failed individuals, etc.). The SNPPIT input file name was “SNPPIT_MY2012_CHNK.txt” and it was only modified by adding in available parental data from the spring Chinook salmon broodstock used in the Klickitat Hatchery for SY2008 and SY2009. We used the following initial threshold values for the parental assignment results: False Discovery Rate = 0.01, P-value = 0.05, and LOD score = 14. For the Chinook salmon harvest datasets, cross checking hatchery records allowed us reasonable confidence to accept all assignments above LOD score = 13.99. This LOD score threshold was also used for the Bonneville Dam spring Chinook salmon mixture.

For Snake River steelhead the SNPPIT file included brood years 2008-2010 compiled into a “cleaned” baseline data file from Jan. 15, 2013, was processed via an R script (i.e. no duplicates, no failed individuals, etc.). The SNPPIT input file name was “STHD_080910.txt” and the complete set of parental data was used for this analysis to assign parentage for unclipped samples from Zone 6. For this dataset, we accepted all assignments above LOD score = 14.00.

The program ONCOR was used to estimate the most likely population-of-origin for the Chinook salmon and steelhead harvest samples. Individuals were assigned using a “best estimate” approach in which the reporting group with highest probability was provided as that individual’s likely population-of-origin.

Results

Accuracy testing of 186 SNP Chinook salmon baseline

The 77 collections were grouped into 17 reporting groups based on the clustering we observed in the phylogenetic analysis (Figure 2). Results from 100% mixture simulations from each of the baseline collections, showed a majority (75 of 77; 97%) of the simulated mixtures were estimated to be composed of greater than 90% proportion of the correct reporting group (Figure 4, lower panel). Two collections, North Fork John Day spring-run and Dworshak spring-run, showed less than 90% proportions, but only the North Fork John Day collection was significantly below 90% (as indicated by the upper 95% confidence interval). This population has been shown likely to be affected by out-of-basin hatchery stock sources (e.g. from straying originating from the Snake R.; Narum et al. 2008). The average correct mixture proportion based on these 100% mixture simulations is provided for each reporting group (Table 4).

Results from the leave-one-out analysis (Figure 4, upper panel), showed lower performance of baseline power, where less than half of the baseline collections displayed greater than 90% correct individual assignment. Among the baseline collections with the lowest correct assignment (below 60%) were North Fork John Day spring-run (36%), SF Salmon R spring-run (46%), Carson_stock_(WNFH) spring-run (48%), John Day R spring run (56%), and Wenaha R. spring run (59%). The average correct assignment based on this leave-one-out analysis is provided for each reporting group (Table 4).

Table 4. Average GSI accuracy for Chinook salmon reporting groups using 186 SNPs.

Reporting Groups	Leave-1- out	100% Sims
	% Correct	
W_Cascade_sp	81.9%	99.5%
W_Cascade_fa	93.5%	99.8%
Willamette_sp	100.0%	99.9%
Spring_Cr_Group_Tule	90.5%	100.0%
Klickitat_sp	94.9%	99.9%
Deschutes_R_sp	90.4%	99.8%
John_Day_sp	58.8%	84.7%
Yakima_sp	100.0%	99.7%
Upper_Columbia_R_sp	62.7%	94.8%
Lower_Snake_sp	73.0%	99.5%
RapidR_Clearwater_sp	76.6%	95.4%
MF_Salmon_sp	89.7%	99.0%
Chamberlain_Cr_sp	91.6%	99.8%
SF_Salmon_sp	73.3%	97.6%
Upper_Salmon_sp	85.1%	99.1%
Interior_Columbia_R_su/fa	98.6%	100.0%
Columbia_Rogue	100.0%	100.0%
Grand Total	84.0%	97.7%

Note: Accuracy is based on results from leave-1-out and 100% mixture simulations performed with the program ONCOR.

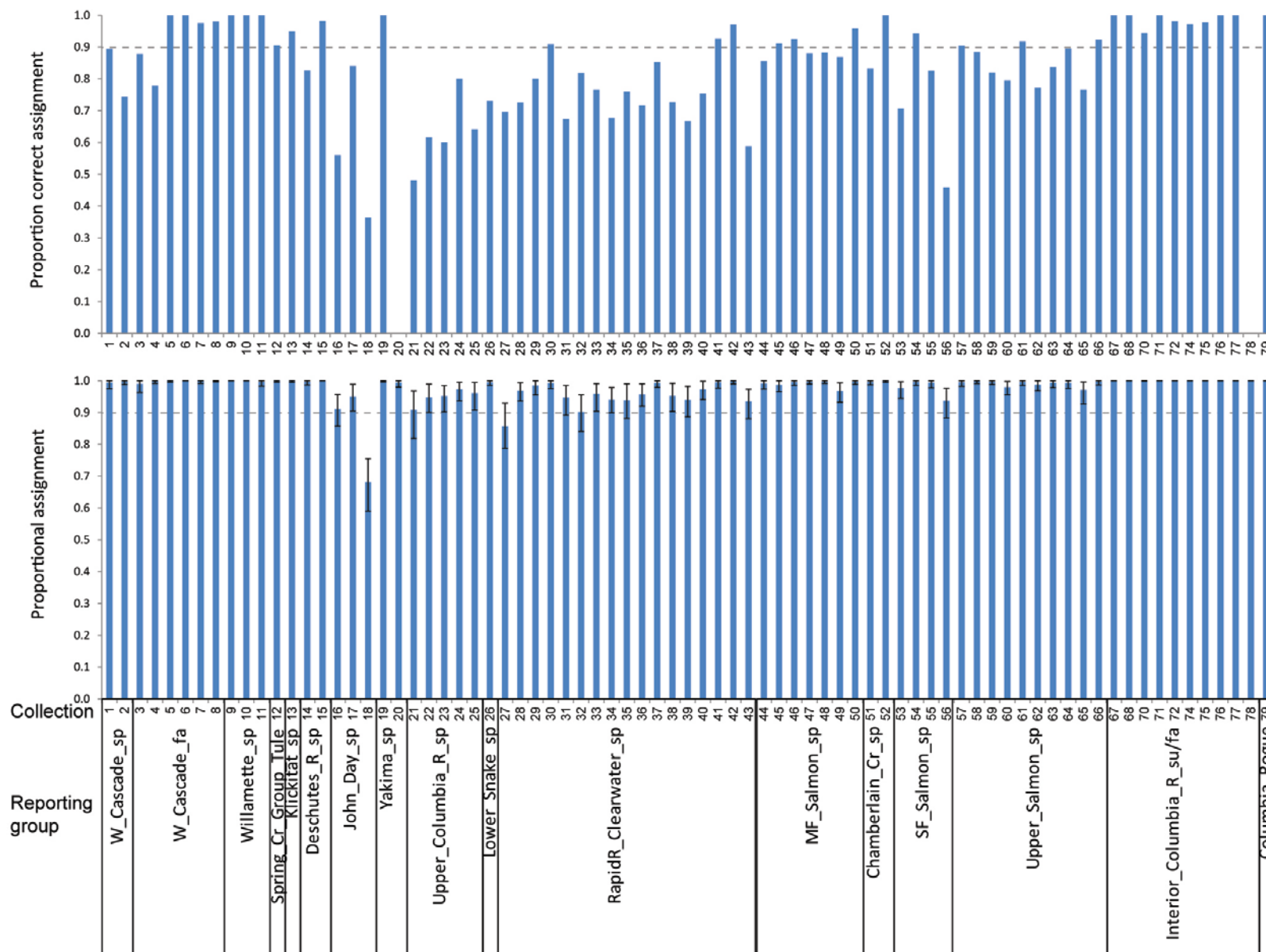


Figure 4. Accuracy of the Chinook salmon GSI baseline. Results plotted in charts are based on correct assignment of baseline individuals back to reporting-group-of-origin from leave-one-out analysis (top), and estimated reporting-group proportions from 100% mixture simulations (bottom) using ONCOR. Estimated mixture proportions include 95% confidence intervals. Both charts show a dashed line at the 90% level, considered to be a guidance threshold for determining whether accuracy of reporting groups is sufficient for fisheries management applications. The Cle Elum (20) and Lyons Ferry (78) collections had too much missing genotypic data to calculate a leave-one-out correct assignment value in ONCOR.

Based on CWT source hatchery data, we estimated concordance of our genetic stock ID individual assignments for 174 CWT recovered from hatchery-origin fish in the fall-run Chinook salmon harvest. The following six genetic stock types were represented in the subset of CWT salmon: W_Cascade_fa, Spring_Cr_Group_Tule, Interior_Columbia_R_su/fa, Columbia_Rogue, California fall-run Chinook salmon, and a label error. We predicted these stocks based on available history of the broodstock that is utilized at each source hatchery (Table 5). Genetic stocks with CWT representation of 10 or more samples showed a high level of concordance (above 90%). There were also stocks that were expected to provide a challenge to genetic ID due to the lack of representation in the reference baseline, e.g. the stocks from California and “steelhead” from Magic Valley that had been mislabeled.

Table 5. Comparison of GSI assignments (genetic origin) of fall-run Chinook salmon with source hatchery information based on coded wire tags (CWTs).

CWT genetic stock	CWT hatchery source	GSI reporting group assignment						Total	%Correct
		W- Cascade- sp	Willamette- sp	W- Cascade- fa	Spring- Cr- Group- Tule	Interior- Columbia- R-su/fa	Columbia- Rogue		
W_Cascade_fa	Washougal H.	1		22				23	95.7%
Spring_Cr_Group_Tule	Big Cr. H.				1	1		2	50.0%
Spring_Cr_Group_Tule	Spring Cr. N.F.H.			1	30			31	96.8%
Interior_Columbia_R_su/fa	Lyons Ferry H.					27		27	100.0%
Interior_Columbia_R_su/fa	Nez Perce H.					20		20	100.0%
Interior_Columbia_R_su/fa	Priest Rapids H.					27		27	100.0%
Interior_Columbia_R_su/fa	Prosser H.			1		13		14	92.9%
Interior_Columbia_R_su/fa	Similkameen H.		1			23		24	95.8%
Spring_Cr_Group_Tule/ Columbia_Rogue	S.F. Klaskanine Pond						1	1	100.0%
Columbia_Rogue	C.E.D.C. Youngs Bay Net		1				1	2	50.0%
CA fall stock	Coleman N.F.H.				1			1	-
CA fall stock	Trinity R. H.						1	1	-
Label error	Magic Valley					1		1	-
	Total	1	2	24	32	112	3	174	88.1%

Note: The fall-run Chinook salmon mixture was constructed by pooling a group of CWT hatchery-origin Chinook salmon harvested in the sport and commercial lower river fisheries. The CWT genetic stock is the expected stock of origin for each of these hatchery

source stocks, and we calculated percent correct assignment (%Correct) based on this expectation (# of correct individual assignments are highlighted in gray). The CWT sources from Coleman and Trinity R. hatcheries are from California and were not represented in our baseline. Magic Valley is actually a steelhead hatchery and this fish was mislabeled in the data collection process.

Parentage based tagging assignments of harvested Chinook salmon

The PBT analysis resulted in 1,316 hatchery-origin salmon that could be assigned back to Klickitat and Snake River hatchery broodstock parents that were spawned in 2008 and 2009 (Table 6). These salmon were found to have originated from the following fifteen sources: Klickitat, S.F. Clearwater, Catherine Creek, Dworshak, Grande Ronde, Imnaha, Johnson Cr., Lookingglass Cr., Lostine, McCall, Nez Perce Tribal Hatchery, Pahsimeroi, Clearwater (Powell), Rapid R., Sawtooth, and Tucannon hatcheries. The broodstock from Rapid R. 2008 yielded the highest number of assignments (N=578), and the Johnson Cr. And Tucannon hatcheries yielded the least (N=1). There were ten 4-year-old fish from the Wind R. fishery that assigned to the following PBT source hatcheries: Catherine, Dworshak, McCall, Rapid, and Sawtooth hatcheries.

Table. 6. Summary information on numbers and origins of Chinook salmon that assigned to the PBT baseline.

PBT source hatchery	PBTpop	Tagging Rate	Fishery:	Bonneville		Lower R.		Warm Springs		Wind R.	Total
				Origin:	Dam	commercial	sport	Ceremonial		sport	
								HOR	NOR		
Klickitat Hatchery	OtsKLKR08S	93.3%									15
	OtsKLKR09S	99.3%									3
	OtsCLWH08S	97.0%									142
LSRCP/IDFG - Clearwater (SF)	OtsCLWH09S	94.1%									2
LSRCP/ODFW - Catherine Creek	OtsCTHW08S	100.0%									12
	OtsDWOR08S	97.6%									254
LSRCP/USFWS - Dworshak	OtsDWOR09S	95.3%									6
LSRCP/ODFW - Grande Ronde	OtsGRUW08S	100.0%									2
	OtsIMNW08S	99.2%									29
LSRCP/ODFW - Imnaha	OtsIMNW09S	98.4%									2
	Johnson Cr.	OtsJHNW08S	100.0%								1
LSRCP/ODFW - Lookingglass Creek	OtsLOOK08S	98.6%									49

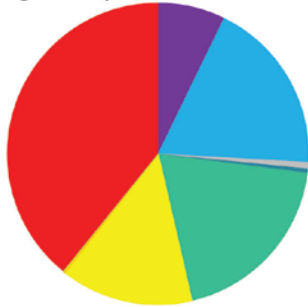
	OtsLOOK09S	97.2%	2							2
	OtsLSTW08S	94.5%	4							4
LSRCP/ODFW/NPT – Lostine	OtsLSTW09S	89.3%		1						1
	OtsMCCA08S	97.6%	36	1		9			1	47
LSRCP/IDFG - McCall (SFSR)	OtsMCCA09S	95.3%	2							2
Nez Perce Tribal Hatchery (NPTFH)	OtsNPFH08S	97.9%		3						3
Idaho Power/IDFG - Pahsimeroi	OtsPAHH08S	98.4%	3			1				4
LSRCP/IDFG - Clearwater (Powell)	OtsPOWP08S	98.6%	25	5	24	15	18	2		89
	OtsRAPH08S	98.4%	144	4	105	258	64		3	578
Idaho Power/IDFG - Rapid River	OtsRAPH09S	96.8%	6							6
	OtsSAWT08S	99.0%	35	11		9			1	56
LSRCP/IDFG - Sawtooth	OtsSAWT09S	98.0%	5			1				6
LSRCP/WDFW - L.F. (Tucannon)	OtsTUCW08S	89.7%		1						1
	Total		441	41	218	454	147	5	10	1316

Note: PBTpop is the abbreviated hatchery source and the digits indicate the spawn year, e.g. 08S is spawn year 2008. Adipose-clipped (HOR) and adipose unclipped (NOR) categories of fish could be separated for the Bonneville Dam and Ceremonial mixtures. All other fishery mixtures were mark-selective (HOR only).

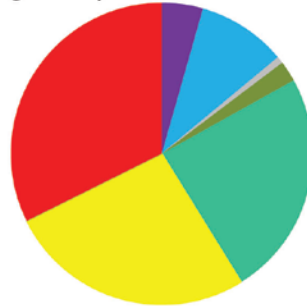
Stock proportions of the spring Chinook salmon fishery sources

Comparisons of stock composition across the different fishery sources generally produced results consistent with our analyses from previous years using the 2010-2011 Chinook salmon harvest. For the spring-run Chinook salmon harvest, we continue to observe large stock proportion differences in the Willamette River spring-run stock and they were most strongly associated with geographic location of the fishery mixture. This stock was present in the commercial and sport fisheries below Bonneville Dam (Regions A and B, Figure 5), but nearly absent at Bonneville Dam (Figure 5, 6) and the Ceremonial and Wind R. fisheries in Zone 6 (Figure 6). Within the commercial and sport fisheries, the Willamette R. stock showed greater proportions in the Region B relative to Region A (Figure 5), and this pattern also has been observed in prior years.

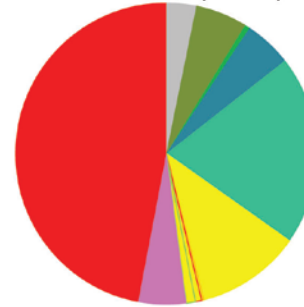
Lower R. commercial fishery
(Region B)



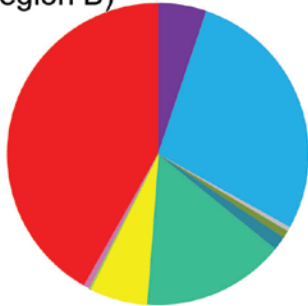
Lower R. commercial fishery
(Region A)



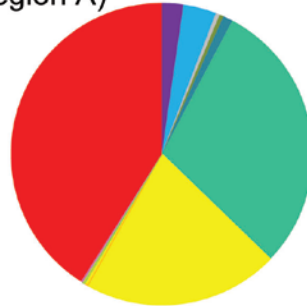
Bonneville Dam
(HOR)



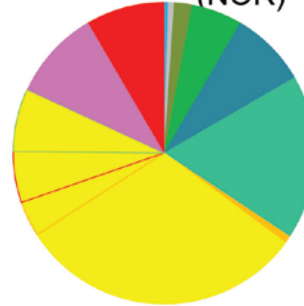
Lower R. sport fishery
(Region B)



Lower R. sport fishery
(Region A)



Bonneville Dam
(NOR)



Genetic stock group

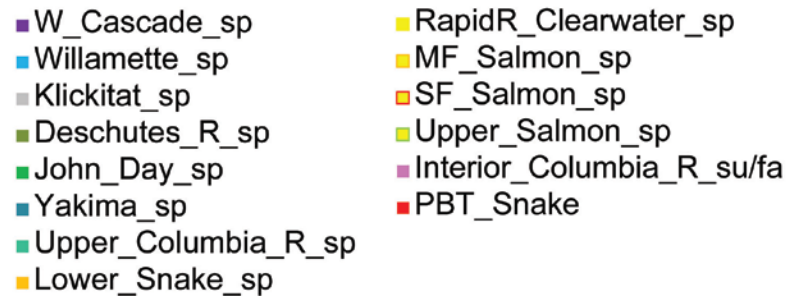


Figure 5. Comparison of stock proportions across all spring-run Chinook salmon fishery sources below Bonneville Dam.

Included are commercial, sport, and test fisheries in regions B (mouth of the Columbia River to Willamette River) and A (Willamette River to Bonneville Dam). Stock proportions are indicated by different colors that correspond with 14 genetic stock groups as shown.

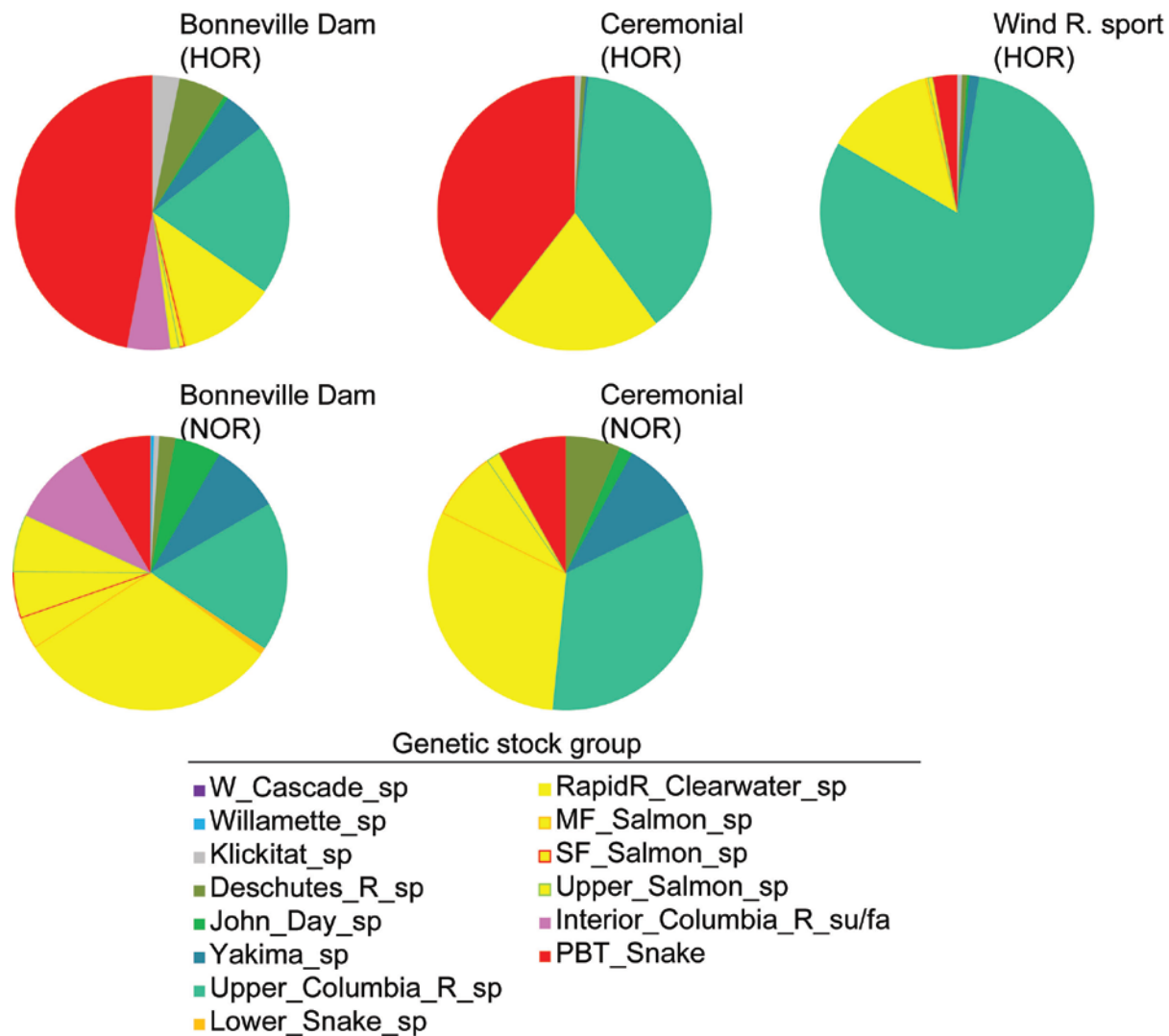


Figure 6. Comparison of stock proportions across all spring-run Chinook salmon fishery sources above Bonneville Dam.

Included are the Warm Springs Ceremonial Harvest (conducted exclusively in the Bonneville pool in 2012) and the Wind R. sport fishery (conducted around the mouth of the Wind R., a tributary in the Bonneville pool). Stock proportions are indicated by different colors that correspond with 14 genetic stock groups as shown.

The 2012 Chinook salmon harvest dataset was also examined for stock composition differences between adipose-clipped (hatchery-origin, HOR) and unclipped (natural-origin, NOR) Chinook salmon. These two groups could be analyzed separately for the Bonneville Dam and Ceremonial harvest mixtures, which sample both hatchery-marked and unmarked wild fish unlike the mark selective commercial and sport fisheries. One of the key differences between these NOR samples and most of the HOR samples was a relatively smaller proportion of PBT assigned Snake R. fish in the NOR samples (Figure 6). The one exception was the HOR sample from the Wind R. sport fishery (3%) which was less than half the portion of PBT-assigned fish in the NOR samples from Bonneville Dam (8%) and the Ceremonial (8%) harvest. The source hatcheries that contributed to these PBT-assigned NOR samples were generally representative of those that contributed to the HOR samples, however in the Bonneville Dam NOR mixture (Table 6), Sawtooth hatchery showed a relatively high proportion (27%) versus its proportion in the Bonneville Dam HOR mixture (8%). In general, the Wind R. sport fishery was different across some of the upriver stock proportions. A combined stock proportion of the PBT-assigned Snake R. fish and the Rapid R./Clearwater R. stock was less than half that of all the other fishery mixture sources (Wind R. = 16%). Further, the Upper Columbia R. stock proportion (81%) was more than twice that of any of the other fishery mixture sources.

The fishery samples were not taken from the same group of weeks. The lower river commercial and sport samples were harvested during the earliest weeks of the fishing season (10-17), the Ceremonial harvest was conducted over three weeks 17-19, and the Bonneville Dam and Wind R. harvest overlapped during the latter weeks (18-25) of the fishing season (Table 1). Therefore, if the Wind R. fishery were representative of the total run of Chinook salmon that was occurring during the time period of the fishery, it should appear most similar to the Bonneville Dam HOR sample. However, the Wind R. fishery was the most distinctive among all fishery samples in our dataset.

Accuracy testing of 180 SNP steelhead baseline

The 131 steelhead reference collections were grouped into 14 steelhead reporting groups including one outgroup, Quinault Hatchery on the Washington coast (Table 3, Figure 3). Results from the leave-one-out analysis, a relatively conservative accuracy test, showed 3/4 of the baseline collections (96) achieved above 75% correct individual assignment to reporting-group-of-origin. The following two of the 14 reporting groups showed an average correct individual assignment below 75%: Upper Columbia R. (UPPCOL, 62.9%) and Upper Salmon R. (UPSALM, 61.0%). Across all 14 reporting groups, an average correct assignment of 83.4% was achieved. These reporting groups were formed by merging some of the reporting groups that have been used in the past (Hess et al. 2012, Section 4).

Results from 100% mixture simulations for each of the baseline collections, showed that 91% of collections (119 of 131) produced estimates greater than 90% proportion of the correct reporting group (Table 3). When these proportions were averaged for each reporting group, all fourteen reporting groups yielded averages above 90%.

Parentage based tagging assignments of harvested steelhead

There were 514 tissues for steelhead in the zone 6 harvest mixture, however 5 tissues failed to genotype with less than 10% missing data, 22 tissues were identified as pure cutthroat (determined by positive genotypes for at least 2 of the 3 species diagnostic loci), and 14 tissues were found to be duplicate pairs of genotypes. We excluded 41 samples because of these issues, and for the duplicate pairs, we excluded one individual from each pair (14 total). These exclusions left 473 zone 6 harvest samples and 6 high-seas samples for analysis. Of these 479 total samples, 118 fish assigned to PBT source hatcheries, and one of these fish came from the high-seas collection (Table 7). The ten hatcheries were Wallowa, Tucannon, Little Sheep Cr., Lyons Ferry, Dworshak, Squaw, Oxbow, E.F. Salmon R., Pahsimeroi, and Sawtooth hatcheries. The one PBT-assigned fish from the high-seas harvest was a 1-ocean aged fish from Sawtooth hatchery (Table 8).

Table 7. Summary information on numbers and origins of steelhead that were assigned using a combination of GSI and PBT.

		Zone 6 harvest											
Genetic stock		Statistical week										High-	
		35	36	37	38	39	40	41	42	43	44	seas	Total
Natural-origin	LOWCOL								2			1	3
	SKAMAN	1	2									2	5
	WILLAM								1				1
	BWSALM		1									1	2
	KLICKR	1			1		1	1	5	1	1	1	12
	MGILCS	25	17	59	23	39	12	14	25	4	7		225
	YAKIMA	1		1		1			1				4
	UPPCOL	2	1	7	2	2	3		1	1	1		20
	SFCLWR			4	7	4	2	2	1		2		22

	UPCLWR	1		11	4	5	2	2	1	1		27	
	SFSALM			6	1	4		1				12	
	MFSALM	2	1	4	2			1				10	
	UPSALM	3	2	7		3	2		1			18	
Snake R. hatchery	WALL	1			1			1	1			4	
	TUCW	1		2	1		1					5	
	LSCR		1									1	
	LYON			1		1						2	
	DWOR	1	3	7	7	8	7	31	4	2	2	72	
	SQUW			1	1			1				3	
	OXBO		2	1		1			2			6	
	EFSW				3			1				4	
	PAHH		3	2		2			1			8	
	SAWT		2	2	1	6		1			1	13	
Excluded	cutthroat	1	1	2				18				22	
	duplicate		7	4		1		2				14	
	failure			3					2			5	
Total		40	43	124	54	77	30	76	48	9	13	6	520

Note: Statistical week 35 begins 8/20/2012 and week 42 ends on 10/14/2012. Natural-origin individuals were assigned using GSI to the stock with the highest probability (best estimate). Snake River hatchery stocks were assigned using PBT and include broodyears from 2008 to 2010. The zone 6 fishery samples could be broken into weekly strata, but the collection dates were not available for the high-seas sample.

Table 8. Summary information on numbers, spawn-year, and origins of steelhead from each fishery mixture that assigned to the PBT baseline

PBT source hatchery	PBTpop	Tagging Rate	Fishery:	Zone	High-	Total
				6	seas	
LSRCP/ODFT - Little Sheep Cr. FH	LSCR10S	89.1%		1		1
LSRCP/WDFW-Lyons Ferry	LYON09S	95.5%		1		1
	LYON10S	100.0%		1		1
LSRCP/WDFW-L.F. (Tucannon)	TUCW09S	84.6%		2		2
	TUCW10S	77.9%		3		3
LSRCP/ODFW-Wallowa F.H.	WALL09S	91.6%		4		4
LSRCP/IDFG/USFWS Dworshak/C.W.	DWOR08S	72.9%		4		4
	DWOR09S	97.4%		67		67
	DWOR10S	97.1%		1		1
LSRCP/IDFG Sawtooth (USB/Squaw)	SQUW10S	95.6%		3		3

LSRCP/IDFG Sawtooth (EFSR)	EFSW09S	100.0%	3		3
	EFSW10S	100.0%	1		1
Idaho Power/IDFG, Oxbow F.H.	OXBO09S	88.0%	6		6
Idaho Power/IDFG, Pahsimeroi F.H.	PAHH09S	95.1%	3		3
	PAHH10S	97.1%	5		5
LSRCP/IDFG Sawtooth (IDFG & SBT)	SAWT09S	99.6%	7		7
	SAWT10S	99.8%	5	1	6
	PBT total		117	1	118
	Unassigned total		397	5	402
	Grand total		514	6	520

Note: PBTpop is the abbreviated hatchery source and the digits indicate the spawn year, e.g. 08S is spawn year 2008. The tagging rate indicates the percent of broodstock genotyped from a particular hatchery for that spawn year.

Stock composition of the steelhead fishery sources

The high-seas harvest likely included many steelhead stocks that were not represented in our baseline, which is almost exclusively from the Columbia River. This fact can lead to spurious GSI assignment and so requires some caution for interpretation of the stock assignments. The Lower Columbia, Skamania, Big White Salmon, and Klickitat stocks were all represented in this high-seas harvest. However, the assignment to the Big White Salmon is especially questionable because it is a stock of very low abundance that would not be expected to be observed. What is certain is that 1/6 of this collection could be assigned to a Snake R. hatchery, which was very surprising given the small sample size from this harvest.

The zone 6 unclipped steelhead harvest was comprised of nearly 25% of hatchery-origin fish (Figure 7). There are many Snake River hatcheries that release unclipped smolts, and so this proportion is in line with expectations. The largest proportion of this harvest was derived from the MGILCS reporting group (48%), which is also the most broadly geographically distributed and includes portions of the Snake River basin. All other stocks were less than 10%, and only the S.F. Clearwater (5%) and upper Clearwater R. (6%) were above 5% proportion.

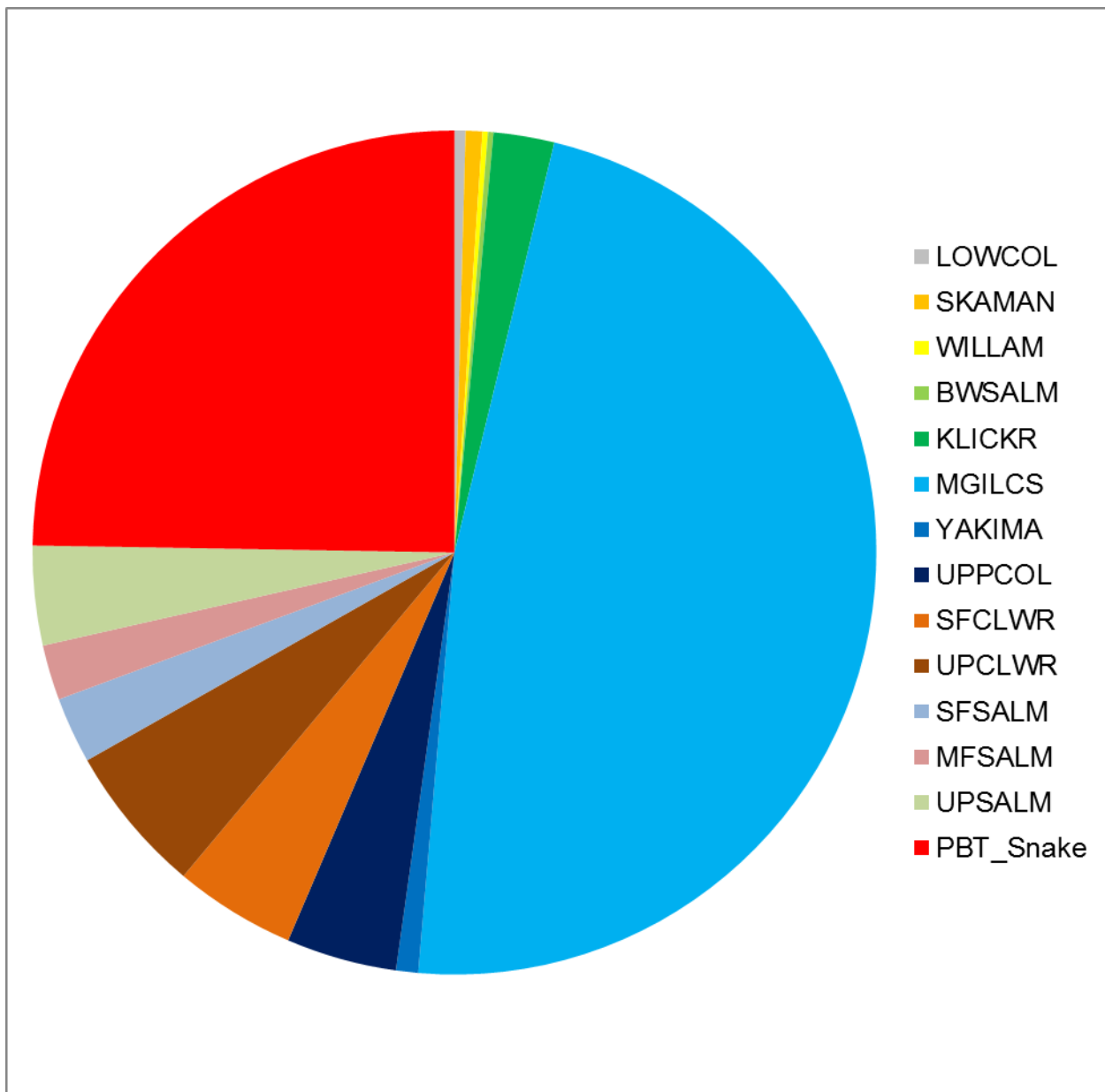


Figure 7. Stock proportions of the zone 6 adipose unclipped steelhead based on the “compiled” results from combining PBT and GSI analyses.

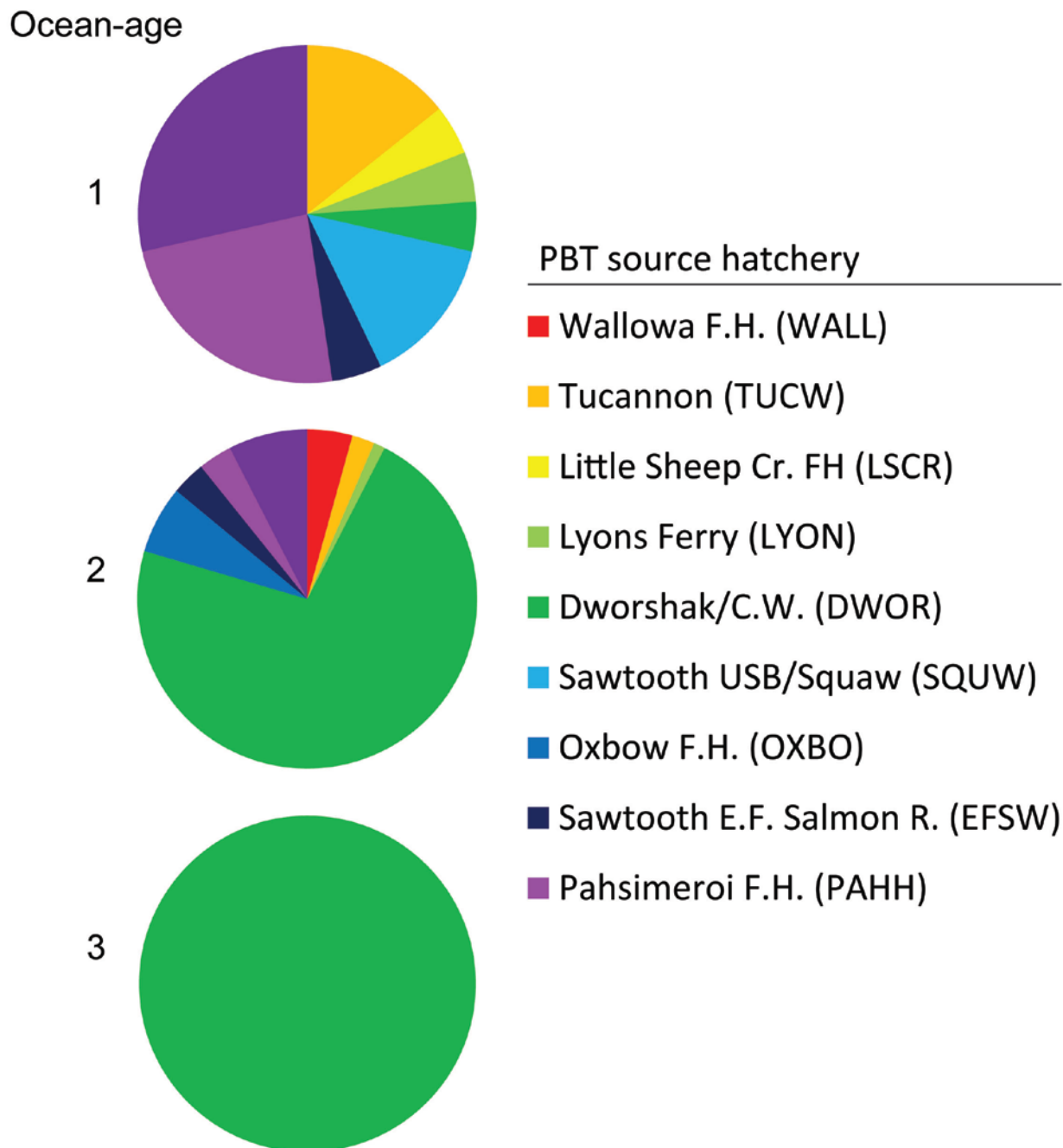


Figure 8. Stock proportions of PBT-assigned 1-, 2-, and 3-ocean-age steelhead harvested in the zone-6 fishery.

The PBT assigned fish from the zone 6 unclipped steelhead harvest were aged according to the year in which their parents were spawned. The 1-ocean age fish were the most representative of all PBT source hatcheries, but dominated by the Sawtooth (29%) and Pahsimeroi (24%) hatcheries (Figure 8). The 2-ocean age fish were mostly from Dworshak hatchery (72%), and all four 3-ocean fish were from the Dworshak hatchery.

Discussion

Management implications

This study demonstrates the substantial improvement in accuracy and increase in information that can be achieved by using two types of analyses in combination, genetic stock identification (GSI) and parentage based tagging (PBT), for estimating stock composition of mainstem Columbia River Chinook salmon and steelhead fisheries. Our Chinook salmon and steelhead GSI baselines discriminate 17 and 14 reporting groups, respectively. Now, we can further discriminate the Snake River hatchery origin Chinook salmon and steelhead to provide information on the year an individual was spawned, and the hatchery in which the parents were spawned. The PBT baseline is continually expanding and for the first time, we were able to assign spring Chinook from the Klickitat hatchery, which is the only hatchery outside of the Snake River. We analyzed a small number of fall-run Chinook salmon that all had coded-wire tags and were used to test the accuracy of the fall-run Chinook salmon reporting groups. The three reporting groups West Cascade, Spring Creek group tule, and interior Columbia R summer/fall-run showed greater than 90% concordance with the coded wire tags. If this level of discrimination is useful to managers, the baseline is sufficient to provide accurate stock composition. However, currently coded wire tags can more finely discriminate the interior Columbia R summer/fall-run reporting group, and so unless fisheries managers have specific questions that GSI can address, we will reduce our emphasis on fall-run Chinook salmon. When the expansion of the PBT baseline has made it possible to assign returning adult fall-run Chinook salmon (~2015), we will begin analyzing larger portions of these samples again.

In this report, we had a specific objective to test which was initiated following a change in the fishing boundary of the Wind R. sport fishery. By extending this boundary further away from the mouth of the Wind R. and into the mainstem of the Columbia R., there was concern whether the stock composition of this harvest would show higher proportions of upriver stocks than in previous years. We were able to show that the number of PBT-assigned Snake R. fish that were harvested in the Wind R. sport fishery was less than all other fishery samples we analyzed, including the natural-origin sample from Bonneville Dam that was collected during the same set of weeks. We will likely continue to monitor this fishery in future years and examine how consistent this stock composition remains.

We observed consistent patterns in stock proportions of spring-run Chinook salmon harvest in the lower mainstem of the Columbia River. The spring-run Chinook salmon harvested in the commercial and sport fishery were primarily composed of three adipose-clipped stocks: Rapid River Hatchery/Clearwater R., Upper Columbia R. (i.e., Carson stock), and Willamette R. The PBT-assignments made it possible to further discriminate fish by their hatchery-of-origin (ten total hatcheries represented), which is a vast improvement over the GSI assignments that would have mostly been to the Rapid River Hatchery/Clearwater R. stock (a very broadly distributed reporting group). In both the commercial and sport fisheries, the Willamette River stock appears in highest abundance in region B, which is closer to the mouth of the Columbia R. This result continues to follow expectations as the mouth of the Willamette R. is located at the upstream boundary of region B, and so most fish destined for this tributary will only be captured in region B.

The incorporation of steelhead was a novel aspect of this year's harvest analysis. There were two fishery mixtures we included in the analysis, the bulk of the samples were unclipped steelhead from the zone 6 tribal fishery and a small portion of samples were taken by a high-seas

fishing vessel as by-catch. We demonstrated that we could in fact assign one of the high-seas fish to Snake River hatchery parents, which may support devoting some resources to a more thorough examination of high-seas harvest of Snake River steelhead in the future. The unclipped steelhead from zone 6, turned out to be a quarter hatchery-origin fish from the Snake R. Half of these fish were from the larger reporting group that spans much of the middle Columbia R. and lower Snake R. tributaries (MGILSC), and another quarter were mostly from Snake R. (natural-origin).

Future directions

Basic information is available for each individual including collection date and location, length, and genetic sex of fish. The incorporation of PBT into our genetic stock analysis will help to characterize the biological attributes of particular hatcheries that are contributing to each fishery harvest. To make this information relevant to fisheries management, it will be important to establish a protocol for expanding these PBT stock proportions and estimate total abundance of harvested stocks. This protocol is currently in progress and will be available for future years.

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Section 4: Characterization of steelhead and Chinook and sockeye salmon run-timing and abundance at Bonneville Dam

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, habitat degradation, hydropower, hatchery practices, and over-harvesting. 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 genetic stock identification (GSI) analyses 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 conduct a study that samples a representative mixture of all hatchery- and natural-origin stocks at a fixed location to accurately estimate abundance and characterize run-timing distributions of stocks. In addition to information on abundance and run-timing, biological data including fork length and age can be examined with estimated stock of origin in order to characterize life history differences among stocks. This type of examination is especially important for steelhead, which has been managed using two life-history categories, A- and B-run. These life-history categories are defined by run-timing at Bonneville Dam (e.g. B-run arrives after Aug. 25th), fork length (e.g. B-run is greater than 78 cm), and ocean age (e.g. B-run spends 2 or more years in saltwater) and all of these types of data have been collected for steelhead in this study.

Here we analyze fish across the entire run of steelhead and Chinook and sockeye salmon from April to October to estimate temporally stratified proportions of stocks and extrapolate abundance using a daily census that is conducted at the Bonneville Dam fish counting window. We examine steelhead and Chinook salmon using two sets of species-specific SNP assays for a combined total of 192 loci per set. GSI of sockeye salmon requires fewer markers, and a set of 96 SNP loci can accurately resolve the fewer number of sockeye stocks that are present in the basin relative to other salmonids. Although there are some methodological differences among these species-specific applications (e.g. different temporal strata), the general approach to estimating abundance and characterizing run-timing distributions was applied consistently across species. For all three species, we demonstrate the accuracy of the genetic baselines that we utilized for GSI, though these details are provided for Chinook salmon and steelhead in the previous section (Section 3). Previous reports have demonstrated application of SNP loci for GSI in both steelhead and Chinook salmon, but this report represents the first analysis using SNP loci for GSI analysis of Bonneville Dam sockeye salmon mixtures. We compare the genetic results for sockeye salmon with the results from PIT-tag stock composition analysis.

Analysis of the 2011 dataset by Hess et al. (2012) was the first year we were able to apply an additional genetic tool, referred to as Parentage Based Tagging (PBT), to assign a portion of Snake River hatchery-origin spring-run Chinook salmon and summer-run steelhead back to their hatchery parents (Steele et al. 2011). This new and powerful genetic tool provides the opportunity to obtain additional types of data including accurate age of fish, quantification of the number of unmarked (non-adipose clipped) hatchery fish, and precise assignments of fish to

their source hatchery. The ability of PBT to identify a fish's source hatchery is a much finer spatial distinction as compared to the stock identity provided by GSI. However, these tools can provide the greatest benefit when applied in combination, as GSI has the ability to provide information on wild fish and hatchery fish that originate from outside the Snake River basin. Steelhead passing Bonneville in 2012 represent the first year in which the Snake River PBT program can provide information on the three major year classes (1-, 2-, and 3- ocean ages) of steelhead and both jacks and 4 year old for Chinook salmon. We have taken steps to integrate the information of PBT and GSI to provide the greatest amount of stock information ever available for hatchery and wild steelhead and Chinook salmon passing Bonneville Dam.

The aim of this study was to use GSI and PBT to estimate stock abundance and discriminate Columbia River steelhead and Chinook and sockeye salmon stocks according to their peak run-timing. Since Bonneville Dam is the most downstream dam on the Columbia River, the fishery 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 abundance of steelhead and Chinook and sockeye salmon and provide this data to fisheries managers.

Methods

Sample Collection

Tissue samples were obtained from adult steelhead (n=1,483), Chinook (n = 3,485) and sockeye salmon (n=1,647) adults in 2012 during migration runs at Bonneville Dam. Based on numbers of fish collected, samples were pooled into weekly strata for Chinook (Table 1) and sockeye salmon (Table 2) and monthly strata for steelhead (Table 3) spanning the majority of the run-year from April to October. 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 on Whatman filter paper (Lahood et al. 2008) before being shipped to the Hagerman Fish Culture Experiment Station for DNA extraction and analysis. In addition, scales are collected for estimating the number of years spent in freshwater and saltwater for each fish. 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 that were DNA sampled or tallied for abundance at Bonneville Dam in 2012.

			Sample						
	Statistical	Fish	Ad-				Total	rate	
			clipped(HOR)		unclipped(NOR)				
			Non-PBT	PBT	Non-PBT	PBT			
Management period	week	count							
	Spring	18	15411	57	37	23	3	120	0.8%
		19	21197	89	82	44	6	221	1.0%
		20	70676	108	131	102	9	350	0.5%
		21	34677	82	64	83	6	235	0.7%
		22	10047	33	40	51	4	128	1.3%
		23	14324	27	30	65	8	130	0.9%
		24	12332	35	38	34	5	112	0.9%
	25	17576	31	19	36		86	0.5%	
	Summer	26	15221	15	10	30	1	56	0.4%
		27	11942	18	2	15		35	0.3%
		28	10416	24	2	20		46	0.4%
		29	8563	31	3	25		59	0.7%
		30	5550	12		10		22	0.4%
31		4396	12		4		16	0.4%	
Fall	32	4309	7		14		21	0.5%	
	33	5582	4		7		11	0.2%	
	34	7404	6		5		11	0.1%	
	35	22091	38		101		139	0.6%	
	36	80169	79		158		237	0.3%	
	37	107671	75		191		266	0.2%	

38	116856	101		216	317	0.3%
39	64286	108		261	369	0.6%
40	34593	59		168	227	0.7%
41	16249	41		121	162	1.0%
42	8344	27		82	109	1.3%
Total/avg	719882	1119	458	1866	42	3485 0.6%

Note: For reference, statistical week 18 is 4/23/12-4/29/12 and 42 is 10/08/12-10/14/12. “Fish count” is based on tallies of Chinook salmon adults and jacks provided by the Fish Passage Center (<http://www.fpc.org>) as observed at their fish counting window. Genetic sample numbers (N) above 20 are highlighted in gray and these weeks were the only ones deemed sufficient to estimate stock abundance with GSI. The total sum of all samples for a given week was used to calculate sample rate. The management periods approximate the date ranges from April to June 15th, June 16th to July 31st, and August 1st to October which are used to categorize spring-, summer-, and fall-run Chinook salmon.

Table 2. Sample numbers by weekly strata for sockeye salmon that were DNA sampled or tallied for abundance at Bonneville Dam in 2012.

Statistical week	Fish count	Sample	
		N	rate
20	1	0	0.0%
21	0	0	-
22	6	0	0.0%
23	598	0	0.0%
24	6703	65	1.0%
25	53481	201	0.4%
26	148375	408	0.3%
27	192052	426	0.2%
28	88670	374	0.4%
29	23089	118	0.5%
30	1917	24	1.3%
31	554	15	2.7%
32	150	0	0.0%
33	48	0	0.0%
34	21	0	0.0%
35	2	0	0.0%
36	0	0	-
37	3	0	0.0%
38	0	0	-
39	3	0	0.0%
Total†	514841	1631	0.3%

Note: For reference, statistical week 20 is 5/07/12-5/13/12 and 39 is 9/17/12-9/23/12. “Fish count” is based on tallies of sockeye salmon adults provided by the Fish Passage Center (<http://www.fpc.org>) as observed at their fish counting window. Genetic sample numbers (N) above 15 are highlighted in gray and these weeks were the only ones deemed sufficient to estimate stock abundance with GSI. The # of samples for a given week was used to calculate sample rate. †Indicates this total represents the sum of samples and total count during weeks 24-31 in which genetic sample sizes were deemed sufficient.

Table 3. Sample numbers by monthly strata for steelhead that were DNA sampled or tallied for abundance at Bonneville Dam in 2012.

		Fish count				Sample					
		Statistical week	Statistical month	Ad- clipped	unclipped	Ad-clipped(HOR)		unclipped(NOR)		Total	rate
						Non-PBT	PBT	Non-PBT	PBT		
Management period	Skamania	18		181	67	0		1		1	0.40%
		19	19_22	163	73	3		1		4	1.69%
		20		211	70	2	2	2		6	2.14%
		21		324	162	2		6		8	1.65%
		22		282	101	2	5	2		9	2.35%
		23	23_26	486	161	9	3	1		13	2.01%
		24		629	235	8	1	2		11	1.27%
		25		1102	455	3	5	3		11	0.71%
		26		1584	775	4	1	4		9	0.38%
	Summer	27		2243	1348	3	3	5	2	13	0.36%
		28	27_30	3214	2853	3	6	7		16	0.26%
		29		6618	6458	18	23	43	1	85	0.65%
		30		11294	10286	17	44	64	4	129	0.60%
		31	31_34	14941	11242	26	72	74	5	177	0.68%
		32		14982	9659	12	65	61	5	143	0.58%
		33		15716	8458	13	45	34	3	95	0.39%
		34		13792	6592	6	19	14		39	0.19%
		35	35_38	7818	3975	21	79	53	5	158	1.34%
		36		11077	4628	14	90	33	8	145	0.92%
		37		9831	3936	6	28	13	7	54	0.39%
		38		9242	3908	3	22	7	4	36	0.27%
		39	39_42	7764	2988	8	51	14	9	82	0.76%
		40		5346	2043	3	46	8	13	70	0.95%
		41		3274	1240	1	56	12	19	88	1.95%

	42	1927	801	4	56	14	7	81	2.97%
Total/avg		144041	82514	191	722	478	92	1483	0.65%

Note: For reference, statistical week 18 is 4/23/12-4/29/12 and 42 is 10/08/12-10/14/12. “Fish count” is based on tallies of adipose-clipped and unclipped adult steelhead provided by the Fish Passage Center (<http://www.fpc.org>) as observed at their fish counting window. Genetic sample numbers (N) above 15 per stratum are highlighted in gray and these statistical months were the only ones deemed sufficient to estimate stock abundance with GSI. The total sum of all samples for a given week was used to calculate sample rate. The management periods approximate the date ranges from April 1st to June 30th and July 1st to October 31st which are used to categorize Skamania and summer steelhead, respectively.

Molecular markers

We used both the GSI-96 and PBT-96 SNP panels (Hess et al. 2012; Section 1, Appendix 1) for a total of 192 SNP loci to genotype Chinook salmon mixtures, and we removed six loci for reasons described in Section 3 which leaves 186 for all GSI analyses. For steelhead, we also used the GSI-96 and PBT-96 SNP panels (Appendix 2, Section 2, Hess et al. 2011) for a total of 192 SNP loci. However, we removed three loci that are used to detect cutthroat hybrids, the sex determination marker, and six involved in linkage resulting in a combined total of 180 SNP loci for GSI applications (Section 2-3). For sockeye, we genotyped fish with the 96 SNP sockeye loci (Section 2), however we used 95 loci for GSI after having excluded locus *One_UCA-24* which had some HWE deviations (Section 2) and was missing entirely in the Redfish Lake collection.

Statistical analyses

Steelhead and salmon comprised of several upriver mixed stocks were non-lethally sampled at the Adult Fish Facility (AFF) located on the northern most (Washington shore) ladder of Bonneville Dam. Sampling occurred at the AFF from April through October, 4-5 days per statistical week (except when reduced due to restrictions on trap use or low run size at the beginning and end of the run), and for 4-6 hours per day. A picket weir was used to divert migrating fish, ascending the Washington shore fish ladder, into the AFF collection pool. An attraction flow was used to draw fish through a false weir where they were selected for sampling. After sampling was completed and fish recovered from the anesthetic, they were returned to the Washington Shore Fish ladder above the picket weir. Samples from each fish included caudal fin tissue for genetic analyses, scales for aging, and metadata associated with each fish including date and fork length to the nearest 0.5 cm.

Stock proportions of these mixed collections were estimated using a recently implemented Bayesian inference module provided by Eric Anderson to the program *gsi_sim* (Anderson et al. 2008) that also generated 1,000 posterior probabilities for each stock proportion of a fishery mixture using the following parameters to run the Markov chain Monte Carlo method: Burn-in = 10,000, number of sweeps = 100,000, and thinning interval = 100. These probabilities were summed across all time strata (per week or month) mixtures for a given year and then the 95% distribution was used as the confidence interval for each stock's point estimate. The program ONCOR v1.0 (available at <http://www.montana.edu/kalinowski>) was also used to assign mixture individuals according to highest probability ("best estimate") to the baseline reporting groups to provide a way to utilize individual metadata such as sex, PIT-tag detections, length, adipose marks, and age composition.

Abundance was estimated for each stock using the following equation:

$$N = \sum_i B_i G_i$$

where N is the estimated abundance for a particular reporting group, i is a statistical week, B_i is the number of fish counted passing Bonneville Dam during week i , and G_i is the proportion of fish sampled at the Adult Fish Facility (AFF) during week i that assigned to the reporting group for which abundance was being estimated. G_i was used only for those consecutive statistical weeks (or months for steelhead) in which a minimum of 15 fish were sampled. For example, statistical weeks with sufficient sampling for Chinook salmon occurred during (weeks 18 to 29) from the timeframe of April 23rd to July 15th, and the next time window with sufficient sampling occurred during weeks 35 to 42 which equates to dates August 20th to October 14th. The period from July 16 to August 19 coincides with the highest water temperatures at Bonneville Dam and the adult trap is shut down when temperatures exceed 22.2° C while trap operations are more restricted between 21.1° and 22.2° C. The time window of weeks (or months for steelhead) with insufficient sampling ($n < 15$) was omitted from analyses estimating abundance and run-timing. The sample rate of adult salmonids at the AFF varies during the course of the run for each

species (e.g. for Chinook salmon the rate varied between 0.1%-1.3% in 2012, Table1), therefore the abundance equation relies on weekly stock proportions to correct for this uneven sampling bias. If the sampling rate were even, the alternative approach would be to use a single stock proportion from a total mixture sampled across the entire run. The component B_i of the equation included the tallies provided by the Fish Passage Center (<http://www.fpc.org>) as observed at the Bonneville Dam fish counting window. We characterized the distributions of run-timing for each stock by multiplying G_i uniformly across daily fish counts for week i and observed which days marked the passing of 5%, 25%, 50%, 75%, and 95% of total stock abundance. For Chinook salmon and steelhead, each year's samples were first divided into two datasets of hatchery- and natural-origin fish based on absence and presence of an adipose fin, respectively. Analyses were conducted on each of these datasets to estimate stock abundance and run-timing distributions for all hatchery and natural-origin steelhead stocks separately.

Estimation of Chinook salmon stock composition utilized the baseline and 17 reporting groups described in Section 3, Table 2. Estimation of steelhead stock composition utilized the baseline and 14 reporting groups described in Table 3. Sockeye stock composition utilized a baseline comprised of four reference collections discussed in this Section. Similar to the accuracy testing that was conducted with the Chinook salmon and steelhead baselines in Section 3, we performed both Leave-one-out assignment tests and 100% mixture simulations for the sockeye baseline using ONCOR.

Parentage based tagging (PBT) analysis was used to identify the source hatcheries of 3- and 4-year old Klickitat and Snake River spring-run Chinook salmon and 3-, 4-, and 5- year old (spawn age) Snake River steelhead. Genotypes of parents and offspring were analyzed with SNPPITv1.0, a software program developed for large PBT datasets based on SNP markers (Anderson 2010). Analyses with SNPPIT were performed separately on the spawn year 2008-2009 (SY08-SY09) Chinook salmon and spawn year 2008-2010 (SY08-SY10) steelhead parental broodstock baselines (Steele et al. 2011), to assign any offspring that were included in the 2012 Bonneville Dam mixtures of Chinook salmon and steelhead, respectively. PBT assignments were based on marker exclusion and a LOD score threshold of 14 was used to ensure high confidence in all parent matches. Due to this high confidence in the identity of an individual's hatchery parents, we were able to accurately age these Chinook salmon and steelhead. Further we quantified the percent of unmarked (non-adipose clipped) fish that were hatchery-origin using PBT assignments. Finally, similar to the methods we used to estimate abundance and run-timing of GSI reporting groups, we estimated these metrics at the scale of PBT hatchery sources. We first estimated the total abundance of all adipose-clipped PBT-assigned fish using the proportion of the adipose-clipped samples that were found to assign to any of the PBT source hatcheries multiplied by total adipose clipped abundance. The proportion of PBT-assigned samples was expanded using the average tagging rate across all PBT source hatcheries. This procedure was repeated using the adipose-unclipped PBT-assigned fish, but in this case, these proportions were applied to the total adipose-unclipped abundance. Next, we combined the estimated adipose-clipped and unclipped PBT-assigned fish abundance to provide a total PBT-assigned fish abundance. Finally, we used the number of PBT assignments that were observed for each PBT source hatchery within each time strata (weekly for Chinook salmon, and monthly for steelhead) to calculate each PBT source hatchery's weekly proportion. These weekly proportions were expanded for each PBT source hatchery's specific tagging rate, and then multiplied by the total PBT-assigned fish abundance. In parallel with the calculations to yield a total PBT-assigned fish

abundance (expanded by average tagging rate), the total abundance estimates for the hatchery-origin (non-PBT-assigned) and natural-origin (non-PBT-assigned) were reduced to account for the expanded PBT-assigned fish in either category.

Results

Estimated abundance of Chinook salmon stocks in 2012

There were nine adipose clipped Chinook salmon stocks passing Bonneville Dam that we estimated abundance greater than 1,000 fish in the season (Table 3, Figure 1). The nine major stocks in order of increasing magnitude were Klickitat R. spring (4,300), Yakima R. spring (6,500), Deschutes R. spring (6,600), Spring Creek group tule fall (6,800), South Fork Salmon R. spring/summer (6,900), upper Salmon R. spring/summer (9,900), upper Columbia R. spring (28,000), Rapid R./Clearwater R. spring (69,700), and interior Columbia R. summer/fall (153,100). These estimates include abundance estimated from PBT-assigned fish that were mostly adipose clipped, however a portion of the PBT-assigned fish were found to have their adipose intact. Therefore PBT assignments improved our ability to accurately identify hatchery-origin fish and estimate total stock abundance (Figure 1). Further, using PBT assignments we can now provide abundance, run-timing, and size and age information at a spatial scale of a particular hatchery (Table 5, Figure 2). At the level of hatchery, we estimated less than 1,000 fish for Grande Ronde GRUW, Johnson Creek JHNW, Nez Perce Tribal Fish Hatchery NPFH, Pahsimeroi PAHH, and Catherine CTHW hatchery stocks. However, the following ten hatchery stocks produced greater than 1,000 fish: Lostine LSTW (1,400), Klickitat KLKR (2,900), Lookingglass LOOK (3,300), Powell POWP (4,800), Imnaha IMNW (5,200), McCall MCCA (6,200), Clearwater CLWH (6,200), Sawtooth SAWT (7,200), Dworshak DWOR (12,300), and Rapid RAPH (23,200).

Table 4. Basic information on run-timing distributions of hatchery-origin (based on adipose-clips and PBT assignments) Chinook salmon stocks passing Bonneville Dam.

Reporting group	mean	Estimated abundance				Run-timing distribution						
		Total	Before	June 16 -	After	1st	3rd	5th	95th	Median	Interquartile	
		95% C.I.	15-Jun	July 31	August 1	Median	quartile	quartile	percentile	percentile	date	range (d)
W_Cascade_sp	0	0 - 489	0	0	0	-	-	-	-	-	-	-
W_Cascade_fa	42	19 - 991	0	0	42	264	262	265	261	267	20-Sep	3
Willamette_sp	0	2 - 599	0	0	0	-	-	-	-	-	-	-
Spring_Cr_Group_Tule	6822	4170 - 9780	0	0	6822	250	246	253	240	259	6-Sep	7
Klickitat_sp	4272†	3345 - 5716	3781	491	0	148	136	160	131	179	27-May	24
Deschutes_R_sp	6587	4752 - 8318	6177	410	0	136	130	143	116	177	15-May	13
John_Day_sp	831	27 - 2234	831	0	0	130	129	134	125	154	9-May	5
Yakima_sp	6497	4742 - 8454	6156	340	0	135	130	140	123	177	14-May	10
Upper_Columbia_R_sp	27980	26184 - 33666	27714	266	0	130	125	135	115	152	9-May	10
Lower_Snake_sp	208†	208 - 559	208	0	0	130	129	132	128	134	9-May	3
RapidR_Clearwater_sp	69654†	66472 - 73097	66193	3461	0	130	127	136	115	167	9-May	9
MF_Salmon_sp	195	27 - 1179	195	0	0	117	115	124	114	127	26-Apr	9
Chamberlain_Cr_sp	106	9 - 647	106	0	0	152	151	154	149	155	31-May	3
SF_Salmon_sp	6943†	6107 - 7847	5298	1646	0	156	148	167	132	192	4-Jun	19
Upper_Salmon_sp	9908†	8802 - 11524	8549	1359	0	143	133	158	127	174	22-May	25
Interior_Columbia_R_su/fa	153136	147198 - 154367	5460	20202	127474	252	242	260	170	272	8-Sep	18
Columbia_Rogue	0	0 - 294	0	0	0	-	-	-	-	-	-	-
Total	293181		130668	28175	134338							

Note: These summary statistics of run-timing distributions were calculated using a method to estimate abundance of each stock based on weekly stock proportions and total numbers of Chinook salmon that were observed passing Bonneville Dam at the fish counting window. These run-timing distributions are plotted in Figure 3. †Combined with PBT estimated abundance.

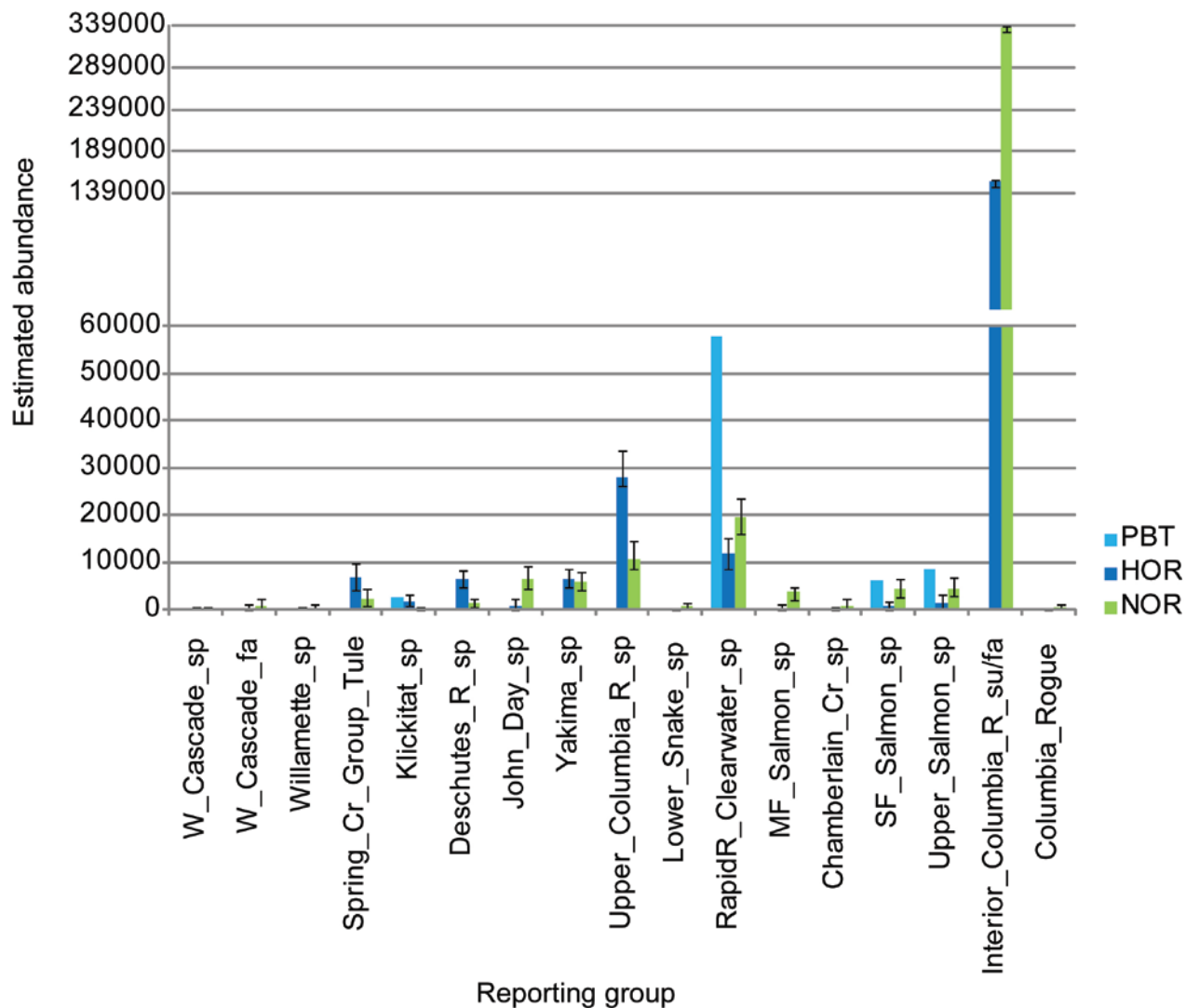


Figure 1. Estimated abundance of reporting groups for Chinook salmon passing Bonneville Dam in 2012. This data was generated by splitting the daily tallies of Chinook salmon at the Bonneville Dam fish counting window into three categories: adipose clipped fish and non-adipose clipped fish and all fish that were PBT-assigned (combined adipose and non-adipose clipped fish). Reporting group proportions for each of these three categories were estimated on weekly pooled mixtures of Chinook salmon passing Bonneville Dam and then these proportions were multiplied with weekly tallies of Chinook salmon at the Bonneville Dam fish counting window. Abundance that was observed during weeks 30 to 34 was not accounted for due to insufficient sample numbers of Chinook salmon.

Table 5. Basic information on run-timing distributions of unclipped and clipped PBT-assigned (hatchery origin) Chinook salmon stocks passing Bonneville Dam.

Broodstock collection	Tagging rate	Total	Estimated abundance			Run-timing distribution							Sex and size					
			Before 15-Jun	June 16 - July 31	After August 1	Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Interquartile range (d)	Female N	Female Length	Male N	Male Length	Total N	Total Length
CLWH08S	97.0%	6080	6080	0	0	130	128	134	116	156	9-May	6	25	71.0	12	72.6	37	71.5
CLWH09S	94.1%	201	201	0	0	156	146	160	142	162	4-Jun	14	-	-	2	51.5	2	51.5
CTHW08S	100.0%	947	947	0	0	130	128	132	115	134	9-May	4	3	68.7	2	72.5	5	70.2
DWOR08S	97.6%	11743	11743	0	0	130	127	134	115	151	9-May	7	46	73.3	28	73.7	78	73.3
DWOR09S	95.3%	682	682	0	0	136	133	138	129	140	15-May	5	-	-	4	49.9	4	49.9
GRUW08S	100.0%	190	190	0	0	150	146	153	142	155	29-May	7	2	71.0	-	-	2	71.0
IMNW08S	99.2%	4787	3182	1604	0	163	148	169	130	179	11-Jun	21	15	74.8	14	73.0	29	73.8
IMNW09S	98.4%	322	251	71	0	165	161	167	156	169	13-Jun	6	-	-	2	52.0	2	52.0
JHNW08S	100.0%	386	111	274	0	171	155	174	150	176	19-Jun	19	1	76.0	1	-	2	76.0
KLKR08S	93.3%	1984	1909	75	0	147	138	158	135	167	26-May	20	5	71.1	10	67.5	15	68.7
KLKR09S	99.3%	724	372	352	0	162	156	180	135	183	10-Jun	24	-	-	4	60.0	4	60.0
LOOK08S	98.6%	2961	2961	0	0	130	127	135	115	158	9-May	8	12	69.0	7	67.3	20	68.0
LOOK09S	97.2%	327	255	72	0	165	154	167	150	169	13-Jun	13	-	-	2	49.5	2	49.5
LSTW08S	94.5%	1253	434	819	0	171	160	175	146	189	19-Jun	15	5	71.3	2	76.0	7	73.6
LSTW09S	89.3%	88	88	0	0	145	143	146	142	148	24-May	3	-	-	1	50.5	1	50.5
MCCA08S	97.6%	5096	4100	996	0	156	149	165	137	175	4-Jun	16	23	75.2	17	75.3	40	75.2
MCCA09S	95.3%	601	226	375	0	191	165	194	144	197	9-Jul	29	-	-	4	47.8	4	47.8
NPFH08S	97.9%	440	440	0	0	127	117	130	114	134	6-May	13	3	67.3	-	-	3	67.3
PAHH08S	98.4%	632	332	300	0	167	154	186	144	190	15-Jun	32	2	67.0	2	65.0	4	66.3
POWP08S	98.6%	4833	4762	71	0	131	129	135	122	161	10-May	6	16	73.6	13	76.4	30	75.0
RAPH08S	98.4%	22155	22155	0	0	129	125	133	115	139	8-May	8	80	69.1	59	70.4	148	69.7
RAPH09S	96.8%	871	871	0	0	133	129	139	125	160	12-May	10	1	71.0	5	48.4	6	52.2
SAWT08S	99.0%	6877	6111	766	0	142	135	157	129	172	21-May	22	27	74.6	21	69.4	48	72.2
SAWT09S	98.0%	843	563	280	0	148	142	171	129	175	27-May	29	-	-	6	47.9	6	47.9
TUCW08S	89.7%	228	228	0	0	130	129	132	128	134	9-May	3	1	67.5	-	-	1	67.5
Total		75254	69199	6055	0								267	71.7	218	69.1	500	70.5

Note: These summary statistics of run-timing distributions were calculated using a method to estimate 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 4.

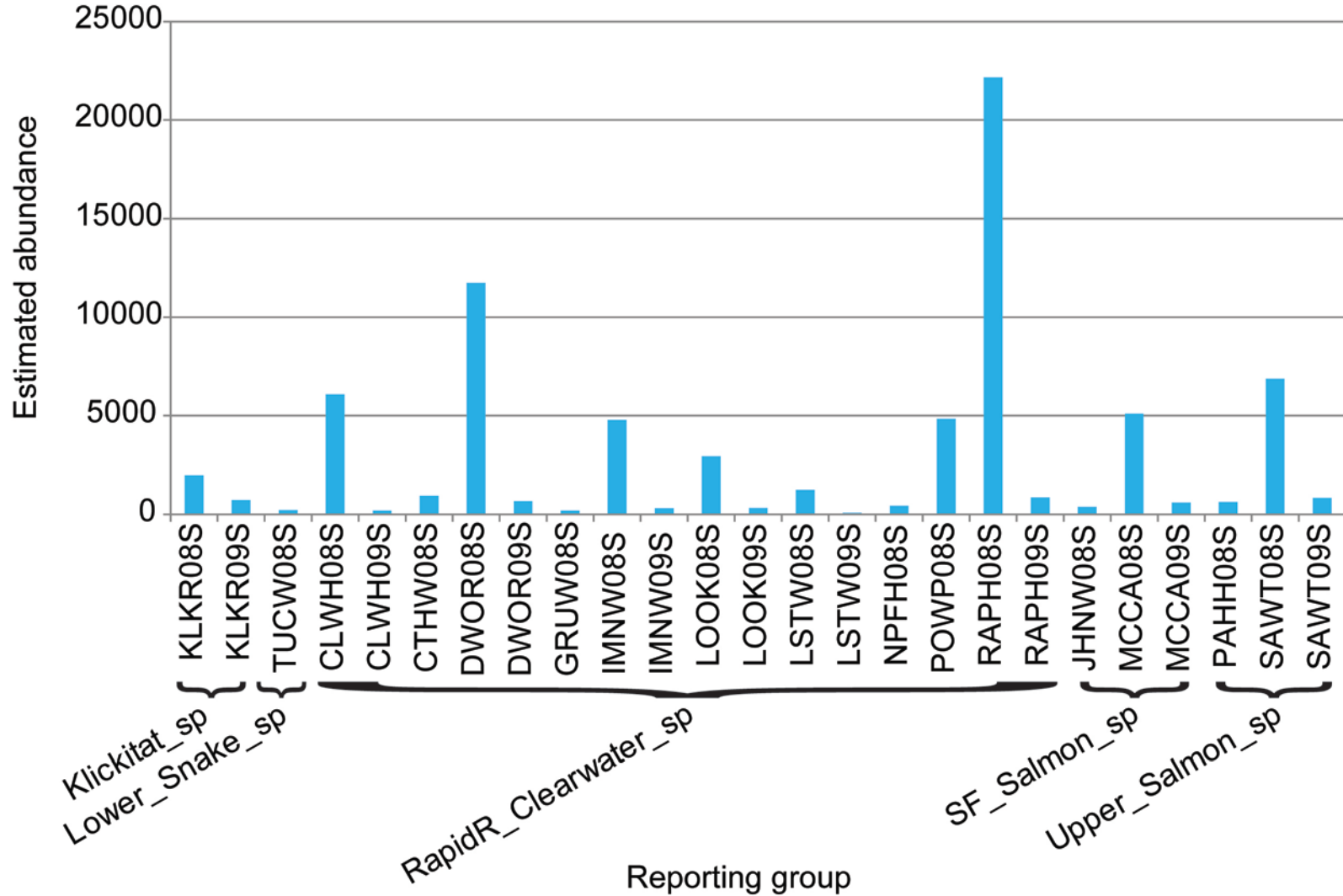


Figure 2. Estimated abundance of broodstock sources for Chinook salmon passing Bonneville Dam in 2012. Hatchery broodstock source abbreviations are listed in Table 7, and they are categorized here into the equivalent GSI reporting group.

There were ten non-adipose-clipped Chinook salmon stocks estimated with abundance greater than 1,000 fish (Table 6, Figure 1). These non-adipose clipped stocks in order of increasing magnitude were Deschutes R. spring (1,300), Spring Cr. group tule fall (2,400), Middle Fork Salmon R. spring/summer (3,700), South Fork Salmon R. spring/summer (4,300), upper Salmon R. spring/summer (4,400), Yakima R. spring (5,900), John Day R. spring (6,500), upper Columbia R. spring (10,700), Rapid R./Clearwater R. spring (19,600), and interior Columbia R. summer/fall (337,400). These stock abundance estimates were based on the stock proportions that were estimated in GSI_sim across weekly strata, and were multiplied with the total abundance of Chinook salmon that was tallied on a daily basis at the Bonneville Dam fish counting window (Table 1).

Table 6. Basic information on run-timing distributions of unclipped (natural origin) Chinook salmon stocks passing Bonneville Dam.

Reporting group	Estimated abundance					Run-timing distribution						
	mean	Total 95% C.I.	Before 15-Jun	June 16 - July 31	After August 1	1st Median	3rd quartile	5th quartile	95th percentile	Median percentile	date	Interquartile range (d)
W_Cascade_sp	0	0 - 569	0	0	0	-	-	-	-	-	-	-
W_Cascade_fa	836	338 - 2286	0	0	836	243	240	245	236	246	30-Aug	5
Willamette_sp	314	85 - 1210	245	69	0	165	154	167	150	169	13-Jun	13
Spring_Cr_Group_Tule	2393	880 - 4517	0	0	2393	251	249	253	247	259	7-Sep	4
Klickitat_sp	110	8 - 505	110	0	0	152	151	154	149	155	31-May	3
Deschutes_R_sp	1296	613 - 2371	1296	0	0	136	130	142	126	154	15-May	12
John_Day_sp	6462	4331 - 9169	6388	73	0	134	130	138	126	159	13-May	8
Yakima_sp	5924	4183 - 7888	5924	0	0	133	129	137	122	153	12-May	8
Upper_Columbia_R_sp	10665	8555 - 14592	10339	327	0	133	129	139	116	162	12-May	10
Lower_Snake_sp	671	164 - 1549	671	0	0	130	129	134	124	139	9-May	5
RapidR_Clearwater_sp	19633	16182 - 23597	17465	2168	0	134	129	150	116	174	13-May	21
MF_Salmon_sp	3653	1881 - 4773	3571	83	0	134	130	141	123	154	13-May	11
Chamberlain_Cr_sp	910	324 - 2170	499	411	0	167	163	170	136	175	15-Jun	7
SF_Salmon_sp	4293	2684 - 6575	3580	713	0	154	144	164	128	173	2-Jun	20
Upper_Salmon_sp	4408	3037 - 6863	3971	436	0	141	135	153	129	175	20-May	18
Interior_Columbia_R_su/fa	337363	331469 - 338511	5572	19627	312164	253	246	261	182	275	9-Sep	15
Columbia_Rogue	528	118 - 1244	0	0	528	271	270	273	268	285	27-Sep	3
Total	399459		59631	23908	315921							

Note: These summary statistics of run-timing distributions were calculated using a method to estimate 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.

Run-timing of Chinook salmon stocks in 2012

We were able to obtain sufficient sample sizes to characterize the run-timing distributions of fourteen hatchery-origin adipose-clipped Chinook salmon stocks (Table 4, Figure 3). We included all PBT-assigned Chinook salmon in this abundance estimate regardless of whether they were adipose-clipped or not. The Chinook salmon management periods divide the run into the following three date ranges: April to June 15th (Spring-run), June 16th – July 31st (Summer-run), and August 1st - October (Fall-run). The following six hatchery-origin stocks were found to terminate within the Spring management period (i.e. the 95th percentile of their run distribution occurred on or before June 15th): John Day R., upper Columbia R., lower Snake R., Rapid R./Clearwater R., Middle Fork, and Chamberlain Cr. Note that although the John Day R., Middle Fork, and Chamberlain Cr. reporting groups are listed here, there are no hatchery programs that exist in these tributaries and their estimated abundance (all were less than 1000 fish) was likely due to misassignment error in GSI. The following five hatchery-origin stocks were found to terminate within the summer management period (i.e. the 95th percentile of their run distribution occurred after June 15th and on or before July 31st): Klickitat R., Deschutes R., Yakima R., and South Fork and upper Salmon R. The remaining summer/fall-run hatchery-origin stocks could be ordered by median date as follows: Spring Cr. group tule (Sep 6th), interior Columbia R. summer/fall (Sep 8th), West Cascade (Sep 20th).

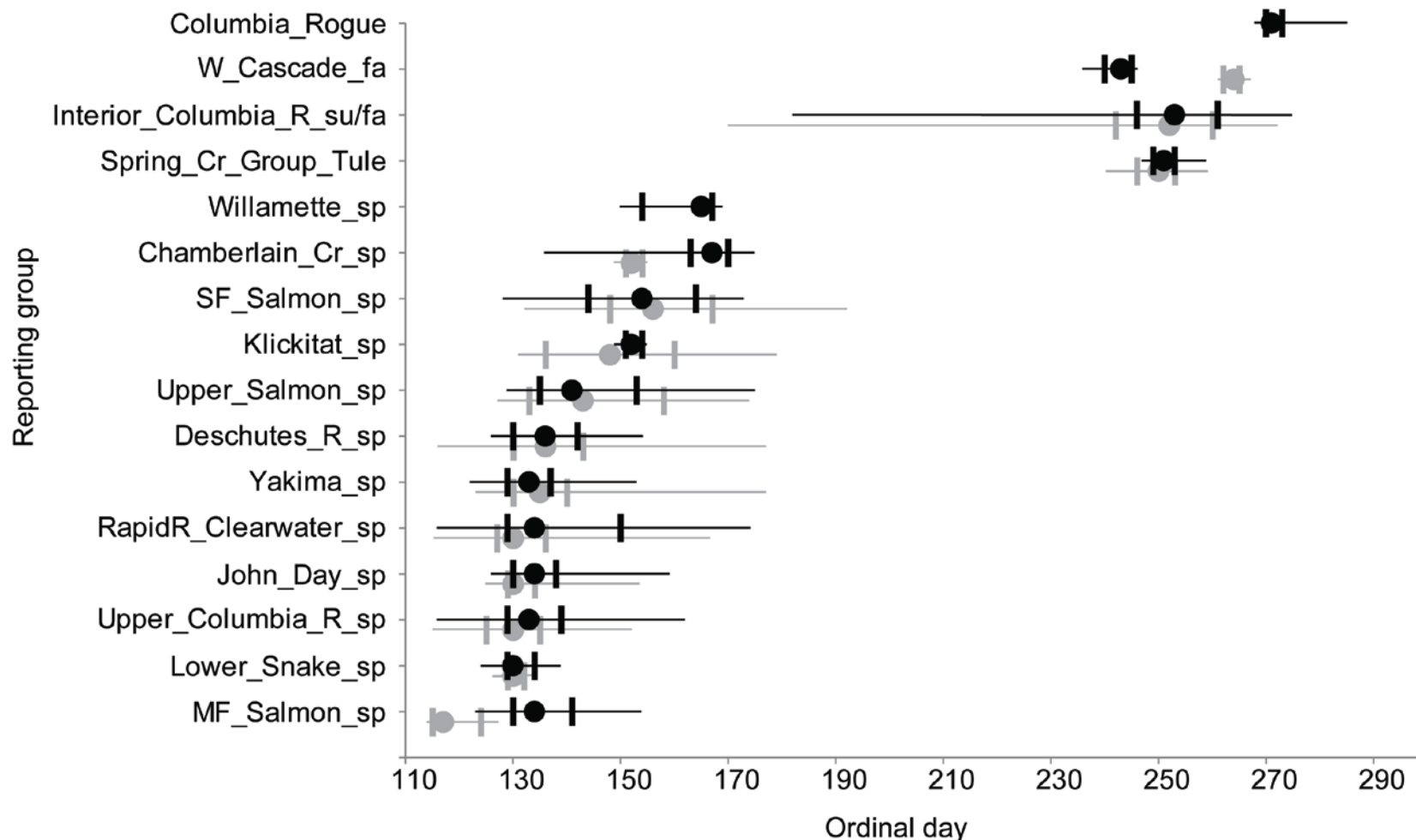


Figure 3. Columbia River Chinook salmon hatchery-origin (gray) and natural-origin (black) stock timing distributions at Bonneville Dam in 2012, including median (circles), inter-quartile (bars), and 5th and 95th percentile dates (horizontal lines). The distributions were based on the weekly estimated reporting group proportions that were applied to the total abundance of Chinook salmon tallied at the Bonneville Dam fish counting window. This method for estimating run-timing distributions minimizes bias imposed by uneven sampling. Hatchery-origin run-timing distributions include stock abundance estimated from PBT and GSI assigned fish.

The run distributions of 16 natural-origin non-adipose-clipped Chinook salmon stocks were similarly characterized (Table 6, Figure 3). The following seven natural-origin stocks were found to terminate within the Spring management period (i.e. the 95th percentile of their run distribution occurred on or before June 15th): Klickitat R., Deschutes R., Yakima R., John Day R., upper Columbia R., lower Snake R., and Middle Fork Salmon R. The following five natural-origin stocks were found to terminate within the summer management period (i.e. the 95th percentile of their run distribution occurred after June 15th and on or before July 31st): Willamette R., Rapid R./Clearwater R., Chamberlain Cr., and South Fork and upper Salmon R. The remaining summer/fall-run hatchery-origin stocks could be ordered by median date as follows: West Cascade (Aug 30th), Spring Cr. group tule (Sep 7th), interior Columbia R. summer/fall (Sep 9th), and Columbia Rogue (Sep 27th). Although these categories differ in composition of stocks depending whether we examine hatchery- or natural-origin fish, comparison to previous analyses of these stocks demonstrates some consistent patterns, e.g. Salmon R. stocks and Klickitat R. have relatively late runs. It may be of interest to managers that we observed more than 4,000 natural-origin and 8,000 hatchery-origin fish from spring-run Chinook salmon reporting groups (mostly Snake R. origin) that are estimated to return during the summer management (Tables 4, 6).

Using the PBT-assigned Chinook salmon, we also characterized the run-distributions at a very fine scale according to hatchery and spawn year (Table 5, Figure 4). This decomposition of broodstock sources showed that fish that assigned to the following 11 sources were found to terminate within the summer management period (i.e. the 95th percentile of their run distribution occurred after June 15th and on or before July 31st): Imnaha (IMNW08S and IMNW09S), Johnson Cr. (JHNW08S), Klickitat (KLKR09S), Lookingglass (LOOK09S), Lostine (LSTW08S), McCall (MCCA08S and MCCA09S), Pahsimeroi (PAHH08S), and Sawtooth (SAWT08S and SAWT09S). The fish that assigned to the remaining 14 broodstock sources terminated within the spring management period. One pattern worth noting is that the 3-year-old jack Chinook salmon (fish that assigned to broodstock that were spawned in 2009), were found to arrive later than the 4-year-old salmon from the same hatchery source. There were 9 hatcheries which had assignments for both age classes (3- and 4- year old), and for most of these hatcheries (8 of 9), the 3-year old fish had a later median day relative to the 4-year olds (Figure 4). The one exception was the Lostine, but in this case, the 3-year old abundance was estimated from a single fish.

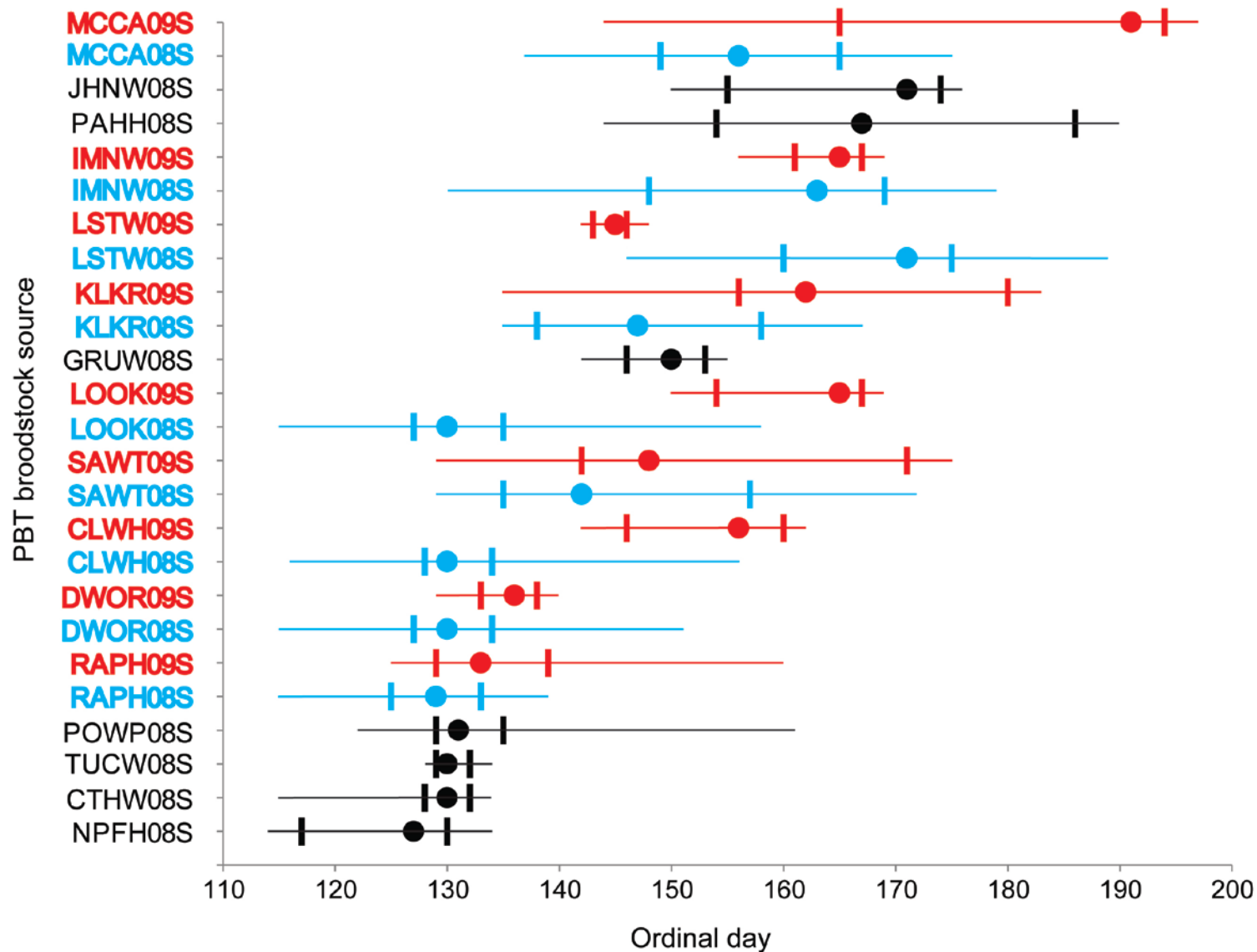


Figure 4. Columbia River PBT-assigned hatchery-origin Chinook salmon run timing distributions at Bonneville Dam in 2012, including median (circles), inter-quartile (bars), and 5th and 95th percentile dates (horizontal lines). Hatchery source abbreviations (Table 7) include spawn year, either 2008 (08S) or 2009 (09S). Sources that have representation in two spawn years (3- and 4-year old returning adults) are indicated in blue (2008) and red (2009). Ordinal day of passage includes dates April 19th (110) to July 18th (200).

Parentage based tagging analyses of Chinook salmon in 2012

We were able to assign 500 Chinook salmon sampled at Bonneville Dam in 2012 to the 2008 and 2009 spring-run Chinook salmon broodstock from fifteen different Snake River hatcheries and the Klickitat Hatchery (Table 7). The fourteen Snake River hatchery sources identified by PBT were aggregated into the appropriate GSI reporting group in order to integrate the abundance estimates from this analysis with those abundance estimates from GSI analyses. Tucannon hatchery was placed in the lower Snake R. reporting group. Johnson Cr. and McCall hatcheries were placed in the South Fork Salmon R. reporting group. Pahsimeroi and Sawtooth were placed in the Upper Salmon R. reporting group, and all other hatcheries were grouped into the Rapid R./Clearwater R. reporting group. Marking rates vary across source hatcheries, for most hatcheries with sample sizes above 15 fish, the rate was near or below 10% unmarked. Two exceptions were Powell and Sawtooth hatcheries which had a sample sizes above 30 fish but were 16.7% and 20.4% unmarked, respectively. Sex (determined by a genetic marker) and length information were available for these PBT-assigned fish and were used to characterize the broodstock sources (Table 5).

Table 7. Summary information on the PBT Chinook salmon source hatcheries and numbers of assignments

PBT source hatchery	Broodstock source abbreviation	Tagging rate	Spawn year	Age of PBT assigned adults	# of assignments
Yakama Nation - Klickitat Hatchery	KLKR08S	93.33%	2008	4-year	15
	KLKR09S	99.27%	2009	3-year (jack)	4
LSRCP/IDFG - Clearwater (SF)	CLWH08S	97.00%	2008	4-year	37
	CLWH09S	94.10%	2009	3-year (jack)	2
LSRCP/ODFW - Catherine Creek	CTHW08S	100.00%	2008	4-year	5
	DWOR08S	97.60%	2008	4-year	78
LSRCP/USFWS - Dworshak	DWOR09S	95.30%	2009	3-year (jack)	4
	GRUW08S	100.00%	2008	4-year	2
LSRCP/ODFW - Grande Ronde	IMNW08S	99.20%	2008	4-year	29
	IMNW09S	98.40%	2009	3-year (jack)	2
Johnson Cr.	JHNW08S	100.00%	2008	4-year	2
	LOOK08S	98.60%	2008	4-year	20
LSRCP/ODFW - Lookingglass Creek	LOOK09S	97.20%	2009	3-year (jack)	2
	LSTW08S	94.50%	2008	4-year	7
LSRCP/ODFW/NPT – Lostine	LSTW09S	89.30%	2009	3-year (jack)	1
	MCCA08S	97.60%	2008	4-year	40
LSRCP/IDFG - McCall (SFSR)	MCCA09S	95.30%	2009	3-year (jack)	4
	NPFH08S	97.90%	2008	4-year	3
Nez Perce Tribal Hatchery (NPTFH)	PAHH08S	98.40%	2008	4-year	4
Idaho Power/IDFG - Pahsimeroi	POWP08S	98.60%	2008	4-year	30
LSRCP/IDFG - Clearwater (Powell)					

Idaho Power/IDFG - Rapid River	RAPH08S	98.40%	2008	4-year	148
	RAPH09S	96.80%	2009	3-year (jack)	6
LSRCP/IDFG - Sawtooth	SAWT08S	99.00%	2008	4-year	48
	SAWT09S	98.00%	2009	3-year (jack)	6
LSRCP/WDFW - L.F. (Tucannon)	TUCW08S	89.70%	2008	4-year	1
		96.94%			500

Estimated abundance of steelhead stocks in 2012

There were seven stocks represented in the total estimated abundance (N=145,363) of hatchery steelhead passing Bonneville Dam in 2012. These stocks in order of magnitude were lower Columbia R. (94), Yakima R. (108), Skamania summer-run (5557), Upper Columbia R. (9615), South Fork Clearwater R. (17,945), MGILCS-mega complex (42,179), and Upper Salmon R. (69,865) (Table 8, Figure 5). These estimates include abundance estimated from PBT-assigned fish that were mostly adipose clipped, however a large portion of the PBT-assigned fish were found to have their adipose intact. Therefore PBT assignments improved our ability to accurately identify hatchery-origin steelhead and estimate total stock abundance (Figure 5), particularly for three reporting groups MGILCS, South Fork Clearwater R., and Upper Salmon R. Further, using PBT assignments we can now provide abundance, run-timing, and size and age information at a spatial scale of a particular hatchery (Table 9, Figure 6). At the level of hatchery, we estimated less than 1,000 fish for Little Sheep Cr. LSCR, Tucannon TUCW, Touchet TOUW, and Squaw SQUW. However, the following eight hatchery stocks produced greater than 1,000 fish: E.F. Salmon EFSW (1,400), Catherine CGRW (5,500), Lyons Ferry LYON (13,300), Wallowa WALL (13,700), Dworshak (16,800), Oxbow OXBO (20,000), Sawtooth SAWT (21,400), and Pahsimeroi PAHH (24,400).

Table 8. Basic information on run-timing distributions of hatchery-origin (based on adipose-clips and PBT assignments) steelhead stocks passing Bonneville Dam in 2012.

Reporting group	Estimated abundance				Run-timing distribution						
	Total		Management period								
	mean	95% C.I.	Skamania Apr. 1 - Jun. 30	Summer Jul. 1 - Oct. 31	Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Inter- quartile range (d)
WCOAST	0	0 - 43	0	0	-	-	-	-	-	-	-
LOWCOL	94	1 - 345	2	93	223	214	262	206	277	10-Aug	48
SKAMAN	5557	4070 - 6933	2316	3241	191	171	201	156	217	9-Jul	30
WILLAM	0	0 - 221	0	0	-	-	-	-	-	-	-
BWSALM	0	0 - 39	0	0	-	-	-	-	-	-	-
KLICKR	0	0 - 291	0	0	-	-	-	-	-	-	-
MGILCS	42179†	39173 - 44109	2321	39858	216	201	232	181	258	3-Aug	31
YAKIMA	108	0 - 273	8	100	197	191	201	180	204	15-Jul	10
UPPCOL	9615	7817 - 12180	419	9196	220	209	230	186	255	7-Aug	21
SFCLWR	17945†	17858 - 18158	16	17929	263	249	271	235	283	19-Sep	22
UPCLWR	0	0 - 325	0	0	270	264	275	261	284	26-Sep	11
SFSALM	0	0 - 198	0	0	-	-	-	-	-	-	-
MFSALM	0	0 - 352	0	0	-	-	-	-	-	-	-
UPSALM	69865†	68270 - 71756	606	69259	224	212	242	196	259	11-Aug	30
Total	145363		5688	139677							

Note: These summary statistics of run-timing distributions were calculated using a method to estimate abundance of each stock based on stock proportions and total numbers of steelhead that were observed passing Bonneville Dam at the fish counting window. This information is also plotted in Figure 6. † These estimates were combined with PBT-estimated stock abundance.

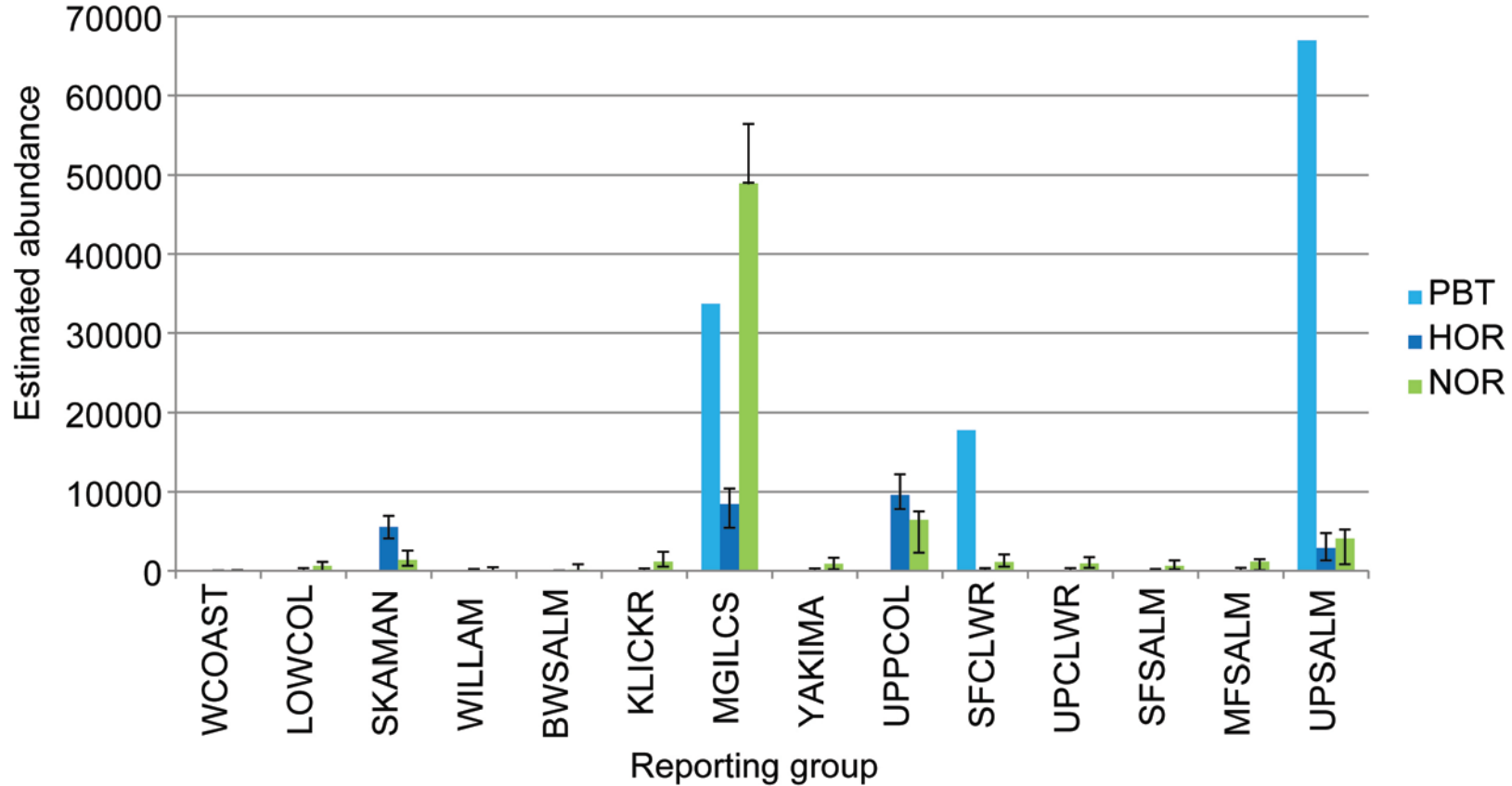


Figure 5. Estimated abundance of reporting groups for steelhead passing Bonneville Dam in 2012. This data was generated by splitting the daily tallies of the adipose clipped (HOR) and unclipped (NOR) steelhead at the Bonneville Dam fish counting window into two categories: those fish that were either assigned using PBT analyses or could not be assigned with PBT. Reporting group proportions were then estimated for the following three categories: PBT-assigned (includes both adipose clipped and unclipped fish) or the fish not assigned with PBT, which were either adipose-clipped (HOR) or unclipped (NOR). Reporting group proportions were estimated on monthly pooled mixtures of steelhead passing Bonneville Dam and then these proportions were multiplied with monthly tallies of steelhead at the Bonneville Dam fish counting window.

Table 9. Basic information on run-timing distributions of unclipped and clipped PBT-assigned (hatchery origin) steelhead stocks passing Bonneville Dam.

GSI reporting group	Broodstock collection	Tagging rate	Estimated abundance		Run-timing distribution								Sex and size					
			Total	Skamania	Summer	1st		3rd	5th	95th	Median	Interquartile	Female		Male		Total	
				Apr. 1 - Jun. 30	Jul. 1 - Oct. 31	Median	quartile	quartile	percentile	percentile	date	range (d)	N	>78cm length	N	>78cm length	N	>78cm length
MGILCS	CGRW09S	98.0%	2607	106	2501	204	197	221	183	248	22-Jul	24	10	0.0%	3	0.0%	13	0.0%
	CGRW10S	100.0%	2871	0	2871	229	217	247	208	261	16-Aug	30	5	0.0%	13	0.0%	18	0.0%
	LSCR10S	89.1%	160	0	160	247	241	253	234	259	3-Sep	12	0	-	1	0.0%	1	0.0%
	LYON09S	95.5%	6119	1044	5074	202	191	216	165	232	20-Jul	25	31	0.0%	8	12.5%	39	2.6%
	LYON10S	100.0%	7227	576	6651	207	197	226	177	252	25-Jul	29	15	0.0%	23	0.0%	39	0.0%
	TOUW09S	92.5%	299	0	299	221	213	230	207	275	8-Aug	17	2	0.0%	0	-	2	0.0%
	TOUW10S	67.5%	211	0	211	247	241	253	234	259	3-Sep	12	0	-	1	0.0%	1	0.0%
	TUCW09S	84.6%	498	18	480	207	198	219	184	229	25-Jul	21	1	0.0%	0	-	2	0.0%
	WALL09S	91.6%	10355	146	10209	220	208	235	192	258	7-Aug	27	40	0.0%	12	0.0%	53	0.0%
SFCLWR	WALL10S	97.6%	3311	0	3311	226	215	244	207	263	13-Aug	29	6	0.0%	14	0.0%	20	0.0%
	DWOR08S	72.9%	486	0	486	270	264	275	261	284	26-Sep	11	6	100.0%	1	100.0%	7	100.0%
	DWOR09S	97.4%	15011	0	15011	264	252	272	238	283	20-Sep	20	138	63.0%	81	93.8%	222	74.3%
	DWOR10S	97.1%	1340	0	1340	253	239	266	213	280	9-Sep	27	1	0.0%	14	0.0%	15	0.0%
	SQUW09S	100.0%	723	15	708	244	204	255	189	272	31-Aug	51	4	25.0%	2	0.0%	6	16.7%
UPSALM	SQUW10S	95.6%	106	0	106	270	264	275	261	284	26-Sep	11	1	0.0%	1	0.0%	2	0.0%
	EFSW09S	100.0%	368	0	368	227	216	243	207	258	14-Aug	27	1	0.0%	1	0.0%	2	0.0%
	EFSW10S	100.0%	982	15	967	222	208	242	191	261	9-Aug	34	2	0.0%	4	0.0%	6	0.0%
	OXBO09S	88.0%	18481	253	18228	222	209	241	192	258	9-Aug	32	61	0.0%	30	0.0%	92	0.0%
	OXBO10S	95.5%	1482	0	1482	221	214	230	207	253	8-Aug	16	4	0.0%	3	0.0%	7	0.0%
	PAHH09S	95.1%	8581	31	8549	228	215	245	205	259	15-Aug	30	28	0.0%	20	0.0%	48	0.0%
	PAHH10S	97.1%	15862	107	15755	226	214	245	198	262	13-Aug	31	44	0.0%	52	0.0%	96	0.0%
	SAWT08S	98.1%	145	0	145	247	241	253	234	259	3-Sep	12	1	0.0%	0	-	1	0.0%
	SAWT09S	99.6%	6650	60	6590	222	211	239	196	258	9-Aug	28	31	0.0%	6	0.0%	37	0.0%
Total	SAWT10S	99.8%	14647	89	14557	226	214	244	199	259	13-Aug	30	35	0.0%	49	0.0%	85	0.0%
			118520	2461	116059								467		339		814	

Note: GSI reporting groups indicate which group the broodstock sources are most genetically similar. Collection abbreviations can be found in Table 11. These summary statistics of run-timing distributions were calculated using a method to estimate 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 5. The date ranges listed under “Skamania” and “Summer” were chosen by steelhead fishery managers, and for each hatchery source stock we provide the abundance that has passed within these time periods. Under “sex and size” we provide number of each sex as determined by a genetic marker and length shows the percent of fish larger than 78 cm for each category.

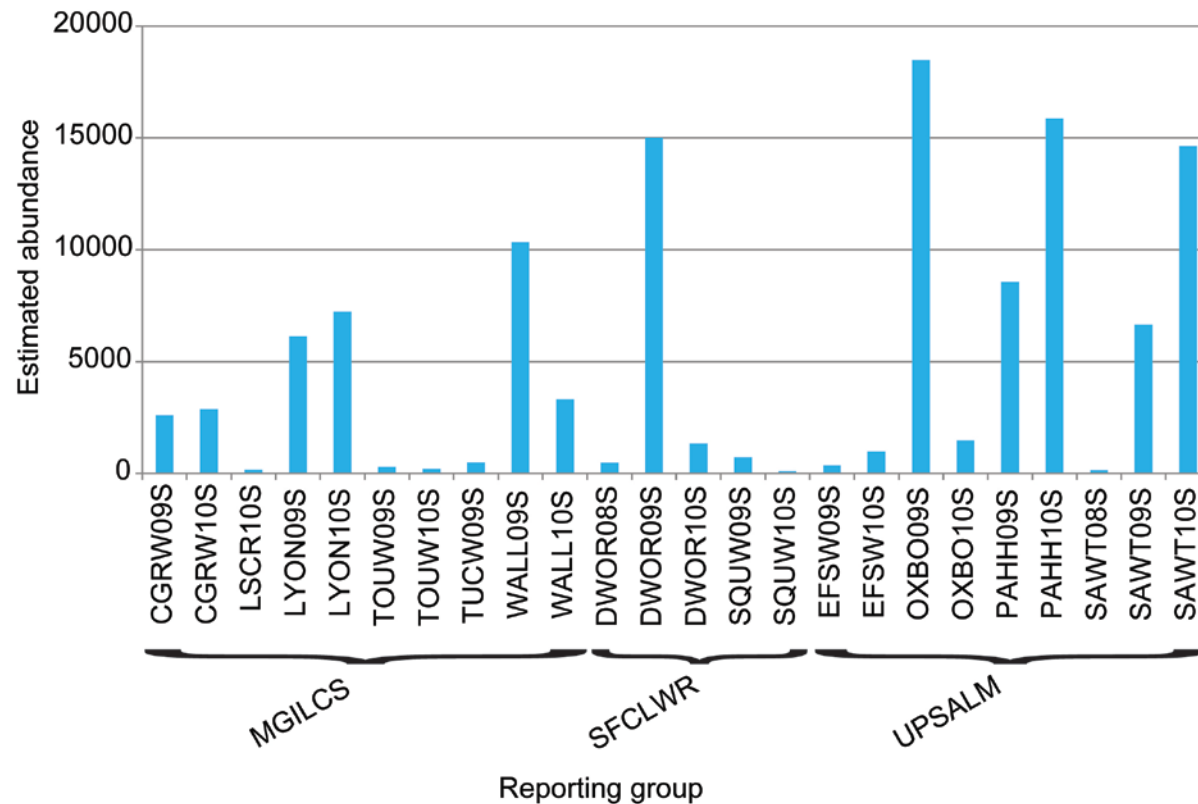


Figure 6. Estimated abundance of broodstock sources for steelhead passing Bonneville Dam in 2012. Hatchery broodstock source abbreviations are listed in Table 11, and they are categorized here into the equivalent GSI reporting group.

There were twelve stocks represented in the total estimated abundance (n=67,499) of wild steelhead (Table 10, Figure 5). These stocks in order of magnitude were Big White Salmon R. (126), lower Columbia R. (615), South Fork Salmon R. (642), Yakima R. (890), upper Clearwater R. (939), South Fork Clearwater R. (1,100), Klickitat R. (1,136), Middle Fork Salmon R. (1,165), Skamania (1,389), Upper Salmon R. (4,102), Upper Columbia R. (6,454), and MGILCS-middle Columbia R. (48,941).

Table 10. Basic information on run-timing distributions of natural-origin (adipose unclipped) steelhead stocks passing Bonneville Dam in 2012.

Reporting group	Estimated abundance		Management period		Run-timing distribution						
	Total		Skamania	Summer	1st		3rd	5th	95th	Median	Interquartile
	mean	95% C.I.	Apr. 1 - Jun. 30	Jul. 1 - Oct. 31	Median	quartile	quartile	percentile	percentile	date	range (d)
WCOAST	0	0 - 47	0	0	-	-	-	-	-	-	-
LOWCOL	615	5 - 1098	10	606	214	202	228	189	253	1-Aug	26
SKAMAN	1389	635 - 2549	48	1341	201	195	209	184	226	19-Jul	14
WILLAM	0	0 - 445	0	0	-	-	-	-	-	-	-
BWSALM	126	0 - 829	0	126	246	240	253	234	259	2-Sep	13
Klickr	1136	508 - 2398	10	1125	214	207	222	193	231	1-Aug	15
MGILCS	48941	49014 - 56365	724	48217	213	202	226	189	252	31-Jul	24
YAKIMA	890	179 - 1654	35	854	199	194	204	183	255	17-Jul	10
UPPCOL	6454	2292 - 7508	98	6355	219	202	242	189	258	6-Aug	40
SFCLWR	1100	501 - 2050	0	1100	233	216	258	207	276	20-Aug	42
UPCLWR	939	378 - 1701	0	939	245	236	254	211	269	1-Sep	18
SFSALM	642	169 - 1322	0	642	242	227	254	209	271	29-Aug	27
MFSALM	1165	66 - 1436	11	1154	234	210	247	193	259	21-Aug	37
UPSALM	4102	813 - 5211	50	4053	220	205	242	191	259	7-Aug	37
Total	67499		986	66511							

Note: These summary statistics of run-timing distributions were calculated using a method to estimate abundance of each stock based on stock proportions and total numbers of steelhead that were observed passing Bonneville Dam at the fish counting window. This information is also plotted in Figure 6.

Run-timing of steelhead stocks in 2012

We were able to obtain sufficient sample sizes to characterize the run-timing distributions of 8 hatchery steelhead stocks and 12 wild steelhead stocks in 2012 (Figure 7). Results for the hatchery stocks indicate three main run-timing categories of stocks. An early run-timing category has in the past been occupied primarily by the Skamania summer-run (Median date Jul. 9th), however this year the Yakima R. also appeared early (Median date Jul. 15th). An intermediate run-timing category includes the following four hatchery steelhead stocks (ordered by median dates): MGILCS (Aug. 3rd), upper Columbia R. UPPCOL (Aug. 7th), lower Columbia R. LOWCOL (Aug. 10th), and upper Salmon R. UPSALM (Aug. 11th). Finally, a late run-timing category includes South Fork Clearwater R. SFCLWR (Sep. 19th), and upper Clearwater R. UPCLWR (Sep. 26th). The late run-timing category is typically thought to be characteristic of B-run steelhead that return after August 25th at Bonneville Dam.

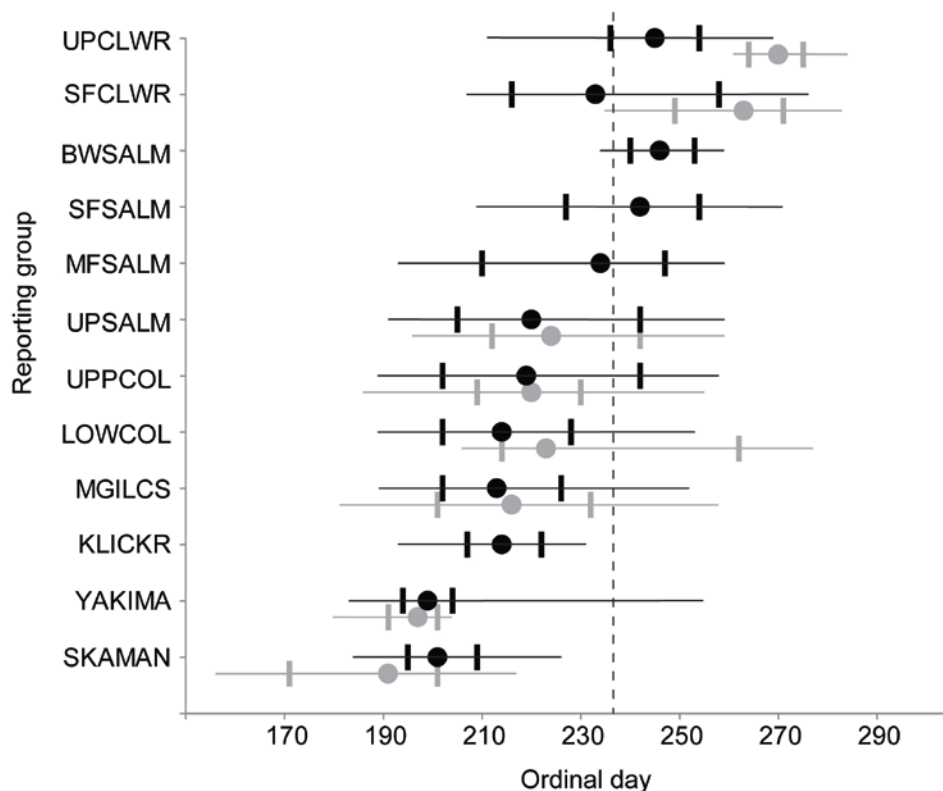


Figure 7. Columbia River steelhead hatchery-origin (gray) and natural-origin (black) stock timing distributions at Bonneville Dam in 2012, including median (circles), inter-quartile (bars), and 5th and 95th percentile dates (horizontal lines). August 25th (dashed vertical line) classifies A-run (before the 25th) and B-run (after the 25th) steelhead. Reporting groups were organized from top to bottom in order of average median return day across hatchery/natural origins. The distributions were based on the weekly estimated reporting group proportions that were applied to the total abundance of steelhead tallied at the Bonneville Dam fish counting window. This method for estimating run-timing distributions minimizes bias imposed by uneven sampling. Hatchery-origin run-timing distributions include stock abundance estimated from PBT and GSI assigned fish.

The wild steelhead stocks generally fit these same categories but insufficient sample sizes prevented analysis of the one of the first statistical months (weeks 23-26) that was possible to analyze for hatchery steelhead. Therefore, the total range in run-distributions of all wild stocks is slightly narrower than the hatchery stocks. The wilds stocks ordered by median date were Yakima R. (Jul. 17th), Skamania (Jul. 19th), MGILCS (Jul. 31st), lower Columbia R. (Aug. 1st), Klickitat R. (Aug. 1st), upper Columbia R. (Aug. 6th), Upper Salmon R. (Aug. 7th), S.F. Clearwater R. (Aug. 20th), M.F. Salmon R. (Aug. 21st), S.F. Salmon R. (Aug. 29th), upper Clearwater R. (Sep. 1st), and Big White Salmon R. (Sep. 2nd).

Using the PBT-assigned steelhead, we also characterized the run-distributions at a very fine scale according to hatchery and spawn year (Table 9, Figure 8). This decomposition into broodstock sources allowed us to group them into categories according to their median date. The following broodstock sources all had median dates before August: Lyons Ferry 2009-2010, Grande Ronde Cottonwood 2009, and Tucannon 2009. Broodstock sources with median dates between August 1st – 24th were: Wallowa 2009-2010, Touchet 2009, Oxbow 2009-2010, Sawtooth EFSR 2009-2010, Pahsimeroi 2009-2010, Sawtooth 2009-2010, and Grande Ronde Cottonwood 2010. Finally, broodstock sources with median dates occurring after August 25th were the following: Squaw 2009-2010, Little Sheep Cr. 2010, Touchet 2010, Sawtooth 2008, and Dworshak 2008-2010.

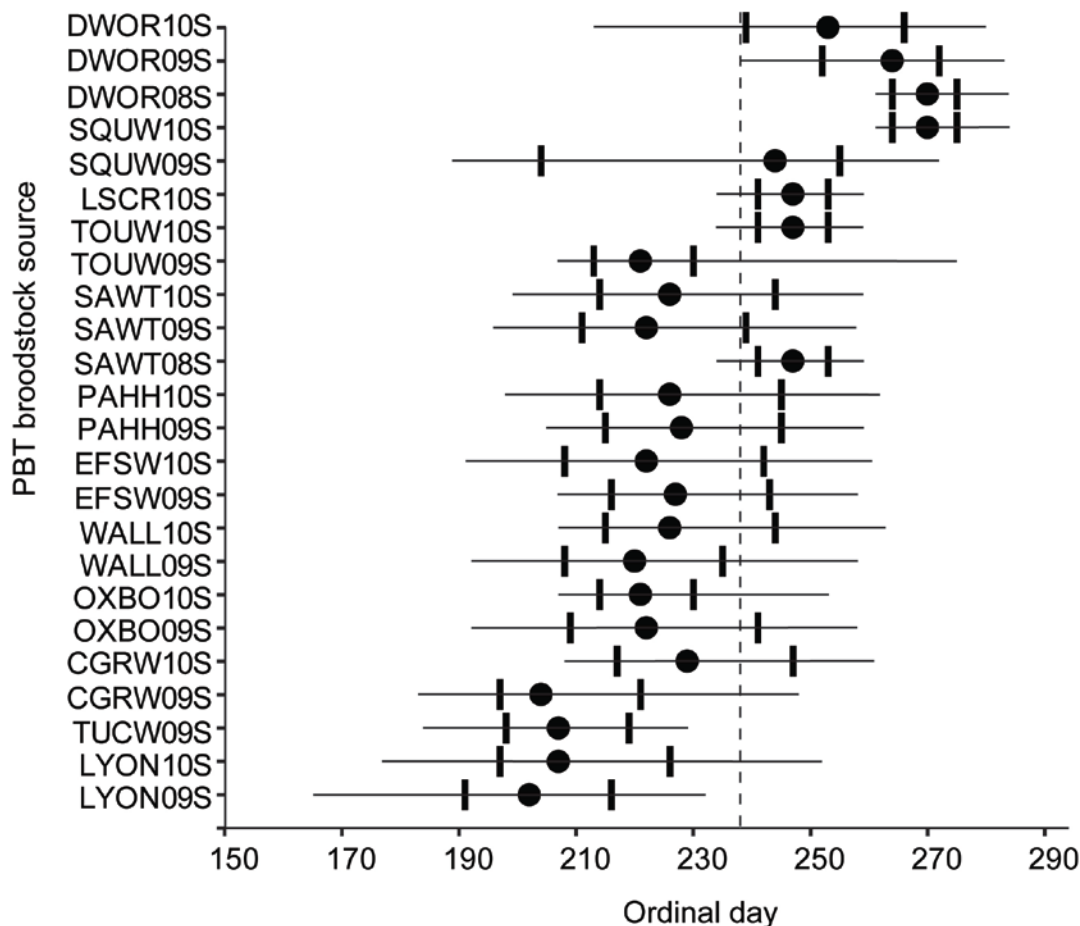


Figure 8. Columbia River PBT-assigned hatchery-origin steelhead run timing distributions at Bonneville Dam in 2012, including median (circles), inter-quartile (bars), and 5th and 95th percentile dates (horizontal lines). August 25th (dashed vertical line) classifies A-run (before the 25th) and B-run (after the 25th) steelhead. Reporting groups were organized from top to bottom in order of average median return day across spawn years of each hatchery source. The distributions were based on the weekly estimated reporting group proportions that were applied to the total abundance of steelhead tallied at the Bonneville Dam fish counting window. This method for estimating run-timing distributions minimizes bias imposed by uneven sampling. Hatchery source abbreviations (Table 11) include spawn year, either 2008 (08S), 2009 (09S) or 2010 (10S). Returning adults that assigned to 2008, 2009, and 2010 spawn years spent 3, 2, and 1 year in the ocean, respectively. Ordinal day of passage includes dates May 29th (150) to October 16th (290).

Parentage based tagging analyses of steelhead in 2012

We were able to assign 814 steelhead sampled at Bonneville Dam in 2012 to the 2008-2010 steelhead broodstock from twelve different Snake River hatcheries (Table 11). The largest portion of the PBT-assigned fish originated from the Dworshak Hatchery (n=244, 30%), and the second greatest portions originated from the Pahsimeroi (18%) and Sawtooth (15%) hatcheries. Using these known hatchery-of-origin steelhead, we compared the individual assignments based on GSI analysis and used these assignments to help classify them into the most genetically similar reporting group (Table 11). Those groupings were used to combine results of PBT-hatchery abundance estimates with the GSI estimated abundance of hatchery stocks (Figure 5).

We examined which of the hatchery sources was contributing the size range of fish typically classified as B-run steelhead (Table 9). Fish with a fork length greater than 78 cm were found to only originate from the following three different broodstock sources: Lyons Ferry 2009 (% of fish greater than 78 cm = 2.6%), Dworshak 2008-2009 (100%, 74.3%), and Squaw 2009 (16.7%). The brood years 2009 and 2008 represent 2- and 3-ocean age adults, respectively. These ages are typical of B-run life history. Further, the regions of the South Fork and Upper Clearwater R. and Middle Fork and South Fork Salmon R. are generally thought to be the largest sources of B-run steelhead. Dworshak and Squaw broodstock fit within the South Fork Clearwater R. genetic stock and so are expected to produce large, older steelhead. Lyons Ferry is expected to generally produce smaller steelhead.

Table 11. Summary information for Snake River steelhead broodstock sources and numbers of assignments of Bonneville Dam steelhead in 2012.

GSI reporting group		PBT source hatchery	Broodstock source abbreviation	Tagging rate	Spawn year	Age of PBT assigned adults	# of assignments
MGILCS	LSRCP/WDFW-L.F. (G.R. cottonwood)	CGRW09S	98.0%	2009	2-ocean	13	
		CGRW10S	100.0%	2010	1-ocean	18	
	LSRCP/ODFT - Little Sheep Cr. FH	LSCR10S	89.1%	2010	1-ocean	1	
	LSRCP/WDFW-Lyons Ferry	LYON09S	95.5%	2009	2-ocean	39	
		LYON10S	100.0%	2010	1-ocean	39	
	LSRCP/WDFW-L.F. (Touchet)	TOUW09S	92.5%	2009	2-ocean	2	
		TOUW10S	67.5%	2010	1-ocean	1	
	LSRCP/WDFW-L.F. (Tucannon)	TUCW09S	84.6%	2009	2-ocean	2	
	LSRCP/ODFW-Wallowa F.H.	WALL09S	91.6%	2009	2-ocean	53	
		WALL10S	97.6%	2010	1-ocean	20	
SFCLWR	LSRCP/IDFG/USFWS Dworshak/C.W.	DWOR08S	72.9%	2008	3-ocean	7	
		DWOR09S	97.4%	2009	2-ocean	222	
		DWOR10S	97.1%	2010	1-ocean	15	
	LSRCP/IDFG Sawtooth (USB/Squaw)	SQUW09S	100.0%	2009	2-ocean	6	
		SQUW10S	95.6%	2010	1-ocean	2	
UPSALM	LSRCP/IDFG Sawtooth (EFSR)	EFSW09S	100.0%	2009	2-ocean	2	
		EFSW10S	100.0%	2010	1-ocean	6	
	Idaho Power/IDFG,Oxbow F.H.	OXBO09S	88.0%	2009	2-ocean	92	
		OXBO10S	95.5%	2010	1-ocean	7	
	Idaho Power/IDFG, Pahsimeroi F.H.	PAHH09S	95.1%	2009	2-ocean	48	
		PAHH10S	97.1%	2010	1-ocean	96	
	LSRCP/IDFG Sawtooth (IDFG & SBT)	SAWT08S	98.1%	2008	3-ocean	1	
		SAWT09S	99.6%	2009	2-ocean	37	
		SAWT10S	99.8%	2010	1-ocean	85	
Total			93.85%			814	

Note: The GSI reporting group is the group that is most genetically similar to the listed hatchery broodstock sources. The hatchery abbreviations include digits that indicate the spawn year, e.g. 08S = 2008 spawn year. The tagging rate is the percent of broodstock parents that were genotyped. Assignments refer to the number of adult steelhead passing Bonneville Dam in 2012 that were assigned to parents from a particular broodstock source.

Power analysis of 95 SNP sockeye baseline

Four *O. nerka* reference collections were used to represent three sockeye populations and one outgroup, Whatcom Lake, which is a major kokanee stock that is used for transplanting across a large geographic area. We combined collections 18 and 19 for Wenatchee (collection #s in Section 2, Appendix 1c), 20 and 21 for Okanogan, and used 22 and 4 for Lake Whatcom and Redfish Lake populations, respectively. Only one locus was excluded, *One_UCA-24*, because the Redfish Lake collection was missing 100% of genotypes. Results from the leave-one-out analysis, a relatively conservative accuracy test, showed all four baseline collections achieved above 99% correct individual assignment to population-of-origin (Table 12). This level of accuracy that is consistent across all reference collections makes this baseline the most powerful for GSI applications that we have demonstrated to date. The relatively small size of the baseline and highly differentiated reference collections are the factors influencing this power. Results from 100% mixture simulations for each of the baseline collections, showed that all four collections produced estimates that were near 100% proportion of the correct population (Table 12). Essentially, there is no need for improving the assignment power of this sockeye baseline, at least for discriminating these three sockeye populations.

Table 12. Sample numbers of sockeye baseline collections and population accuracy.

Population	Total N	Complete genotype N	Leave-1-out			100% simulations		
			% Correct	Largest mis- identification pop.	% Allocation	Avg. % Correct	Stan. dev.	95% interval
Wenatchee	345	285	100.0%			100.0%	0.000	100.0% - 100.0%
Okanogan	217	164	99.4%	Wenatchee	0.60%	100.0%	0.000	100.0% - 100.0%
Redfish	86	73	100.0%			100.0%	0.000	100.0% - 100.0%
Whatcom	46	34	100.0%			100.0%	0.000	100.0% - 100.0%
Total	694	556	99.9%			100.0%	0.000	

Concordance between PIT-tag and genetic methods for stock identification of sockeye salmon

An ongoing study employing PIT-tags to estimate sockeye salmon escapement relies on detection of tagged fish at upstream dams in order to identify stocks (Nowinski 2013). For example, detections at Tumwater, Wells, and Lower Granite dams provide identification of Wenatchee, Okanogan, and Redfish Lake sockeye stocks, respectively. However, in many years there is a portion of fish that cannot be assigned due to failure to tag, tag loss, or for reasons related to detection failure (either mortality of the fish or equipment sensitivity at the dam detection arrays). In 2012, there were 394 unassigned sockeye (24%) out of the total 1637 that

were PIT-tagged (Table 13). In addition, 27 (2%) sockeye were sampled at the Adult Fish Facility but were never PIT-tagged. GSI can provide the stock information missing for over a quarter of the fish sampled at the Adult Fish Facility at Bonneville Dam. Further, there is a chance that some detections of sockeye at a particular dam would be stray fish that originated from a population different than the one immediately upstream of that dam. Thus we had the following two goals for applying GSI to the sockeye salmon that were sampled at the Adult Fish Facility at Bonneville Dam: 1) Identify all PIT-tag unassigned sockeye, and 2) Estimate concordance of stock identification between PIT-tag and genetic methods.

The concordance between methods was observed to be high, 97.9% to 99.5% for Wenatchee and Okanogan stocks, respectively (Table 13). This level of concordance indicates high precision of stock identification based on these two methods, and so use of either method should yield nearly equivalent results. The small difference between methods may be accounted for by a relatively low stray rate (0.5 to 2.1%). These concordance results allow us to compile the stock identification results from both PIT-tag and genetic methods and use them in a combined analysis. For example, we primarily relied on the genetic stock ID, and in the absence of this genetic ID, we utilized the PIT-tag stock ID. The results from combining PIT-tag and genetic stock ID provided stock information for the entire set of 1,637 fish that were sampled at the Adult Fish Facility in 2012. In order to employ genetic software for generating mean stock proportion estimates and 95% confidence intervals, any fish that was identified to stock exclusively using PIT-tag results was replaced with a genotype from a fish known to have originated from that stock based on GSI. This replacement applied to 102 fish, of which 100 were Wenatchee stock and 2 were Okanogan stock based on PIT-tag results.

Table 13. Concordance of PIT-tag and genetic stock identification of sockeye salmon

PIT_Stock	Genetic_Stock	N	Proportion
Okanogan	1020 (1018 genotyped)		
	Okanogan	1013	99.5%
	Wenatchee	5	0.5%
	not genotyped	2	
Wenatchee	196 (96 genotyped)		
	Okanogan	2	2.1%
	Wenatchee	94	97.9%
	not genotyped	100	
Unknown	394		
	Okanogan	301	76.4%
	Redfish	2	0.5%
	Wenatchee	91	23.1%
Not tagged	27		
	Okanogan	19	70.4%
	Redfish	1	3.7%
	Wenatchee	7	25.9%
Grand Total		1637	

Note: The gray highlighted rows show the concordance rate between stock identification methods. The large number of samples not genotyped among the PIT-tag category of Wenatchee fish was intentional to minimize analysis costs.

Table 14. Basic information on run-timing distributions of sockeye salmon stocks passing Bonneville Dam in 2012.

Reporting group	Estimated abundance				Run-timing distribution						
	Total		PIT-tag "unknown"		1st		3rd	5th	95th	Median	Interquartile
	Mean	95% C.I.	Mean	prop. of total	Median	quartile	quartile	percentile	percentile	date	range (d)
Wenatchee	98881	88609 - 109097	31160	31.5%	179	175	183	170	190	27-Jun	8
Okanogan	415491	404170 - 425241	96595	23.2%	185	170	199	158	209	3-Jul	29
Redfish	470	249 - 2032	274	58.3%	207	205	210	192	211	25-Jul	5
Whatcom	0	43 - 1306	0	0.0%	-	-	-	-	-	-	-

Note: The PIT-tag “unknown” category pertains to sockeye that were PIT-tagged but never detected at their terminal dam and so may reflect survivorship of each stock. The run-timing distributions are indicated by ordinal day of passage in which a specific proportion of the total abundance was estimated to have passed Bonneville Dam. The interquartile range is marked in days.

Estimated abundance of sockeye salmon stocks in 2012

Abundance was estimated during a span of eight statistical weeks in which sample numbers were 15 or greater per week (Table 14). The three sockeye salmon stocks passing Bonneville Dam were estimated with the following abundance in decreasing magnitude: Okanogan (415,500), Wenatchee (98,900), and Redfish Lake (500) (Table 14). When we calculated the proportion of each stock that was not possible to assign due to lack of detections of fish at their terminal dam, we found Redfish Lake with 58%, Wenatchee with 32%, and Okanogan sockeye salmon with 23% of their total abundance not detected. These proportions may indicate differential survivorship of these stocks, with Redfish Lake and Wenatchee sockeye salmon having lower survivorship relative to the Okanogan stock.

Run-timing of sockeye salmon stocks in 2012

We characterized the run-timing distributions of the three sockeye salmon stocks (Table 14, Figure 9). These stocks could be ordered by median date as follows: Wenatchee (Jun. 27th), Okanogan (Jul. 3rd), and Redfish Lake (Jul 25th).

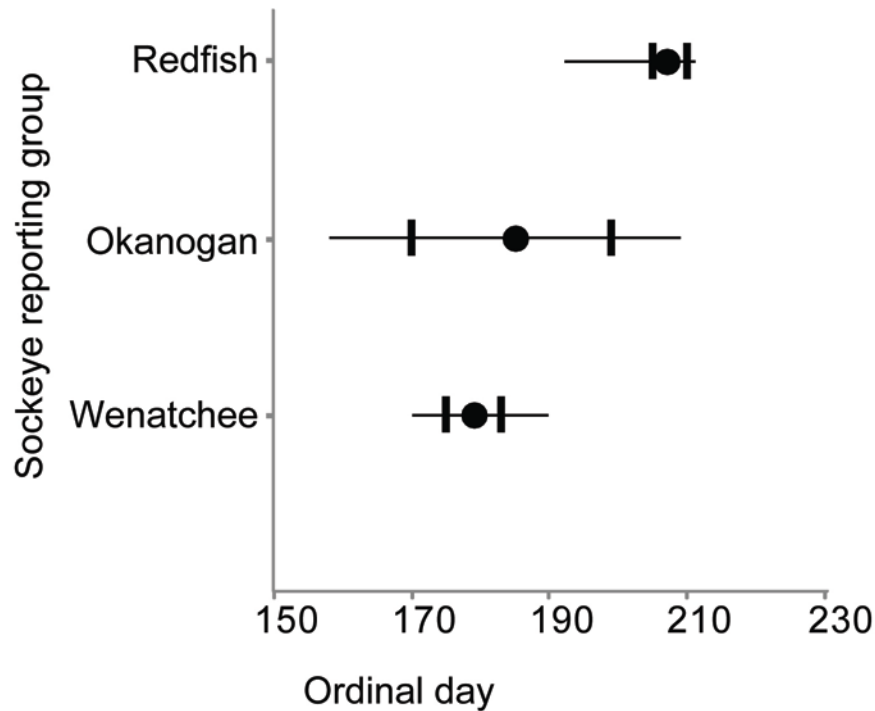


Figure 9. Columbia River sockeye salmon stock run-timing distributions at Bonneville Dam in 2012, including median (circles), inter-quartile (bars), and 5th and 95th percentile dates (horizontal lines). Reporting groups were organized from top to bottom in order of average median return day across populations. The distributions were based on the weekly estimated reporting group proportions that were applied to the total abundance of steelhead tallied at the Bonneville Dam fish counting window. This method for estimating run-timing distributions minimizes bias imposed by uneven sampling.

Discussion

Parentage based tagging (PBT) provides a substantial increase in information when combined with genetic stock identification and used to analyze Columbia River Chinook salmon and steelhead passing Bonneville Dam. PBT improves the accuracy for defining hatchery-origin and natural-origin stocks. In the past, we have had to rely solely on adipose-markings, which we know are not always used on all hatchery stocks especially some steelhead and summer- and fall-run Chinook salmon stocks. PBT can further discriminate the reporting groups that we use for GSI analyses, and so we can now characterize different age-classes from particular hatcheries by run-timing distributions and estimate their abundance at Bonneville Dam. GSI continues to complete the information that PBT cannot provide, which is identification of all non-Snake River hatchery stocks as well as all natural-origin stocks.

Our results indicate there were seven hatchery stocks and seven wild stocks of spring-run Chinook salmon estimated to have greater than 2,000 fish pass Bonneville Dam in 2012. It may interest fisheries managers to know that the run-timing of these stocks contributed to the total abundance of Chinook salmon that pass through the Columbia River mainstem in two management periods, spring and summer. In fact, we observed more than 4,000 natural-origin and 8,000 hatchery-origin fish from spring-run Chinook salmon reporting groups (mostly Snake R. origin) that are estimated to return during the summer management period (June 16 – July 31). There were some consistent run-timing results with those from previous analyses, e.g. Salmon R. stocks and Klickitat R. have relatively late runs compared to other spring stocks. Spring Creek tule and interior Columbia R. summer/fall-run stocks were the only two summer- and fall-run Chinook salmon stocks that we can distinguish with GSI and were estimated to be greater than 2,000 fish. Based on coded wire tag stock origin information, we showed our GSI accuracy was high for distinguishing the following three stocks of fall-run Chinook: West Cascade, Spring Creek tule, and interior Columbia R. summer/fall-run stocks. PBT analysis revealed a potentially interesting run-timing difference between jacks and 4-year olds from the same hatchery. In most cases in which the two age classes could be compared from a single hatchery the jacks were found to come later than the 4-year olds. We will need to examine data from a complete generation to understand whether this is an age-effect or a year-effect (i.e. ocean conditions).

For steelhead, there were seven wild steelhead stocks with an estimated abundance greater than 1000 fish passing Bonneville Dam in 2012. Five hatchery steelhead stocks were also estimated above this abundance level. We described three run-timing categories which were most distinctive among hatchery stocks and included an early Skamania summer-run (and Yakima R.), an intermediate run-timing category that contains most wild and hatchery steelhead stocks, and a late run-timing category that arrives after August 25th and includes South Fork Clearwater R., and upper Clearwater R. Characteristics of the steelhead that assigned to Snake River steelhead hatchery broodstock sources generally support the typical A-run and B-run steelhead life history categories. The relatively large (greater than 78 cm) steelhead came predominately from Dworshak and Squaw hatchery broodstock. These fish were also relatively old (2- and 3-ocean-age) and were derived from the Clearwater R. which is one of the regions expected to produce “B-run” steelhead.

This was the first year we were able to analyze sockeye salmon using GSI, and our results provided some useful insight. Typically, sockeye salmon abundance is estimated with a

mark-recapture (recapture via detection arrays) approach using PIT-tagging. However, each of the PIT-tagged fish must survive and be detected at its terminal dam for the method to successfully assign all individuals to a particular stock. Therefore, there is often an “unknown” group of fish that cannot be assigned to stock using this PIT-tag method alone, which provides a potentially useful role that GSI can fill. We demonstrated in this study that the PIT-tag and GSI methods provide nearly equivalent information on the identity of fish to a particular stock. Further, the additional information that GSI provided for the “unknown” category of fish helped to improve abundance estimates on the three sockeye populations from the Okanogan River, Wenatchee River, and Redfish Lake. Wenatchee and Redfish Lake sockeye salmon appear to be experiencing a disproportionate amount of mortality based on the proportion of each stock in the “unknown” PIT-tag category (i.e. never detected at their terminal dam). We also found these two stocks arrive at the beginning and end of the sockeye fishing season, respectively, which suggests one possible explanation is that harvest is disproportionately impacting the front and back end of the fishing season, leading to higher Wenatchee (early run) and Redfish (late run). Tribal and recreational harvest in Zone 6 above Bonneville could account for 45,800 sockeye salmon in 2012, which would be approximately 35.8% of the missing fish that were estimated. The remainder of the missing fish may be lost to other spawning grounds (e.g. Deschutes R., Yakima R., and Snake R.) and harvests further upstream in the upper Columbia R. (Jeff Fryer, CRITFC, pers. comm.). In future years, we will explore stock composition of the sockeye harvest in zone 6 in order to better understand this disproportionate survivorship of Wenatchee and Redfish Lake sockeye salmon.

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