GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS

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Executive Summary

This project combines four inter-related studies from the Accords Agreement that address the following current and future objectives:

Objective 1) discover and evaluate SNP markers in salmon and steelhead and other anadromous fishes. In the sixth year of this project we have continued our use of GT-seq protocols for SNP discovery. Our laboratory has designed SNP panels for five study species (Chinook salmon [*Oncorhychus tshawytscha*] – 299 loci; Steelhead trout [*O. mykiss*] – 269 loci; Sockeye salmon [*O. nerka*] – 93 loci; Coho salmon [*O. kisutch*] – 257 loci; Pacific lamprey [*Entosphenus tridentata*] – 308 loci) and early development is ongoing for a sixth species (White Sturgeon [*Acipenser transmontanus*] – 117 loci). An additional 476 SNP loci have been selected for expansion of the current Sockeye panel and roughly 75% of these should be retained after testing and optimization. The expanded panel is expected to provide necessary statistical power to perform single parent assignment analyses in Sockeye salmon while also improving genetic stock identification.

Objective 2) expand and create genetic baselines for multiple species including Chinook salmon (*Oncorhynchus tshawytscha*), steelhead (*O. mykiss*), Sockeye salmon and kokanee (*O. nerka*), and Coho salmon (*O. kisutch*). Objective two of this project describes efforts to evaluate genetic diversity among populations that will inform managers in the areas of harvest monitoring, and conservation monitoring. Our approach involves the collection, analysis, interpretation and distribution of genotypic data. These data are being compiled as species-specific reference baselines for characterizing Chinook salmon, steelhead trout, and *O. nerka* population structure specific to the Columbia River Basin. The collaborative, inter-agency application of genetic stock identification (GSI) tools continues to provide invaluable monitoring capabilities to understand relative stock proportions in sport, commercial and tribal harvests, as well as monitoring of stock specific run-timing at Bonneville Dam, Lower Granite Dam and other fish weirs in the basin. Moreover, GSI is being used in concert with parentage based tagging (PBT) to monitor trends in hatchery production, harvest of hatchery fish, and population attributes of specific hatcheries (e.g., stray rates, survival/mortality, migratory behavior, hatchery/wild interactions). We continue to expand our PBT baselines throughout the Columbia River basin, and this is providing the means to assign fish from mixture samples to hatchery broodstock of origin.

Objective 3) implement GSI programs for mainstem Chinook salmon, Sockeye salmon, and steelhead fisheries. In this section, we first evaluate the accuracy of our PBT and GSI baselines for assigning to their hatchery brood (PBT; Chinook salmon, steelhead) or reporting group of origin (GSI; Chinook salmon, steelhead, sockeye salmon). Results of the PBT testing suggest a high degree of accuracy for Chinook salmon (>99%) and steelhead (>99%) when the full suite of baselines that an individual could be assigned to are available. Results of the GSI testing also reveal a high degree of accuracy for assignment of Chinook (Figure 1), steelhead, and sockeye to reporting groups.

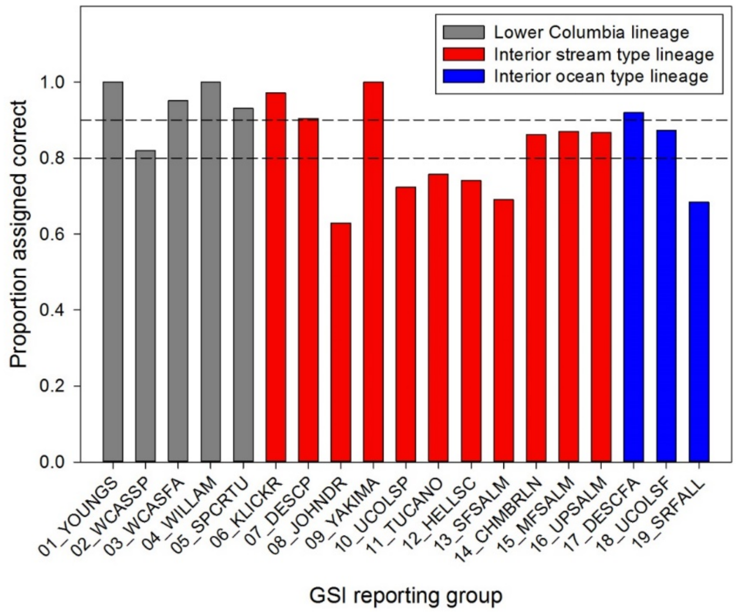


Figure 1: Proportion of Chinook salmon in leave-one-out tests that assigned correctly for each reporting group by lineage. The dashed lines indicate 80% and 90% thresholds for correct assignment.

Upon completion of the accuracy testing, we used a combination of PBT and GSI analyses to determine stock composition of Chinook salmon harvested in sport, commercial, and tribal fisheries in the mainstem Columbia River, and use GSI to estimate stock composition of sockeye salmon harvested above and below Bonneville Dam in commercial, sport, and tribal fisheries during the spring, summer, and fall management periods (Figure 1). We characterized the stock composition of a mark-selective sport fishery from the mouth of the Wind River (above Bonneville Dam) which has recently expanded its fishing boundary into the Columbia River mainstem. We observed that a large proportion (95%) of this fishery assigned to the 10_UCOLSP reporting groups that includes Carson Hatchery and upper Columbia River stocks (Figure 3). Although we did not observe PBT assignments to the Carson Hatchery, our baseline for Carson Hatchery only extends to 2012, and can only assign 3-year-old fish to that hatchery. The 2016 Wind River fishery will provide a better opportunity to observe the proportion of fish from this harvest that can assign to the Carson Hatchery.

Analysis of adipose-clipped Chinook salmon from multiple fishery mixtures in the spring management period (April to June 15th) identified relatively larger proportions of individuals that assigned via PBT to Snake River hatcheries. With the exception of the Wind River sport mark-selective harvest, Chinook salmon from Snake River hatcheries comprised the largest component of each harvest, and accounted for 37-43% of fish harvested in Region B, and 39-64% of fish harvested in Region A from commercial, sport, and Test fisheries (Figure 4).

Analysis of adipose-intact fisheries in the spring management period revealed that PBT assignments to Snake River hatcheries represented 22-34% of the fish harvested; a lower fraction

than that observed for comparable adipose-clipped fisheries, but consistent with assignments for adipose intact fish passing Bonneville Dam (20%) (Figure 5).

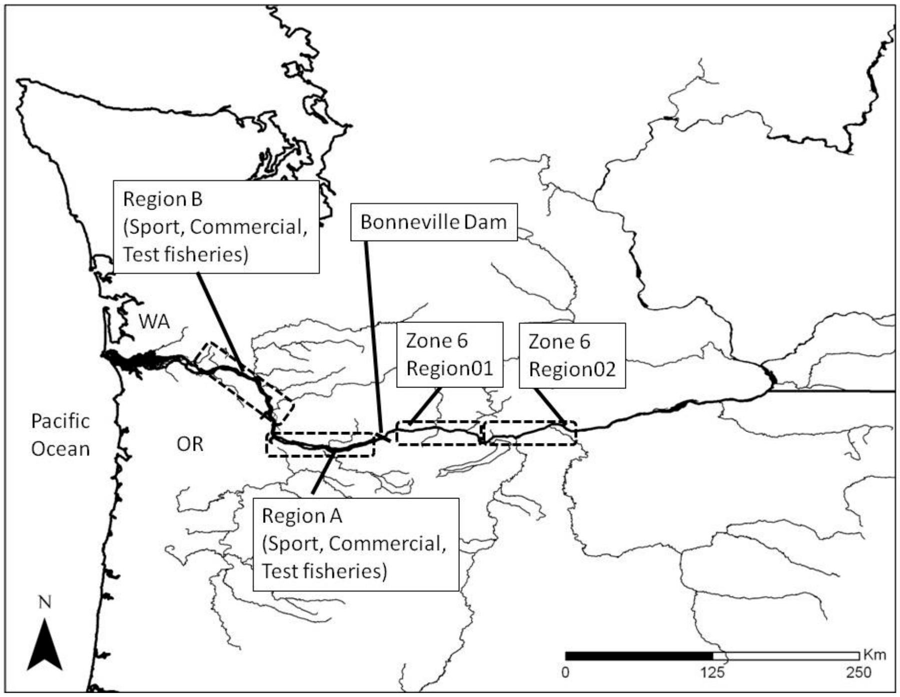


Figure 2: Sources of fishery mixtures in the lower Columbia River mainstem

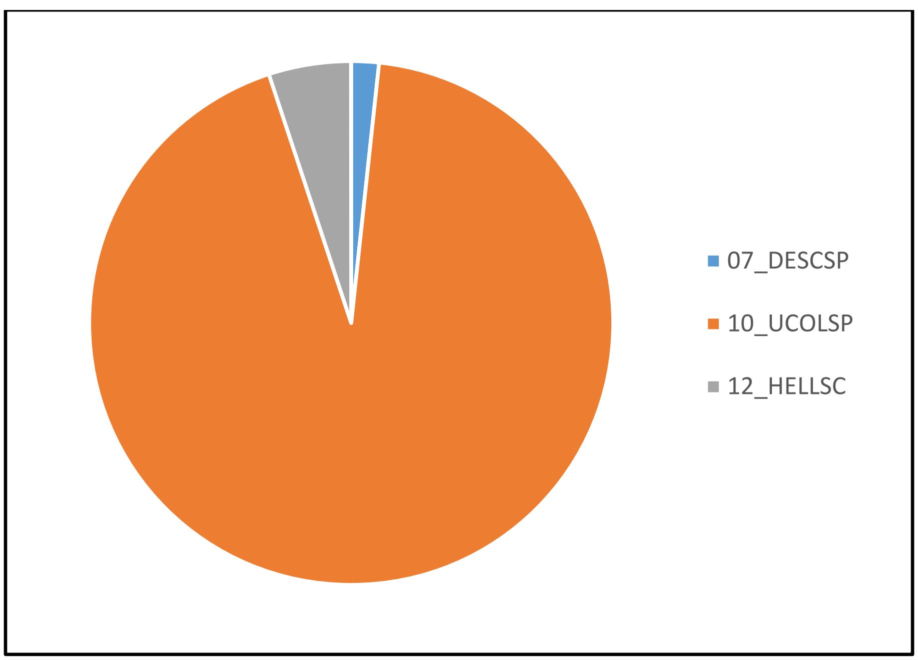


Figure 3: Genetic stock composition of the Wind River sport mark-selective harvest in 2015.

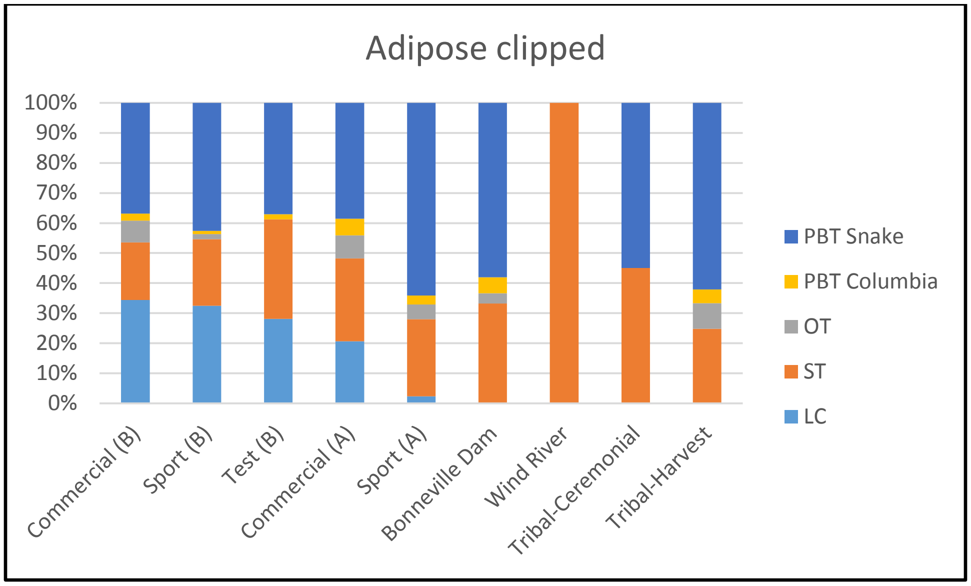


Figure 4**:** Stock composition of spring management period adipose-clipped Chinook salmon harvest mixtures. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

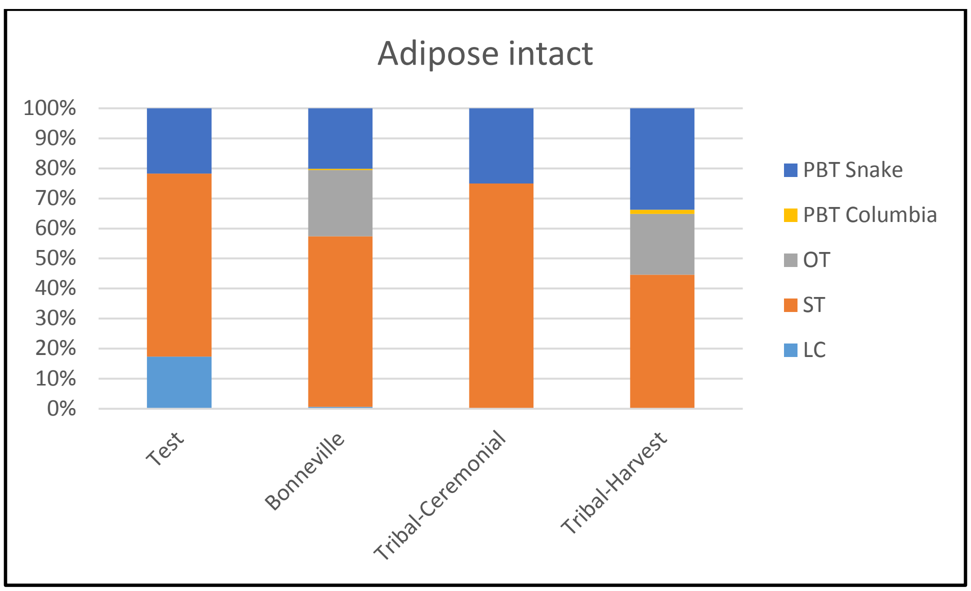


Figure 5: Stock composition of spring management period adipose-intact Chinook salmon harvest mixtures. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

Analysis of Chinook salmon fisheries in the summer management period (June 16 – August 1) focused on understanding the proportion of upriver spring Chinook salmon stocks (ST) that were being harvested. Specifically, we sought to i) estimate the stock composition for sport and commercial fisheries below Bonneville Dam, ii) compare the stock composition of adipose-clipped vs. adipose-intact commercial fisheries below Bonneville Dam, and iii) characterize temporal changes in stock composition across the season.

We observed similar stock compositions for adipose-clipped Chinook salmon taken in Lower Columbia River sport and commercial fisheries from Region B (Figure 6). However, Chinook stocks from the OT lineage comprised a greater proportion of the sport harvest (45%) than the commercial fishery (30%); a similar pattern was observed for Region A (46% vs. 33%, respectively). Additionally, while Chinook salmon stocks from the LC lineage comprised a slighter greater proportion of the commercial harvest (49%) in Region B than the sport fishery (38%), this difference was greater in region A (48% vs. 9%, respectively). A similar proportion of adipose-clipped fish from Region B sport (12%) and commercial (16%) fisheries assigned to Snake River hatcheries (Figure 6). However, for Region A, a greater proportion of adipose-clipped fish from the sport harvest assigned to Snake River hatcheries (41%) compared to the commercial harvest (17%). We observed no appreciable differences between the adipose-clipped and adipose-intact commercial harvest (either within or between regions) in the relative proportion of fish assigned to Columbia River hatcheries or stocks from the ST lineage (Figure 6).

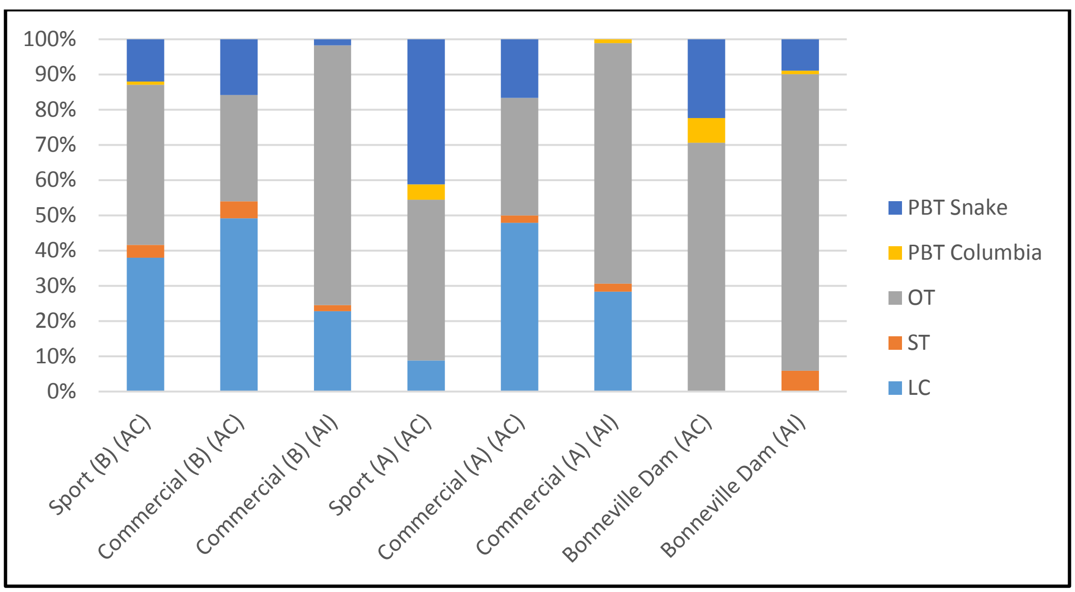
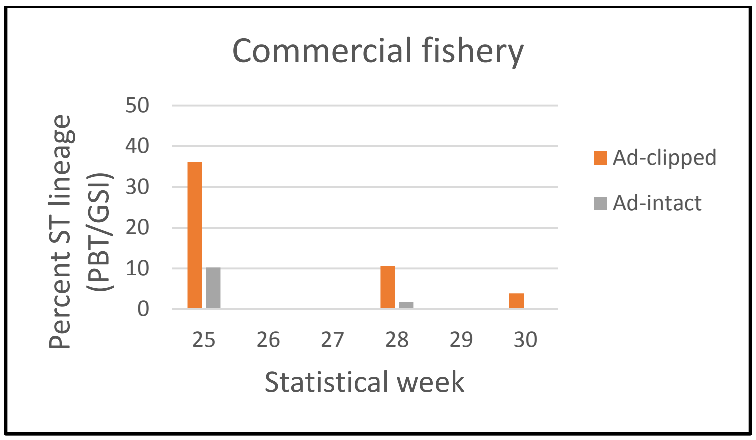


Figure 6: Stock composition of summer management period Chinook salmon fisheries and Bonneville Dam. ‘AC’ is adipose-clipped; ‘AI’ is adipose intact. ‘PBT Snake’ and ‘PBT Columbia’ include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

We compared changes in the % stock composition of the stream-type lineage of adipose-clipped vs. adipose-intact Chinook salmon over the course of the summer management period in the lower Columbia River commercial fishery relative to that passing Bonneville Dam. We detected declines in the proportion of ST lineage Chinook salmon, for both adipose-clipped and adipose-intact fish, in the commercial harvest and at Bonneville Dam over the summer management period (Figure 7). However, there was a modest increase in the proportion of the ST lineage for adipose-clipped fish at Bonneville Dam in statistical week 26. Meaningful comparisons are made challenging by the absence of data continuity over the time series owing to fisheries closures and cessation of sampling at the Bonneville AFF in response to elevated water temperatures.



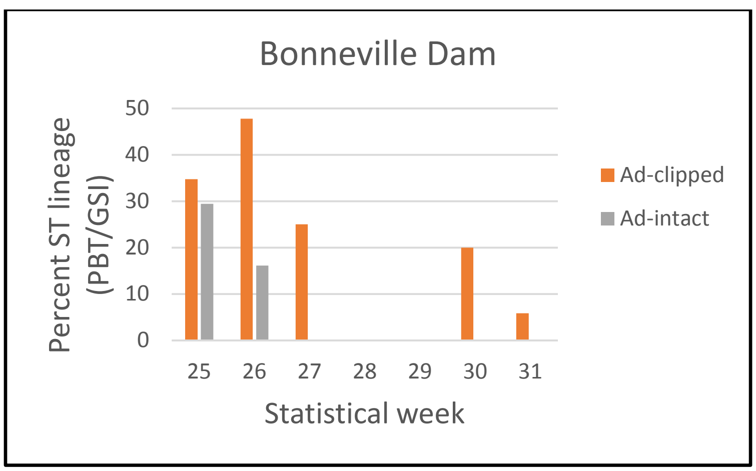


Figure 7: Temporal patterns of the percent of Chinook salmon ST lineage in adipose-clipped and adipose intact mixture samples from the lower Columbia River commercial fishery (top panel) and Bonneville Dam (bottom panel) during the summer management period (June 16-August 31).

We estimated stock composition of the mark selective sport fishery in the lower Columbia River in 2015. Major reporting groups in order of decreasing proportion were: 18_UCOLSF (73%), 03_WCASFA (12%), 19_SRFALL (9%), 05_SPCRTU (6%) (Figure 8). These results are broadly consistent with the 2014 fall sport harvest.

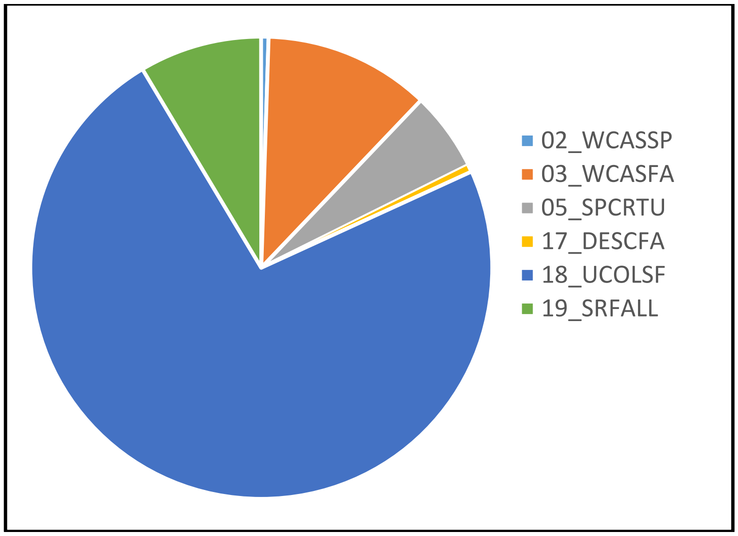


Figure 8: Genetic stock composition of the lower Columbia River fall-run mark-selective Chinook salmon sport harvest in 2015.

Analysis of stock composition of sockeye salmon fisheries included those from the lower Columbia River below Bonneville Dam in sport and commercial fisheries and the Zone 6 tribal fishery. The overall composition of the three stocks of sockeye salmon in these fisheries are shown in Table 1. The proportion of each sockeye salmon stock varied over time (Figure 9).

Table 1: Relative stock composition for sockeye salmon taken in harvests and encountered at Bonneville Dam in 2015.

	Stock proportion		
Mixture source	**Wenatchee**	**Okanagan**	**Snake**
Commercial	31.27%	65.90%	2.83%
Sport	46.30%	52.75%	0.95%
Zone 6	43.67%	54.52%	1.81%
Total Harvest	40.51%	56.72%	2.76%
Bonneville Dam	34.96%	63.48%	1.55%

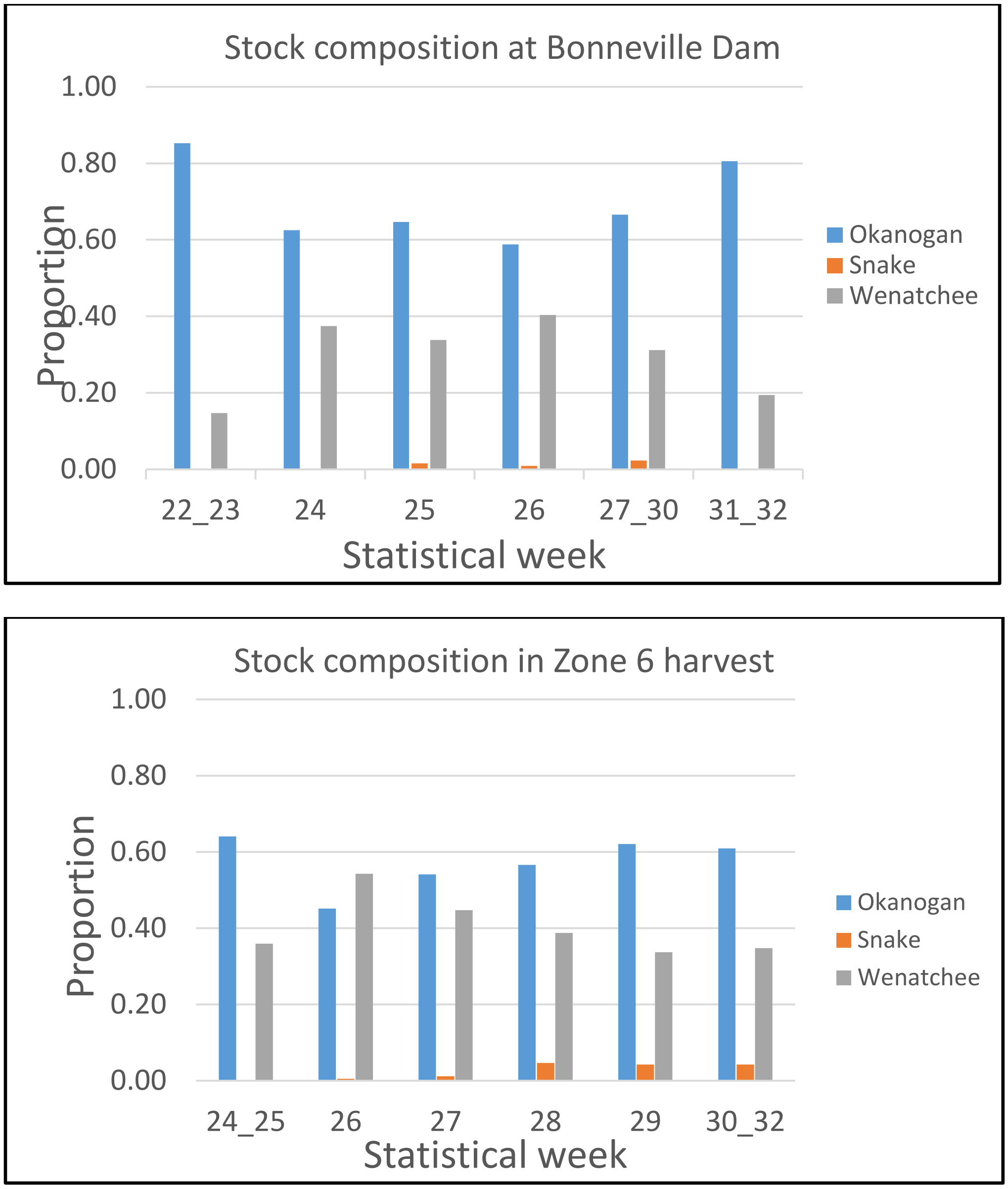


Figure 9: Stock composition of sockeye salmon at Bonneville Dam (top panel) and in the Zone 6 tribal harvest (bottom panel) across weekly strata.

Objective 4) Use PBT and GSI to estimate stock composition of fish passage at Bonneville Dam (steelhead, Sockeye salmon, and Chinook salmon)

Fish were sampled as they migrated past Bonneville Dam. We sampled adult and jack Chinook and adult steelhead during the spring, summer, and fall management periods, and used a combination of GSI and PBT to estimate run-timing distributions and relative abundance of hatchery and wild Chinook salmon and steelhead stocks in 2015.

There were 13 major (i.e., abundance >1000 fish) hatchery origin Chinook salmon stocks represented in the total estimated relative abundance (N=557,403) of hatchery Chinook salmon passing Bonneville Dam in 2015 (Figure 10). The majority of these (n=194,513) assigned to the 18_UCOLSF reporting group. These estimates include relative abundance for PBT-assigned fish (adipose clipped and non-clipped) and adipose clipped fish that were assigned via GSI.

There were 14 major (i.e., abundance >1000 fish) Chinook salmon stocks represented in the total estimated relative abundance (N=818,032) of natural origin (i.e., adipose non-clipped fish that did not assign via PBT) Chinook salmon passing Bonneville Dam in 2015 (Figure 11). The majority of these (n=612,750) assigned to the 18_UCOLSF reporting group.

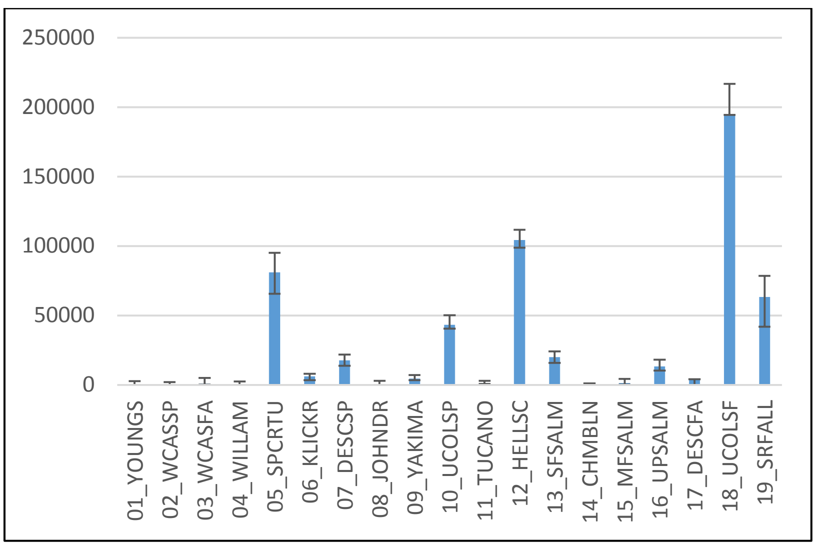


Figure 10: Relative abundance (± 95% CI) of hatchery origin Chinook (adipose clipped and non-clipped) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2015.

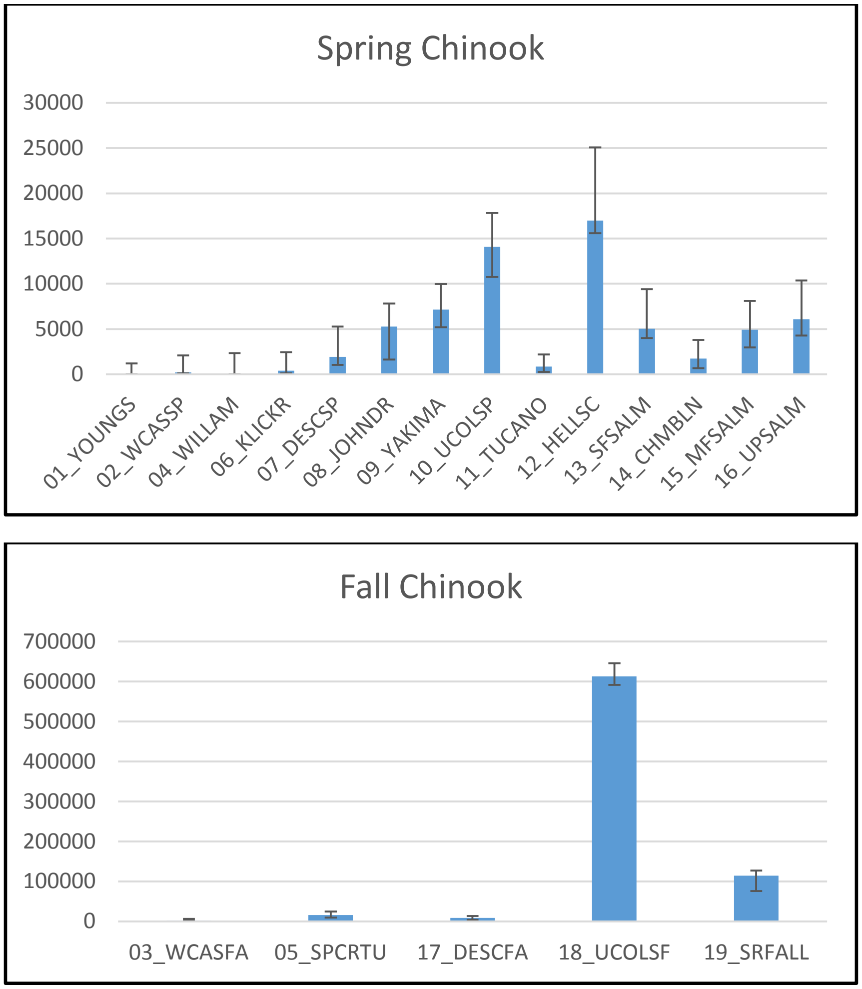


Figure 11: Relative abundance (± 95% CI) of natural origin (adipose non-clipped) Chinook sampled at Bonneville Dam in 2015 assigned to genetic stock of origin. Spring Chinook reporting groups (top panel), and fall Chinook reporting groups (bottom panel) are shown.

We identified five and 11 major stocks (abundance>1000) represented in the total estimated relative abundance of hatchery origin (N=166,201) and natural origin (N=139,120) steelhead passing Bonneville Dam in 2015, respectively (Figure 12). For both hatchery origin and natural origin steelhead, the 07_MGILCS comprised the largest fraction of their abundance (n=63,072, and n=89,315, respectively).

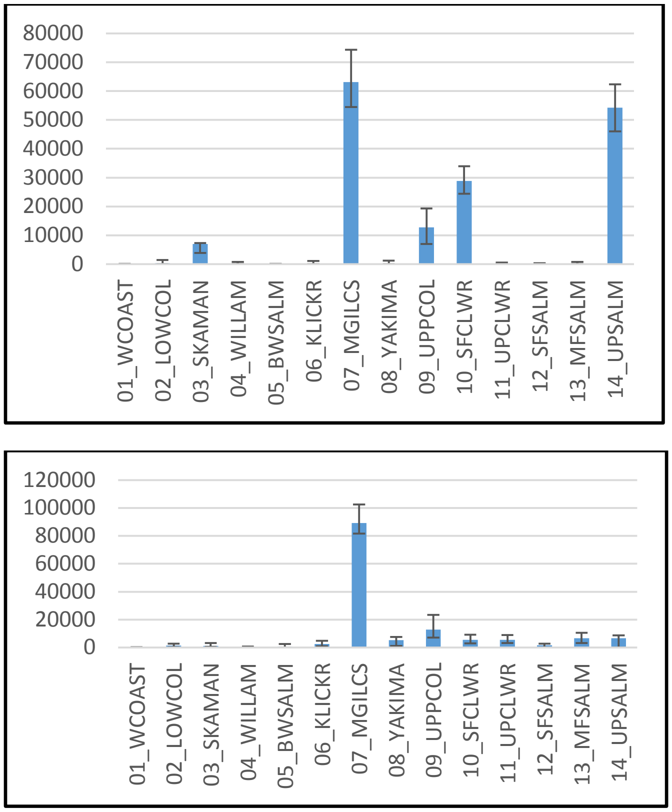


Figure 12: Relative abundance (± 95% CI) of hatchery origin steelhead (adipose clipped and non-clipped) (top panel), and natural origin (adipose non-clipped) steelhead (bottom panel) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2015.

The greatest proportion of sockeye salmon passing Bonneville Dam in 2015 assigned to the Okanogan stock (323,797), followed by the Wenatchee (178,325) and Snake River stock (7,919) (Figure 13).

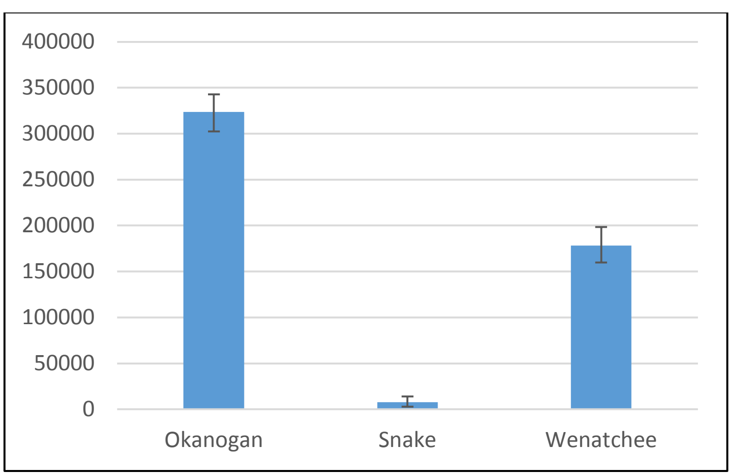


Figure 13: Relative abundance (± 95% CI) of sockeye salmon stocks sampled at Bonneville Dam in 2015.

While the run timing distributions of some hatchery origin and natural origin spring Chinook salmon stocks terminated within the spring management period, several spring Chinook salmon stocks extended well into the summer management period. The run timing distributions for hatchery origin and natural origin fall Chinook salmon stocks all had median dates on or after 8/27/15. For steelhead, we identified an early Skamania summer-run, an intermediate run-timing category that contains most wild and hatchery steelhead stocks, and a late run-timing category with stocks that exhibit median dates after August 25th and includes South Fork Clearwater R. (Dworshak Hatchery), as well as wild stocks from upper Clearwater R, and SF Salmon R. Run timing distributions for sockeye salmon sampled at Bonneville Dam broadly overlapped in 2015.

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Introduction

This project combines four inter-related studies from the Fish & Wildlife Program Accords that address the following current and future objectives: 1) discover and evaluate SNP markers in salmon, steelhead, and lamprey; 2) expand and create genetic baselines for multiple species (Chinook, steelhead, sockeye, and coho); 3) implement Genetic Stock Identification (GSI) sampling programs for mainstem Chinook salmon, sockeye salmon, and steelhead fisheries and 4) GSI of fish passing Bonneville Dam (salmon and steelhead). 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 which can provide information for fisheries management.

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 markers to characterize population variability. These DNA sequence 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), and more recently SNP discovery has become even more efficient for rapid identification of thousands of SNPs using genotyping-by-sequencing technology (e.g., Hess et al. 2013). Mutation rates, mutation models and error rates for SNPs are generally well understood, providing a foundation for estimating genetic divergence between populations. SNP markers offer a more cost-effective and less error-prone alternative to previous 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 lampreys, 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* (including kokanee) in the interior Columbia River Basin. Despite large, representative sample sizes from many populations and high microsatellite allelic diversity, the resolution of specific stocks and populations in these baselines is limited in some cases. For example, Upper Columbia summer and upriver fall Chinook salmon in the Columbia River are closely related and remain impossible 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 (e.g., mainstem Columbia River Chinook fisheries) a finer level of stock discrimination is necessary to match data utilized by managers such as information provided by CWTs. Additional SNP loci will increase stock assignment reliability where greater resolution is desired. Given the difficulty and expense of inter-laboratory standardization, additional microsatellite markers are not the most

efficient choice. In this regard, SNP markers are the preferred option for additional loci since they offer many beneficial characteristics that make them amenable to adding loci to existing baselines.

Objectives 3 & 4) Genetic Stock Identification

Genetic Stock Identification (GSI) methods have proven to be effective in determining the proportion of stock origin in several mixed stock applications (Narum et al. 2008b, Hess et al. 2011, Hess and Narum 2011). This study includes two GSI projects that will utilize genetic baselines: 1) GSI to provide information about harvest; and 2) GSI of fish passing Bonneville Dam.

This study includes GSI analysis of Chinook salmon and Sockeye 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 may include other species such as coho salmon. 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 Confederated Tribes of the Warm Springs of Oregon (CTWSRO). We plan to genotype representative samples from fisheries of primary interest. The GSI estimates may help fill information gaps on wild fish with a different resolution than can be estimated using methods such as CWTs.

The second application of GSI analysis in this study 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 which can be compared to other methods such as using fish PIT tagged as juveniles. 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 in the form of an annual report.

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 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 Snake River Chinook salmon and steelhead hatchery broodstocks (Steele et al. 2011) and we will soon have a baseline that includes most Chinook salmon and steelhead hatcheries located above Bonneville Dam. 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 complimentary, and using them in combination takes full advantage of the strengths of each method, while resolving or minimizing 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. Applications of PBT have been initiated in other species such as Pacific lamprey, and are being used to monitor translocations of lamprey throughout the interior of the Columbia River.

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 stock composition estimates of Chinook salmon, Sockeye salmon and steelhead sampled in mainstem fisheries in 2014. The fourth section includes analysis of run-timing distributions and estimated abundance of adult Chinook salmon, Sockeye salmon, and steelhead stocks migrating over Bonneville Dam in 2014.

Section 1: SNP Discovery

Introduction

Population genetic studies examine variation within the genomes of individuals in order to gain insights into the nature of those populations. For instance, genetic similarities among groups of individuals can indicate relatedness, recent population collapse, or barriers to migration. In the context of salmon conservation, population genetics can answer important questions directly related to fisheries management such as stock exploitation rates, effective population size, and rate of return. Other demographic information such as stock abundance estimates can also be made through analysis of samples taken from fish as they enter the Columbia River through genetic stock identification (GSI). These studies require genotype data from a suitably large and informative set of genetic markers for analysis. Likewise, the number of genotyped individuals must be suitably large to provide accurate results.

Next generation sequencing instruments can provide both a means to identify genetic variation and provide a platform for high-throughput sequencing. Methods such as restriction-site associated DNA sequencing (RAD-seq: https://www.monitoringmethods.org/Method/Details/4144) can be used to identify and genotype thousands of single nucleotide polymorphisms (SNPs) within and among study populations. The most informative SNP loci are chosen for inclusion in high throughput genotyping panels. Genotyping in Thousands by sequencing (GT-seq: https://www.monitoringresources.org/Document/Method/Details/5446) is a high throughput method that uses Illumina sequencers to rapidly genotype thousands of individual samples at hundreds of loci for less than ¼ the cost of previously used TaqMan assays (Campbell et al. 2015). Following the development of GT-seq, our laboratory has designed panels for 5 study species (Chinook salmon [*O. tshawytscha*] – 299 loci; Steelhead trout [*O. mykiss*] – 269 loci; Sockeye salmon [*O. nerka*] – 93 loci; Coho salmon [*O. kisutch*] – 257 loci; Pacific lamprey [*E. tridentata*] – 308 loci) and early development is ongoing for a 6th species (White Sturgeon [*A. transmontanus*] – 117 loci). An additional 476 SNP loci have been selected for expansion of the current Sockeye panel and roughly 75% of these should be retained after testing and optimization. The expanded panel is expected to provide necessary statistical power to perform single parent assignment analyses in Sockeye salmon while also improving genetic stock identification.

The GT-seq method allows for the addition of new loci to existing panels. Soon after new loci are identified, their sequences can be used to design primers for inclusion in the multiplex PCR mix. Additions of only a few primers to the mix require little initial testing before full scale can begin and new SNP loci can be incorporated as they are identified. Such has been the case for inclusion of 8 additional SNP loci associated with adult run timing in Steelhead (Hess et al. 2016). Addition of these loci have now made it possible to genetically

differentiate winter and summer run steelhead which overlap in their return dates in several Columbia River tributaries yet are otherwise largely indistinguishable by GSI.

Methods

For new SNP loci associated with run timing in Steelhead (*N* = 12), the program Primer3 (Rozen and Skaletsky 2000) was used to design primers flanking the target SNP locus for inclusion in existing GT-seq panels. (GT-seq: https://www.monitoringresources.org/Document/Method/Details/5446) Parameters used for primer design are as follows (product size range: 50-80 bases, optimal annealing temperature: 60°C, primer size range: 18-24 bases, optimal GC content: 50%). The designed primers were then modified by including the Illumina sequencing primer sites. The primers were ordered from IDT (Integrated DNA technologies) at a concentration of 200µM at the 25nmole synthesis scale. Testing was done by combining primers from a set of 261 loci for *O. mykiss* that already worked for GT-seq with the newly designed primers. This new primer pool was then used to create a test library containing 96 samples using the GT-seq protocol (Campbell et al. 2015). The test library was "spiked" into an Illumina HiSeq lane with another sequencing library such that the test library produced about 10 million reads of data for analysis. Since the test library uses only a small percentage of the total reads on the flow cell the new library can be sequenced very cheaply. The sequencing reads were analyzed for the presence of significant numbers of hetero-dimers produced in multiplex PCR using custom perl scripts (https://github.com/GTseq/GTseq-Pipeline/). Primers producing large numbers of sequencing artifact reads through primer hetero-dimer interactions were flagged and omitted from the next primer mix. Following this step the primer mix was used for full scale genotyping using GT-seq libraries containing up to 2,500 individual samples for a HiSeq lane or up to 5,000 samples for a NextSeq flow cell.

Results

GT-seq primer pools are being used for all high throughput genotyping projects for 5 target species (Steelhead [O. mykiss] – 268 SNP loci plus sex determination marker, Chinook [*O. tshawytscha*]-298 SNP loci plus sex determination marker, Coho [*O. kisutch*]-257 SNP loci, Sockeye [*O. nerka*]-93 SNP loci, and Pacific Lamprey [*E. tridentata*]-308 SNP loci). An additional 8 loci have been added to the Steelhead panel after dropping 4 of the attempted 12 run timing markers due to sequencing artifacts (Steelhead GT-seq269 panel: Table 1). The remaining primer pools remain unchanged from last year's report but an additional 476 Sockeye SNPs from RAD sequencing data are currently under development (Paired-end data assemblies, primer design, and testing). These GT-seq panels have been used to genotype over 125,000 samples as of Dec. 16th in the 2016 calendar year (Figure 1).

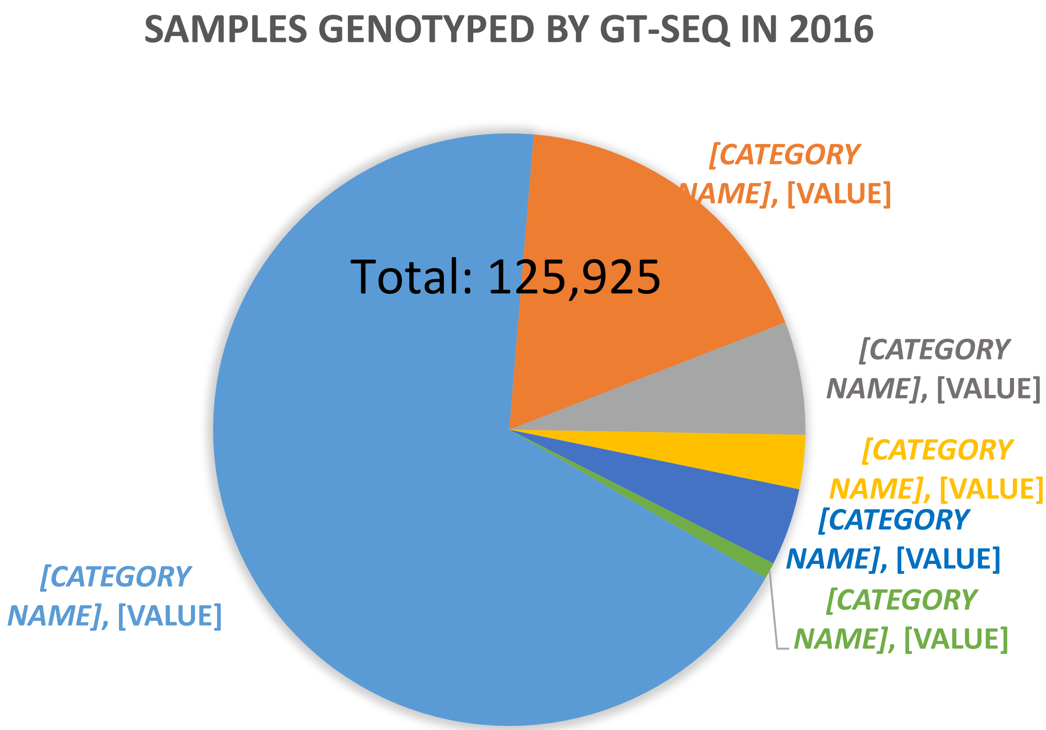


Figure 1: Summary of Columbia River fish samples genotyped using GT-seq in calendar year 2016.

Discussion

The GT-seq genotyping method has allowed for the genotyping of more samples in less time at more loci and at significantly cheaper cost than our previously used method (TaqMan genotyping assays). The total number of samples genotyped using this method is projected to approach 130,000 by the end of the year, greatly exceeding the annual output of any year preceding the use of GT-seq, as well as last year's total of just over 105,000 samples. The inclusion of more loci afforded by this method has also allowed for improved capabilities such as greater ability to discriminate between reporting groups in GSI and single parent assignments in parentage based tagging (PBT) projects. Similarly, we can now take advantage of genetic markers associated with physical and behavioral traits of our study species by including them in our high-throughput panels. An example of this is our ability to distinguish between summer and winter run steelhead by including two SNP loci found to be highly associated with run timing by Hess et al. 2016.

Expansion of our current GT-seq panel for Sockeye salmon is also projected to greatly improve research capabilities in that species. Presently, the panel contains 93 loci which are

sufficient for differentiation of Columbia River populations, but which currently lacks enough statistical power for parentage analysis in this species. Also, this expansion will be the largest panel we've attempted for GT-seq to date (576 target loci) and will give us new insights for the limitations of the technique.

In conclusion, the GT-seq method continues to produce quality genotyping data at a fraction of the cost of TaqMan genotyping assays. The technique uses only general laboratory instrumentation (Thermal cyclers, plate centrifuges, quantitative PCR instrument) for library preparation and the Illumina sequencer itself is used as a high throughput genotyping platform while maintaining its utility for other sequencing studies (whole-genome shotgun, RAD-seq, transcriptome sequencing, synthetic long read, etc.). This is a key feature of the technique since it allows the multipurpose functionality of the laboratory without investment in specialized equipment. Overall, GT-seq is a valuable tool for conservation genetic studies allowing vastly improved statistical power, higher throughput, and prediction of heritable traits at a lower cost.

References

Campbell NR, Harmon SA, Narum SR (2015) Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* **15**, 855-867.

Hess JE, Zendt JS, Matala AR, Narum SR (2016) Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. *Proceedings of the Royal Society B*. **283**, 20153064.

Rozen S, Skaletsky H. (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, editors. *Bioinformatics Methods and Protocols*. Totowa, NJ: Humana Press p. 365-386.

Table 1: Contains the primer sequences, in-silico probe sequences for each allele, and correction values for each allele for each SNP locus included in the O. mykiss GT-seq primer pool. Each forward primer is modified with a 5 prime "small RNA" sequencing primer site (CGACAGGTTCAGAGTTCTACAGTCCGACGATC) and each reverse primer is modified with a 5 prime standard paired-end sequencing primer site (GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT). The additional run timing associated markers are at the end of the list and are indicated by an asterisk.

O. mykiss **GT-seq panel (268 loci plus sex determination marker)**

Assay:	A1:	A2:	FWD:	REV:	A1-Probe:	A2-Probe:	Allele Corrections:
Ocl_gshpx-357	T	G	GAGATCCTGAGGTCCCTGAAGTAT	AAGTGGAAATTTGGGCTCAAAGC	ATCCGTCCAGGAAATG	TCCGTCCCGGAAATG	0,0
Omy_myclarp404-111	T	G	GCTGTGGTGCTCATGGGTAAA	CCAGGGCAGGGTTGTTCTC	CAAAGCCATACGTGGCC	AAGCCATCCGTGGCC	0,0
Omy_Omyclmk438-96	A	C	CCCGACTCTACTTCACTACTTTCCT	GGCCTAGGACAATAGGACTGAAC	TACGCAAATTAGGTTTAAA	CGCAAATTAGGGTTAAA	0,0
M09AAC.055	C	T	GTCTCCGACGTGTGGCT	TGGAACGAACCTGAGAACATAAGG	ACCTCCACGCTGTCC	ACCTCCACACTGTCC	0,0
OMGH1PROM1-SNP1	A	T	TCAAACTGCATTTGATGGAAACAAACAT	AGGACAATTCTAAGTGACCTCAAACTG	TAGTGTTCACTGACTTCA	TAGTGTACACTGACTTCA	0,0
OMS00003	T	G	GTGCCACTGATGAGGATGAGATC	GTAATAAAGCCCTTTTGTGAGGAAAAACTAAT	CTTTACTGTCGACATTTTA	TACTGTCGCCATTTTA	0,0
OMS00008	A	T	CCCTTTAAGGAGGATTTTAAATATGTGAGATAGAA	GGATACAGCGTTTTGGAATGAAACT	CTTCAAATATCCATAATTATATC	TCAAATATCCATAATAATATC	0,0
OMS00013	A	G	GCCTTTGTTCTCCTTGGTGGTTA	AGAAAAGTGTGGACTGAGGTTGAG	CTTCTTTTCCCTTGCTACTC	CTTTTCCCTCGCTACTC	0,0
OMS00014	T	C	CTTACACACAAGGGCTTCATTCTG	GATGTCTCTGGGTGGTTGTCA	TGATTTGATGAATTAAACTTC	TTGATGAATTGAACTTC	0,0
OMS00015	A	T	TCAGACCCTATTTTTGGCACAAGT	GTCTAACTGATCCCACTTCTGCAT	CAAGTCACACTTTTAATGAA	CAAGTCACACTTATAATGAA	0,0
OMS00017	A	G	ATTAAGTTCATACAAAAGTTCATCATAAATATTTTCCTTT	GGAGAACAAAGGGAAAGAGAAGACA	TAGACCTCGGTGCTGTAG	CCTCGGCGCTGTAG	0,0
OMS00018	T	G	AGAGTACATGTGTGGCTGCAA	GTCATAAATCAACACAATTATCTTCTTCACAGAA	AACCACATAATTAATAATTC	CCACATAATTCATAATTC	0,0
Omy_cd28-130	T	C	CACAACTCCACAGAGACAGTGA	GAGGACAAAACTGACCGTATGGT	CCTGTTCATTCACCC	CTGTTCGTTCACCC	0,2.7
OMS00030	T	G	CCTCGTGACTACAGAGCTATACAAC	GATCTGATCGGTCGGGAGAGA	ATGAGGGTCCCTATACAGG	ATGAGGGTCCCTCTACAGG	0,0
OMS00048	T	C	GGAAGAGCTGGAGAACAACGT	TGCAGTTGACAGAGGCTTTCTTT	CAGCTAAACTCAGCAAAA	AGCTAAACTCGGCAAAA	0,0
OMS00052	T	G	TGCGTTTTTCATCCCAATCATTCAC	GGCATCAGGCTCTTCTTCCT	CTTCCTTTTGAGAATAAT	CCTTTTGCGAATAAT	0,0
OMS00056	T	C	TCAGGAAGTAAACTGAAAATTCCAATGTATGA	CCCCAACCATGCTTGTTATTGAAC	TAGCTTGACCAAATAGCA	CTTGACCGAATAGCA	0,0
OMS00061	T	C	AAGTGGAGGCTGACCTGTTG	GCTGATGGCACCTGACAGTTAATT	CATTGCCATTTACAGACTT	TGCCATTTGCAGACTT	0,0
OMS00092	A	C	TCTCCAGGTGTATCTTGAGAAGGT	AGGGTTCACACAGGGAAGATATCAT	CAGCTGAGAATAGGTTC	AGCTGAGAAGAGGTTC	0,0
OMS00096	T	G	CATGAGAATGGATCAGTCTCCACAA	GATGAAATCTGAATGTGTTGACACTACAG	AAAGAGGAAGAGTCTCG	AAAGAGGAAGCGTCTCG	0,0
OMS00087	A	G	GCAAATTTCACCCTTAACGTGGTTT	GATTTGATGTGTGTGTATTACCTCCTCTA	GTTA[CA]AACTGACAAAGTGTG	GTTA[CA]AGCTGACAAAGTGT	0,0
OMS00119	A	T	AGCGGCAGTTGTGTTAATGAGA	CTTCCTAAAGCCTGACAGTCTGT	CCACACAGCTGCCTGT	CACACAGCAGCCTGT	0,0
OMS00129	C	G	GGAGATGATGAAATAAAAATTGAGGAAAAGATGA	TGTCTGGTGAATTATCGCAAATAACCA	TTGAACAACAAGAAAAA	TTGAACAACAACAAAAA	0,0
OMS00133	A	G	GACCACTTCACTCATTCCTCCTTTT	TCCGGTTTACACACTTCATGCA	CGCCTCCATCTTTGTGGT	CGCCTCCATCTCTGTGGT	0,0
OMS00138	T	G	TCGGACCACATGAGCAGTTC	GTTCAACAGGTGCCCACAC	CTAACAATAACCAAAGACTG	CTAACAATAACCACAGACTG	0,0
OMS00149	T	G	GGCATCATTGTTCTTGCTCTGTTTA	CCTGGGAGGGTTTATATCGGAGTAT	GCTAAATGCACAG	GCTAAAGGCACAG	0,0
OMS00151	A	G	CTAACGTCTTCCCAATGATATTTCACAAGATA	ACCGTGGAAATACAATTTTTTATGCCAAT	TCATGACCTTGATAATC	ATGACCTCGATAATC	0,0
OMS00095	A	T	CTCCAATGGCTGTCAACAATTAAATATAAGAC	GTGTGCTGGTCTCTTCTTTTATTCTCA	AGGCAACTATATATTTTTTT	AGGCAACTATATATATTTTT	1.5,0
OMS00169	A	G	AGCACTTGACTCAAACTCACATAAATCA	CTGAGACAGGAAGAACAATGTTAACAAAA	CAAAAAGCATTGATATCAAT	AAAAGCATTGACATCAAT	0,0
OMS00173	T	C	TGGAAGTAGCTACTTAACAGGAAATGG	AACACGTGTGCTTGTTTTGTCAA	CATTAGCTTGTGTATGAACT	ATTAGCTTGTGTGTGAACT	0,0
OMS00176	T	G	GTTGGAAGTTCCGGTGGTAGAG	CTGGGTCCTGAAGGAGCTT	TTCCAGCACTGCTGTC	CCAGCCCTGCTGTC	0,0
Omy_impa1-55	C	T	CGCTGAGAGGATTGTCAA	TTTTCTTTGTTCAGTCTTCTGTCTCTG	CGAGATGATGCGTCTACA	CGAGATGATGCATCTACA	0,0
Omy_103705-558	T	C	CTCCAATCGCAAATACCCAGACT	CGCAGGAGACGGATGCC	AGACTTACCCAGAGTGAGAG	ACTTACCCAGGGTGAGAG	0,0
Omy_105075-162	T	G	GGAGAAGGACAAGGACATTGGTAAT	AAAGCAGACCACACCATACTTCTC	CTTTCTCTCCTACTTTCC	CTTTCTCTCCTCCTTTCC	0,0
Omy_107031-704	C	T	GGCTTTCGGATACTGAGCAACAA	TGAACTCACTGTTGGTATGGACTAGA	TGGACATGATTGCATAGAC	CTGGACATGATTACATAGAC	0,0
Omy_107285-69	C	G	GCCCTTGTGACAATGCACTGTTATA	AGGTCTAGACAGTGTGCCATTTG	ATACGTTACTTTTGACCTTGT	ACGTTACTTTTCACCTTGT	0,0
Omy_110201-359	T	G	GGTAAGGCCTGTCTGACTATTTGA	AGAGGTCAATGGATGCCAGTTT	TTTGGCTATTGAAATTATACATT	TTGGCTATTGAAATTCTACATT	0,0
Omy_CRBF1-1	C	T	AGTTCCGTACGGTAGCCTATTCTA	CGCCCGGGTGAGAGTAATTG	CAGAGTCGCCAAAAT	CCAGAGTCACCAAAAT	0,0

OMS00114	T	G	GGATGATGCTGTGAGTCGAGAAG	ACCTTCGCCACCCATGTTTTATT	AAACGTTTCACATGCACC	AAACGTTTCACCTGCACC	0,0
OMS00143	T	C	GGAGGCACGCCCCAAA	TTTGTTAAAATAGAGCCCTTAGTGGGTTT	CCTGATCCAGAATCTAGA	CCTGATCCAGAGTCTAGA	0,0
OMS00174	A	C	TGACTAACTATGCAGCCTGAAAGG	GGGATACTCTTGTAATAAACTGTTGGTTAGTA	CAAGAACAGG[AC]TAAATGT	CAAGAACAGG[AC]GAAATGT	0,0
Omy_97077-73	T	A	GTGTAAACAAAATGACTCTGGGATTCAG	AGAAGTGGCAATGGTGTGAAGTAT	TGGTGCAATAGAAATA	CATGGTGCAATAGTAATA	0,0
Omy_97865-196	A	G	TCCAGACTTCTGGTTTGTTCCATT	CCAGCCCCTATATTCACAATTAAGTGT	ATTAATTAACAAGCTC	ATTAATTGACAAGCT	0,0
Omy_97954-618	C	T	GCTCTGCTTCCTCGGCAAATA	CACAATTGGTTTTTGCACAAAAGTAAAGTATT	CAACGCTTACCGGTGTGT	CAACGCTTACCAGTGTGT	0,0
Omy_128996-481	T	G	CTCATCCACACTGTACAGTACAAGT	CATGCCTTCGTCTCATCAATAACAC	CAAACCTCAACCAC	CAAACCGCAACCAC	0,0
Omy_aromat-280	T	C	CTCCATTGATTCATGCCGAACATT	GGAGAGGTCAAACATAGCCTGGTA	TCTTGCAAACTCC	TCTTGCGAACTCC	0,0
Omy_aspAT-123	T	C	GCCCATTTCACTGATGCTGTGA	AGGAGACCACTCCAAAGAGAACT	CCTTCCTAGGCAGTCAG	TTCCTGGGCAGTCAG	0,0
Omy_b9-164	T	-	GCACAGAACACAGCCAATATTAACA	GCCTTGACTCTCCCTTCATGAC	CCTACAACTTGATCTAACGTG	CCTACAACTTGATCTACGTG	0,0
Omy_BAC-F5.284	C	T	CCTCATTTACTGTAGGACCATGCA	ACAACGCCAACAACTTTCTCTTG	CAGTAGGGCGGCAAG	ACAGTAGGACGGCAAG	0,0
Omy_BAMBI2.312	G	T	CGAGCTCATGTCCGAAACTCAT	TTTGACAGCCTCAACTTCTAGGG	CCGAAAGTTCAACTTT	CCGAAAGTTAAACTTT	1.7,0
Omy_carban1-264	G	A	GCAAAGCCTCATCTTCAATCATTTGT	GCAAAACACAAGTCAGGAATCACTTA	CATTAATATTGCTAATAACACCAAG	ATTAATATTGCTAATAACACTAAG	0,0
Omy_cd59b-112	C	T	TTTGGATAAGATTGTCTTATATGACTAAAATGTCATGT	GCCAACGTCCTAGATATGGTGTAAT	CTAAAAGCCTATAGCAAACT	CTAAAAGCCTATAACAAACT	0,0
Omy_cin-172	C	T	CGCATGGGACAGGTGTGT	GAGAAAGCCTGTAGAACCATGTCT	CGCTCACCGTGGTTAC	CGCTCACCATGGTTAC	0,0
Omy_cox2-335	T	G	AGCTGGGCTGTATTTGTCAATACTT	CAGCCCGCCACTGTCT	CTTTAAAGACAAAGACTTTAT	TTTAAAGACAAAGCCTTTAT	0,0
Omy_e1-147	G	T	GCACTGACTGTTACCAGGAAAGAG	GTACTGCAGTGTTGAGGCTATATCA	CCATCCTGAATCTGATTAA	CCATCCTGAATATGATTAA	0,0
Omy_g1-103	T	C	CTCAGCAAAAAAGAAACGTCCCTTT	AGTCGTGACAATGAGAAACAGTGTT	CCTTTTACAATGAAGATC	CTTTTACAGTGAAGATC	0,0
Omy_G3PD_2-371	C	A	GCAGGTAAGGTACACCATAGAGACA	CTCCCCCTGCCTTACCAAAC	AGACATGTGGATTGGCA	CAGACATGTGTATTGGCA	0,0
Omy_gadd45-332	T	C	AGAGAAGACTCACTGCTGTTTGC	AAATCAGTTCCCACGCTATGCT	TTGCTCCAAAATGG	TTGCTCCGAAATGG	0,0
Omy_gdh-271	C	T	AGGTCAGTCTACTTACAGTATAAAGCAGT	GTCATGTCAACAGAGTAACATAATAAATCTGC	TCACCCTGAAGTGTAGAC	TCACCCTGAAATGTAGAC	0,0
Omy_gh-475	C	T	AAGTTACCAGAATTTTGCAAACTCAACT	CCATATTTTGAGGTGTAGCTTTACCCT	CTGAAACTCATGGTATACA	CTGAAACTCATGATATACA	0,0
Omy_GHSR-121	T	C	CTGTGTATAAGTTTATACAGTCAGCACAGT	TTCAGAGAGAGAAATGGCAGAAAGG	CCTAATAACCATGATAACAGC	AATAACCATGGTAACAGC	0,0
Omy_hsp47-86	T	A	CACATTAAGCACTCCCAGGGA	TTGCAAAGGCCAAACAGCATT	CAGGAGTGTAAATGTTT	ACAGGAGTGTATATGTTT	0,0
Omy_hsp70aPro-329	A	G	TGCGTATTATTGTTTTTCAAGGACTTTCAAA	TGAATATTTTCAAATACATGCCAATTCTTTCCAA	ACATTCCAATATTCAACTAT	CATTCCAATATCCAACTAT	0,0
Omy_IL1b-163	T	G	GGAACAACAGGATTAAGCCTACTCT	CCTAAAGGCCTAGGAAACTAAACTTCA	CTGAGGTCATAAAAATA	CTGAGGTCATACAAATA	0,0
Omy_inos-97	C	A	GATGGACAGGGTCCTCTTCAC	CCTGTAGATAAAACATGGTACCAGGTC	CCTTTCTTGATGGTATCC	TCCTTTCTTGATTGTATCC	0,0
Omy_LDHB-1_i2	C	T	ACGCACACTTATCCTTGACAATGTT	ACTGTGACAACAAATTCGGTGACA	ATGGGCAGTCATTCA	TGGGCAATCATTCA	0,0
Omy_LDHB-2_e5	T	C	TGCTAGGTGAGTCAGAGGTACATATT	GACTGGAAGGCCACCCATAAG	TTTACCTGTCAACCACTTC	CCTGTCGACCACTTC	0,0
Omy_LDHB-2_i6	G	T	TCCTCGCCAATACCATACATGTC	AGAGTGAAGCTAACACACACATTTCT	CTGTGTTTTGCTTCCCCA	CTGTGTTTTGATTCCCCA	0,0
Omy_lpl-220	C	G	TGACAATCACTGAGCAACTGAACTC	GTCCAGTCTTGCTTCAACTCATTCT	AGTTACTCAGTGACAGTCA	AGTTACTCAGTCACAGTCA	0,0
Omy_mapK3-103	A	T	GAAGTCATTACTGGTCAGTGGTCAA	GCACAAAACATGAGGAAAGTTGAGA	AATTATTAAGCCTATTTTTTT	ATTATTAAGCCTAATTTTTT	0,0
Omy_mcsf-268	T	C	CCAGCATTCGTTCCCATTTCC	CTTTTAATGTAGATTATATTCTTCTGTAGCCACTATGG	AAATAATAGATAAA[CT]CCT	AAATAACAGATAAA[CT]CCT	0,0
Omy_metB-138	T	A	TCTGTCCCTGACGCTATAAAAACG	GAAGTATTTCAGCTTAATTTCACTGTTGAGTT	TTCGCCAAAGAGAAAT	TTCGCCAAAGTGAAAT	0,0
Omy_myoD-178	A	C	TGGCAAAGCTGTCATTCCTTCTAAT	GGTCAAATATTTCATTTACGATTACACTTAGGC	TTTTATGAGATATAATTTCC	TTTTATGAGATATCATTTCC	0,0
Omy_nach-200	A	T	CTCATGAAAAACGGGAGAGCAAAG	CAGCGGCTCTTCAGTAGTCT	AACTGACAGAGTCACAAC	CTGACAGAGACACAAC	0,0
Omy_nxt2-273	C	T	CTTTAGAAAAGCCAAGGTATATTTTAACATACTTCT	CTGCTGCCCTCTAATGGTAAGATAG	AAGGCACAGTAAATGT[CT]GAT	AAGGCATAGTAAATGT[CT]GAT	0,0
Omy_OmyP9-180	C	G	CTGGATGTGTAGTATCGGTGGAAAA	CACTGGGCACCTCTGATCTC	CTGTAGTAGTCCCCATTGT	CTGTAGTAGTCCGCATTGT	0,0
Omy_pad-196	C	T	CAAACAACCACAGTAGTCCTCCAAT	GCTTTTCACCCTTTTGTAAATTAAGCCAAA	AAGACAAAGGTGTAATACC	AAGACAAAGGTATAATACC	0,0
Omy_ppie-232	C	T	CTGTTTTAGATTAGAATGTTTTTGGTCAGGT	CTGAACATAGGCTTTCATTTCAGACAT	AAATAGCGGAGAAAAT	AAAATAGCAGAGAAAAT	0,0
Omy_ca050-64	T	G	GTCATACAGAACTGTTTTGTTGTGTCAA	ACCTTGAATTGGTTCCTAATGCTATTGT	CAGTTTGAAGAATATACTC	CAGTTTGAAGACTATACTC	0,0
Omy_sast-264	G	A	GAAGTAGGGTTTGTTGACCATGTGA	TGGATTCCATTTTAGGCTGTAATACATCTT	CTAGCCAATGCGTCTAA	ATCTAGCCAATGTGTCTAA	0,0
Omy_SECC22b-88	T	C	GGATCCCTCCTTTTAACACAAGACT	CTACAGGATGACTACCTAATTGCTAATAAAACA	CTGTCTGTCCATATATC	CTGTCTGTCCGTATATC	0,0
Omy_sSOD-1	T	G	GCCGGACCCCACTTCAA	CAGACTAACCGAACAGCATCAGTGG	CCACAACAAGACCC	CCACAACCAGACCC	0,0
Omy_star-206	A	G	CGTGTGCCAGCCCTTCT	GACCACTGAGATCATTGCTGTGA	TCTTTGGCACTATATCT	TTTGGCACCATATCT	0,0
Omy_sys1-188	C	A	CTTAAATGGTGCTGGTTGCTGTATT	AGTGATATCTTAGTGGGTCGAGGAAA	AAACATGTACGACCTGTC	TGTAAACATGTACTACCTGTC	0,0

Omy_tlr3-377	C	T	GTCGCTCCGGGTGCTT	GGCCCAAACACTTCCTTCCT	CGTGATTAGGTTCTTC	CGTGATTAGATTCTTC	0,0
Omy_tlr5-205	T	A	GAGCGTATCTGGTATGGTAACAACA	CTCCAGCAGCTTTAGAGAGTTTACA	CAGTAATATTTCAGTGCCCG	CAGTAATATTTCTGTGCCCG	0,0
Omy_hsf1b-241	A	-	AGCCCGAACTATCCTAAAGCATTTT	AAATCAATAGCTCAGAGAATAATGAACACCA	CAGTGTTTTGTTTTTTGTCATT	AGTGTTTTGTTTTTGTCATT	0,0
Omy_u07-79-166	G	T	CCCGCTATATTATTTGATCACCCTTGA	ATTTAAATCCATTTCTAAAAATAAGCAAACCTAACCA	ACTTGGGAATACCCCAGCC	CTTGGGAATAACCCAGCC	0,0
Omy_u09-52.284	T	G	TTTGTGTGTATTGTTGTGACTTG	TGATGTTATTGCAGGTCTAGCGAAA	ACTGCATTGTTGTAGCTAG	CTGCATTGTTGTCGCTAG	0,0
Omy_hus1-52	G	A	CTTGCCGGAGGGTAGCT	CCACAACTTCTCAAATGAATGGAATGT	CCCATCCCTCCTCCTGG	CCCATCCCTTCTCCTGG	0,1
Omy_u09-56.119	T	C	CCAAGGTGGACCCACCAG	GCTGAGTTTATAGGTCAGTCATTATACATATTGA	AGTGAGCTGAAACAGAGCA	TGAGCTGAAGCAGAGCA	0,0
Omy_nips-299	T	-	GACAGGATAGGAACGGTTTCTCAAT	ATCAGAAGTTTAATTCAATATGTACACGATCCT	CTGGATTTCACATGTAATAC	CTGGATTTCACGTAATAC	0,0
Omy_UT16_2-173	C	T	GACTCATTATCACCTTAGTTGTAGCTTCA	AGCTACTTGCTGTATCACATGTTTGT	ACAGTCAACAAGGGACTTAA	ACAGTCAATAAGGGACTTAA	0,0
Omy_vamp5-303	A	-	CTGCTTCCCAATTCAGTATCGTCTT	AGGCTGAAGCATTTCTGAGTATGAA	TGGCCGTAGTAGTTGGTCA	TGGCCGTAGTTGGTCA	0,0
Omy_zg57-91	C	A	CACTCATACACTCACTCACAAAGGA	AGCAGATAAGCCTTGTGAGTGAATCTT	CACAGACTGCACAGCC	CCACAGACTTCACAGCC	0,0
Omy_ndk-152	A	G	AAGAATTGAGGGATAAAAACAAAATAATATATAAACATGA	CAAACCTACATTCATTAAAGTCCAGTTTTGT	CACCCACTTTCAAAAC	ACCCACTCTCAAAAC	0,0
M09AAD.076	T	C	ACTGTTACCACTCTCTCATCAACCT	GGGTCCAGGAGGTTTTTAAACAACAT	CACCAACCACTGGTGAA	CCAACCGCTGGTGAA	0,0
M09AAJ.163	G	A	TCCCATGGCCCTTACTCTATCAA	TTGAGGTGTATGTTGAAAAGTAAACTT	AACAAAGTGAAAGTGTCCTTA	CAAAGTGAAAGTGTCTTTA	0,0
M09AAE.082	T	G	CTATGTGCAGTGCCCTTCTCA	GGCTTACAAGTATGCATGACTAGCT	AGGTTGTTTTACAAATTTAA	AGGTTGTTTTACACATTTAA	0,0
OMS00002	A	C	TTTGATTTGATTTGTATCTGCTTCTT	CCAACATGCCTCACACAAAA	TGTTTTGCAGCGCTC	TGTTTGGCAGCGCT	0,0
OMS00006	T	C	TCCACGTAGGACATAGTTTGAGCTA	TGTGGTGTCATGTTTGCCCTAC	CACTTACAAATACAAAATT	CTTACAAATGCAAAATT	0,0
OMS00024	T	G	CACATACAACCATCACCCTTCCTAA	AGCATTGAGCGAAATTACCAAGAGT	AA[AC]CCCAAATTTTAC	AA[CA]CCCAATTTTAC	0,0
OMS00039	A	G	GTCAGTACTGTGTGTGTCTGTGT	CCATCTACATTGTCAGCAGTGTGA	GTACGTGTCTCTGACC	GTGCGTGTCTCTGACC	0,0
OMS00053	T	C	GGAGCCAGGTCAAGGTGATC	GGATGTCTGGTGTGGCTGTAAA	ATTTATATGTATCAATCA	ATTTATACGTATCAATCA	0,0
OMS00057	T	G	GAGAAAGGGAGCATGAGACAGAG	GTTGGGCTCCGGTACGAT	CTCCACAGAACCTTG	CTCCACAGCACCTTG	0,0
OMS00058	A	G	GTGACATTTGGAGCCACTGC	GCTAGGAGACAGAGGGTGAAAG	CAACACTTTGTACCCCTC	CACTTTGCACCCCTC	0,0
OMS00062	T	C	ACCCTGGGAAGGCTACTGTAC	TGAACAGAGATCTGGAGAGTTGGAT	TTGACCAGCAGATGGTGTA	ACCAGCAGGTGGTGTA	0,0
OMS00064	T	G	GTGGATATGTAGTTCGATGGAACAGT	TTTACAACAATCTTCTTTTAATAAAAATATAGCCACTTAT	CAGGCAACATTTTATATAACTA	CAGGCAACATTTTATCTAACTA	0,0
OMS00068	A	G	GCACTAACTGGACAACATTTTTAAGAATGA	GGCAGTTGAGCATTTTGGGATATT	AATATGCCTCCTTCGTCTC	TATGCCTCCTCCGTCTC	0,0
OMS00070	T	C	CGTTCCTGCGGGACAGT	GTTTCTCTCACGTCCACAGATCT	CAAAATACGGAAATGCAG	AAATACGGGAATGCAG	0,0
OMS00071	A	G	CCGGAGTGACCTCACATTTGG	GCATCGTACAGTTCACCTACCT	CTTGTTTGAGCTTTTTCT	TTGTTTGAGCCTTTTCT	0,0
OMS00072	A	G	GTGGGAGAGCTCGTCTATGG	ACAACAGGTCATTGGATGTGATCAG	TAGAAGGTCCATGTATCTC	AAGGTCCATGCATCTC	0,0
OMS00074	T	G	CCTGTTTATTCATCTAAACCAGTTCTTTAAAAT	AACTTAATTTAGCAAACAAATGTCTGAACAGAA	TGAAACAAAACAAATGTTCC	AAACAAAACACATGTTCC	0,0
OMS00077	C	G	AATACCATCTTGAGCTCATTAGTAATTATTCAA	CCAGACTTTACACACTCTTGACTGA	TTCCGGTGGTGAAGTT	CCGGTGCTGAAGTT	0,0
OMS00078	T	C	GAGGGAAGCAGCCATAAACAGAATA	GTCTCACTATGGTCCATATCTGTGTAGA	TTCACATGCATAAGAGTG	TCACATGCATGAGAGTG	0,0
OMS00079	T	C	GTAACATTATGAATCTATCAGTTTCCCTAGCT	ACCTGCAACGTTAGAGCTGTTTATT	CTACTTTTCACAGTAACACAG	CTACTTTTCACAGTGACACAG	0,0
OMS00111	T	C	CATGCGGACCTGCATAGCT	GCTTAGCCATTGACAGAGCATATCA	CAACCAGACTACCATTC	AACCAGACTGCCATTC	0,0
OMS00089	A	G	GCACCATTTGAATAAAAAATCTGCTTTGT	GCAACCCAATTCAATATTAAGCACATGAT	ATGAATCCCAAATAAGAAC	AATCCCAAACAAGAAC	0,0
OMS00090	T	C	AGGGCACAACACCACTCTAAATT	TCGAAAAGCAACATCTGTCTCAGT	ACAACCACACAAGATT	AACCACGCAAGATT	0,0
OMS00101	A	G	GCGTGTCGTGGGTCAGTTAAATA	GTGCAATCCAACCTATTAGTAGATATGCT	CTCTAGTAGCCTTATAGAAAG	CTAGTAGCCTTACAGAAAG	0,0
OMS00105	T	G	ACATTTGAAGTCAGTATGGGTGTTGAG	GAACCTCACCACAGTACTAAATGCA	CTGCTATTCAAATTGCT	CTGCTATTCACATTGCT	0,0
OMS00106	T	G	CGTGTAGCATTCTTGAGGAAGCTT	TTTCCAACAGATGCCAGAATCCT	TCTGATGGAAACTTTC	TGATGGCAACTTTC	0,0
OMS00154	A	T	GATGTTGGCTGGAGGTGTAGT	TGGGAACACTTTGCCTACCC	ACAGGGCTTCTGATTGA	AGGGCTTCAGATTGA	0,0
OMS00112	A	T	TGGCAGCAAAAGGGATGCA	TCCTGAGCAACCAGTCAACATT	CCGGTTTCAAGTTTACTTGT	CGGTTTCAAGTATACTTGT	0,0
OMS00118	T	G	GCTTATTTAGAGTGCATGCCAGATG	TGGAACCAATGGGACAGTCCTA	GCGGGGTGTGC[AG]CATT	GCGGGGGGTGC[AG]CATT	0,0
OMS00120	A	G	GGCAGAAGAGGAGAGAGATATGATTG	CCTCAAATACCTCTGACATTGAAGGTT	C[GA]CCCACTAAAAC	C[GA]CCCACCAAAAC	0,0
OMS00121	T	C	GGAAGGAGGTCCAGTGTGAGT	AAAATATGCAACACCACTAAAACTGGAAAA	ACAGCGTGATAAATT	CAGCGTGGTAAATT	0,0
OMS00132	A	T	GTTTATGACTCCATTGCCGAAATGATT	ACGCGACCTGCAATTCATCAATA	CAGCAGTCCTCTGTGTGG	AGCAGTCCTCAGTGTGG	0,0
OMS00175	T	C	TTGCGATATGGGACTGTATACATTTATTCC	ACTACCTCCAGTTAAAATAGTGTGGGAAA	ATCACTAGTTCAAATACAA	ATCACTAGTTCAGATACAA	0,0
OMS00179	A	C	GTCATAACAAAATCAGGG	TGGGAGATTTGGGCTGCTT	TGCCTCTTCTCTTTT	CCTCTTCTCTTGTCT	0,0

			CTTTCCAA	TAAA	CTCAT	CAT	
OMS00180	T	G	GCGCCGAATGGCATTAGG	CACATTGCTGTCGTTTAGTTTGACT	CTAAAAGTGCATTAAGCC	CTAAAAGTGCCTTAAGCC	0,0
Omy_101832-195	A	C	TGGCTCTGGACCTGTTGAGA	CGTCACAGCTATTTTAGGCGTAGT	TGTAGTCTTTCAGAGTAGTATG	TAGTCTTTCAGAGGAGTATG	0,0
Omy_101993-189	A	T	ACAAAACACAGTGGAATTACAATTAACGTT	GGAAGTTAAATTTCGCTTCGTCAGAA	CTTGATTTGCAGCTTGTCAA	TGATTTGCAGCATGTCAA	0,0
Omy_102505-102	A	G	CTGCAAACTGACATGGTAGCAAAA	TGCTTGCTTTTTAAAAACAATCTCCCA	AACAGGATGTTTTTGC	CAGGATGCTTTTGC	0,0
Omy_104519-624	T	C	CGTGTGAGTTTGCGGTAAAGAC	TGACGAGTCCGTCTTATCATCCT	CAGCAGGATACATCCGACT	AGCAGGATACGTCCGACT	0,0
Omy_105105-448	C	T	CAATTTGCAAGCAGGGAAAGGTTAT	GTGATGGGCTGCAATTGCTT	AAGGAGAATGCATAATC	TGAAAGGAGAATACATAATC	0,0
Omy_105385-406	T	C	ACCTACCCTCACCTGAACTTCA	CGCTCTTCTGGGCGTATCG	CTTGGAACCATTGCTAC	TTGGAACCGTTGCTAC	0,0
Omy_105714-265	C	T	CCACTCAGTGCAAGCATGGA	GCTTTCAATCCTTGGCTCCAATATC	CTGTTGTTTGAGGTTCAG	TGTTGTTTGAGATTCAG	0,0
Omy_107806-34	C	T	TCTTTGTCCATGCACATTGATATT	AGCACATTTAGTTAGCAGTGATGGA	ATTGGATGTCAGTGTCATT	ATTGGATGTCAATGTCATT	0,0
Omy_108007-193	A	G	GTGAATACCACCCAGGCTTGT	GTCCCTTCCCCAGTTTCACTTAATT	ATGTTTTCTCCCTACTTAAC	TTTTCTCCCCACTTAAC	0,0
Omy_109243-222	A	C	ATGTGCACCTCTTAAATTGTAAGTAAAATGT	ACCCTATATTCAGTGGCAAGATTGC	TGTTCATTAAATTGACTTTTT	TTCATTAAATGGACTTTTT	0,0
Omy_109894-185	T	C	GGGAGGAATTGGAATGACAGATTAAC	CGGTGTCATTATGGTTGTCATTGTG	CTCCCTGATCCCCC	CTCCCTGGTCCCCC	0,0
Omy_110064-419	T	G	GTGCAAGGGACCTAGCTAATCC	TCTGAACTGACACTGAAGAACAAAGAA	ACGTTAGCTTTTAATTTC	AACGTTAGCTTTTCATTTC	0,0
Omy_111383-51	C	T	CACGCGCAATCTCTCGTTTTAC	TCTTTAGGCAACAAGCGTGTCA	AGCAAGCGCACT[AG]GGT	AGCAAGTGCACT[AG]GGT	0,0
Omy_113490-159	C	T	CATAGTACATTTACAGATAATGTTTTAAAGTGCATGT	CGAGATACCAAAATGCCACAGTTACAT	CATCTGTTTTGGTTTAGC	CATCTGTTTTAGTTTAGC	0,0
Omy_114315-438	T	G	CCTCACCGATCTAGTCAACTTCATC	AGGAGGCTGAGGGAGATTCTAG	TTATGGGCTTAAGGGTC	TTATGGGCTTACGGGTC	0,0
Omy_114587-480	T	G	CAGATTACGTTATTACGTTTGGGAAATTTTTAAGT	GTGAAAGAGTGGGAAATATAATTATAAGGTCAGA	CCTGTCCAAAATTGT	CCTGTCCACAATTGT	0,0
Omy_129870-756	C	T	TCGTTATTTTGCCTCGCGGTA	TCCCATGAAGATGTATACATGTTTTGTGA	ACAGGTATTTCGTGAAATG	CAGGTATTTCATGAAATG	0,0
Omy_116733-349	C	T	GAAATGGACATGCCTACAAATTGCT	GATGTGATCAGTTTAGGCAAGGC	AGAGAATCTGATAGTATTTC	AGAGAATCTGATAATATTTC	0,0
Omy_128923-433	T	C	ACGTTTCTTTGGGCTGAGACTTATT	CTATGTCCTTGGCAGAAGTCTACA	CTTCATTTTCATTCACTGTTTT	CATTTTCATTCGCTGTTTT	0,0
Omy_130524-160	C	G	CGAAGGTAGCGATTGGTCGTT	TGTCTGTTCTGCTGTGTGCTT	ATGGCTTGATCCTCA	ATGGCTTCATCCTCA	0,0
Omy_97660-230	C	G	TCAGTTATGTGTAATCTCATTACCTCTCCAA	AACAGAAAAGGTCTCAATGTATTTTTTGCA	ACGTAACTTGTAGCGTTTT	ACGTAACTTGTACCGTTTT	0,0
Omy_99300-202	T	A	CAGTTTGACCCGATGGTGTGA	GATTATGGCGTGGCCTTTTGG	TCAGGCATGAGAGAAA	ATCAGGCATGTGAGAAA	0,0
Omy_aldB-165	C	G	GGGTTAGGTGGATTTGAAGGAGTAA	AGGAAGGTGATGCCTGAGAGA	CTAAAATGAACTC[CT]CCACCA	CTAAAATGAACTCGCCACCA	0,0
Omy_anp-17	C	A	GGTAATGCCACATGCGGTAAATT	GGCGAAATCTGAAAATGTGCTGTTA	CTCTCATTGGTATAGTAACC	CTCATTGGTATATTAACC	0,0
Omy_arp-630	G	A	CTGCACAACTTGTTTCCTGCTATT	ACCAAGTGTCCCTGTAAGCC	CCGCTCCGTCTGCT	CCGCTCTGTCTGCT	0,0
Omy_b1-266	G	T	TCATGTGAACTTTAATTGACTAGGAAGTCG	GATATGAAAATATCTGAAGAGTTATATTTGGGAAATTGAC	TCTATAAACAACATTTTTC	TCTATAAACAAAATTTTTC	0,0
Omy_BAC-B4-324	G	T	CGTACTTTTCTTTTACAAAATTAAGTGGAGGAT	GCCTAATATTGGCCTAATGTCCTTCA	CATTGCCAAATACG	TACATTGACAAATACG	0,0
Omy_ada10-71	C	T	TCTTTGAGCGACAAAGTCCTTGT	ACCCACACATGAACGCAAAAG	CTTCCTGCGTCCAA	CTTCCTGCATCCAA	0,0
Omy_redd1-410	C	T	GTACTCCCACTAACATACAGTAGACTCA	GGCACCATTGTGTTTTAGGATGTAG	AAAATATCCTGCAAGGAAT	AATATCCTGCAAGAAAT	0,0
Omy_cd59-206	C	T	CGATTGGCCCAGATGTTTCCAT	GCTCCGTTGCATAGGTGACT	CAACAATCGAAGGTAAAT	CAACAATCAAAGGTAAAT	0,0
Omy_colla1-525	C	T	CCTCGGCGTGACAACCT	CCCAGAGAATGGTGCGATTAGG	CTGTTGGGAGAAGAG	TGTTGGGAAAAGAG	0,0
Omy_cox1-221	T	A	CACTGAACTGTAAGCCATTGTGATT	GCAACATGGGAATGATTCATAAATGCA	CGGTAAGACCATTAAAA	CGGTAAGACCATTTAAA	0,0
Omy_crb-106	G	T	GCTCAAAAAGATTCTGCCAAATTCACA	ATTACAATGAAAGTACTTGAGTGTTTATGCAAA	TTGCAATGCGTCTTT	TTGCAATGAGTCTTT	0,0
Omy_g12-82	T	C	GATCAATTCGATCGCTCATGAAACTT	CTTCTCTCGTTCTCATTGTGTCTCA	CAAACTCTCAGGATTAG	AAACTCTCGGGATTAG	0,0
Omy_gluR-79	C	T	GACTGTCTATAGCTATTCTTCTCAAACTGT	AGAAACTACCATTGTGATTAACAGATAGAAAATACAT	CAAGTATTTTGCGTAGGAAT	CAAGTATTTTGCATAGGAAT	0,0
Omy_hsc715-80	C	A	CCGGTCTACCCTATAGCTGTTG	AGTCAGTCAATTAGTGGTTTGAAATACTATCA	AACTGTATTTGGGAAAAT	ATAAACTGTATTTGTGAAAAT	0,0
Omy_hsf2-146	A	-	GGAGCAGAAAAAGGATTGGACCTT	CCAACAATTGCAGCCTCATCTTAAT	ATAATCTACTA	ATAATCTAACA	0,0
Omy_IL17-185	G	A	CCACCACACTCTGCAGCTT	TTGACGGGAATCCGAGACTTC	AAGAATCTCACCTGCCCAT	AAGAATCTCACTTGCCCAT	0,0
Omy_Il-1b_.028	T	C	ACTGTCTGGCTAGAGCACATTG	ATCTTCTACCACCGCACTGTTTTAA	CTGAGGCAACTTTTGT	TGAGGCAGCTTTTGT	0,0
Omy_Il1b-198	A	T	TTTAATCTCGGTGCTGAGCTAGTG	CAAGCAAAATTGACTCCAGCCATTA	GGGAATGAAGCAACAACTA	GGGAATGAAGCTACAACTA	0,0
Omy_IL6-320	C	T	CTTGTTCCTCGTTGTCTTCCTTCTA	CGACTGATCTCCTGCAGACATG	CTATAGGAGAGAGGACAACA	ATAGGAGAGAAGACAACA	0,0
Omy_metA-161	T	G	CGCATGCACCAGTTGTAAGAAAG	AGTGCCACCAGCGATAAGAAAA	CAAGTAAGTGGTTATATTCT	CAAGTAAGTGGTTCTATTCT	0,0
Omy_NaKATPa3-50	A	C	GTTGAGCGTGTTATGGGAAAAGAG	TTGCATCGGCTTTCTGAAAACC	CACTCTGTTTCCTTTCTTT	TCTGTTTCCGTTCTTT	0,0
Omy_txnip-343	T	C	CCTTCAAACTAACGCATCATAGACATG	GGTCACTTGGCTAATCCCCTTAT	CCAACTGAAGAGATCTG	CAACTGAAGGGATCTG	0,0

Omy_nkef-241	C	A	AGTGTCATTGATGTCGGCCTATTTT	AAACGAATGTCCACCTCAGATGTT	CTTCTGTATCATTTTTG	TCTTCTGTATAATTTTTG	0,0
Omy_ntl-27	G	A	GGTGTGTTACTGTAGTTGTGTCCTT	TGTGTAGCTAGTGATCCTGATTGTCT	CAGACAAGAGTACCCCAAGAC	CAGACAAGAGTACTCCAAGAC	0,0
Omy_Ogo4-212	T	C	TCCTCTCTCCCATTCAATCACTAATGA	AGACAGTAACAAAGCCTCAAACTTGA	CATTTGATGAGACATCTT	ATTTGATGAGGCATCTT	0,0
Omy_bcAKala-380rd	G	A	TTGCTCTCTTCTGGTTGCCTTA	CTTCAGGAGAAAGCGCTACTGT	CATACCCATCCTATGTCAG	CATACTCATCCTATGTCAG	0,0
Omy_Ots249-227	C	T	CCCCTAGATTAAACCTGTCCAGTCT	CTATCTATCTATCTATCTATCTATCTATCTATCTATCTACTTACTGAGA	CCCTCTGAGAACTAC	CCTCTGAAAACTAC	0,0
Omy_oxct-85	A	T	CGTCACTGAAACATTACTGTAACATCCA	CATCATCACGCTGTTGGTTTCTTAA	CATCGCTTATTTATGC	CATCGCTAATTTATGC	0,0
Omy_p53-262	T	A	CCCCAACATCCAGTATACAGTTTCA	CCCAAATTGGCAATTTTAATAGGATTCAGA	CAAGTAGTATGGAGCTCTAT	AAGTAGTATGGTGCTCTAT	0,0
Omy_rapd-167	G	T	CCCAACATGCTCTATTGCAGCTA	AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT	AAACAATCCCCCCCAAA	AAACAATCCCACCCAAA	0,0
Omy_rbm4b-203	-	T	CTGAAATTTGATGAATGGAAGCTGCA	CGTATTCAAGTCGATATACAGTCACGAT	CACGTTATTATGAAAAGGATGT	ACGTTATTATGAAAAAGGATGT	0,0
Omy_srp09-37	C	T	TAGTTGTATTAACTCTTCTTTGAGTCTAGA	TCATTCCAGCTCCGTTCTCTTC	TTGTGCTATTGACGCCACAG	TTGTGCTATTGACACCACAG	0,0
Omy_stat3-273	G	-	CAGACCTCCTCTATCTCCCTATGAG	ACCTCCTTTAAATTGTGCCCAAGAA	TTTTCCAGACTCCAGTTTG	TTTTCCAGACTCAGTTTG	0,0
Omy_u09-53.469	T	C	ACAGCCTGAGCGTTTGCA	GGAAACTGGGAGAGATCAAAGGA	TTGCAGCCCTTATTGTG	TTGCAGCCCTTGTTGTG	0,0
Omy_u09-54-311	C	T	GTGGCTCCCCAGGAACAAG	AAGTTTCATGTCACATTCCAGTTACCT	TGGTAATTATTCAACAGATCAGT	TGGTAATTATTCAACAAATCAGT	0,0
Omy_U11_2b-154	T	C	GGGAAGCAGAAAAACTGGAAGTT	CCCTCTGTGGGCTTGATATTCA	AATGATACTTTTCAGATTGTAAC	TGATACTTTTCAGGTTGTAAC	0,0
Omy_vatf-406	T	C	TTGCTTCATTTTGTCATAACCTTGGG	TGCATGCTCTGACAAATGTTACACT	TTGCAGATGACTATCCACA	TGCAGATGACTGTCCACA	0,0
OMY1011SNP	C	A	AGGCTGGTTTGGGATTCACTG	CGCCAAACACTAACTCTCTGTCT	CTTTACCTCGAAGACAAT	ACTTTACCTCTAAGACAAT	0,0
OmyY1_2SEXY	X	Y	GCGCATTTGTATGGTGAAAA	GCCTGGCATATGAGTGTTGA		ATGTGTTCATATGCCAG	NA
OMS00041	G	C	GATTCTGTTCCATCCTCTTTCTGTCA	AAACATAAAAAAGGGCATGAAGGTGTC	CCACTCTATGCCTGCCCT	CACTCTATGCGTGCCCT	0,0
OMS00103	A	T	GAGATCACTGTAGGATTGGCTGTTT	CCTCAGAGCAGCTCACAATGGCATC	CTCCACAGTAATTTTTTTTT	CCACAGTAATTATTTTTT	0,0
OMS00116	T	A	GCCTTTCTCCCATATCACATTCGA	AAACGCATCTTACACTGTGTTGTG	CTTTTACATTTTCAATATTCTG	TTTACATTTTCAATTTTCTG	0,0
OMS00127	T	G	CACCTTTCTCTCTCTCTCCATCTCA	AGTGTGCTACACAACCTTAAAAAATATATATCTATT	CACACACCCAAATGTA	ACACACCCCAATGTA	0,0
OMS00128	T	G	ATGAAAGAACTCCCAGACACGTATTTT	ACATTTTAACACAGTAACACTAATACACACCA	ACTCTCAGAATTAATTATG	CACTCTCAGAATTCATTATG	0,0
OMS00134	A	G	GAAACTGAAATGATCCCATCGTGTT	GCTAGCATAACAGCATTGCCATAT	TCTATAGCTGCAGTATATTA	TAGCTGCAGCATATTA	0,0
OMS00153	T	G	ACTTTGCACCATAGGCTTGACAT	TGATAAGGATGATCAAAAAGCTGAAGTATGTA	ACAAAATGTAATTTTCC	CAAAATGTCATTTTCC	0,0
OMS00156	A	T	GAGCAGAACACATAGAGGAAAGACT	GTAATCACCCTCTTAGCCTGTATGG	TGTGTGTCCTGCTGTAACA	TGTGTCCTGCAGTAACA	0,0
OMS00164	T	G	CAGAGGAGAGGAGAGCAAAATACTT	ACAACCTACTCATTGAAACTCATTGGA	CCAGATTCAATTAAATTTA	CAGATTCAATTCAATTTA	0,0
Omy_1004	A	T	GAGAATCGGAGCTAATCTTAGTTATTGTGA	CACTTTATTGAGCTACATGGCAAATCTG	CATGTGATGTTTTTTTGC	ATGTGATGATTTTTGC	0,0
Omy_101554-306	T	C	GCCTGTATTTCTCCTGTATGTGCAT	TCAACTTTTGCAAACTTTTTTATTCTTTGTCATTT	TGCTTCTCACATTTTTA	TGCTTCTCACGTTTTTA	0,0
Omy_102867-443	T	G	CATTTGTTTAATTTGATTTGGCACAACTTCA	CCCTAGTTCTGTAACACAAGACGTAA	TTTGGGTACATAATTTTT	TGGGTACATCATTTTT	0,0
Omy_104569-114	A	C	CCGAGGCCGACGTGATC	GCGCCTCGCTCATCATCA	CGCCACTCCGACGCC	CCACGCCGACGCC	0,0
Omy_107336-170	C	G	GCCCTCTCACTCATGACATCAAC	GCTCCAGCCACTCGCA	CACTCCTGGGTGCAGAA	ACTCCTGCGTGCAGAA	0,0
Omy_109525-403	A	G	CCTCATTCTCATTGGTGAGTTGTCT	TGTAAGATCTGACCACATGAGTATAACCA	CCTACACCTCTTTTTTCCACA	CCTACACCTCTTTTCTCCACA	0,0
Omy_110362-585	G	A	GCAGCCAAGATGAACGAAAACTTC	CCGGCCTGGGTCTCAATG	CACCGCCCTGCCCGT	CACCGCCTTGCCCGT	0,0
Omy_110689-148	A	C	GTGTGTGGCAGAGAACTAACTGAT	GGTTAAGACATTAACATAACACTGGACTCT	CAAATGAACACATTATTTATC	ATGAACACATGATTTATC	0,0
Omy_111084-526	A	C	CACCACACCAAGCAACTATTTCATT	ACCCAACTACTGTCCCATTTTTCAT	CCAGTGAAATTTATTTTT	CAGTGAAATGTATTTTT	0,0
Omy_111666-301	T	A	GGGTGAAAAGAGTGGGACATTTACA	GTCAATTTCAAGGCACCAGACAAT	AGTATAACACAGTAAGACAAT	AGTATAACACAGTTAGACAAT	0,0
Omy_112301-202	T	G	GTAAACCCTGCCCACATAATTAGGT	CTGAGACACTGCTCCAAGGT	AATGCGAAGACAAACT	AATGCGAAGCCAAACT	0,0
Omy_112820-82	G	A	CCTTTCCTTTTGCATTTCCTCTACTTATTTATTT	AAATGAACTCACGTTGACCTCTGA	CGCCGCCAAGTTA	CGCCGCTAAGTTA	0,0
Omy_114976-223	T	G	GACAAACAGCACTTCATTGCAGTAA	GTTGCTCCAGCACCAGGT	ACCGATGGAACAATC	CCGATGGCACAATC	0,0
Omy_116938-264	A	G	GTTCATTCATGTTGAAGTGCGACAT	CTCTGCATGCTCCCATCCT	CCTTGTCTCAATTTTTCCTCT	CTTGTCTCAATTTCTCCTCT	0,0
Omy_117286-374	A	T	TGATGTGTTGTTCCTCATGGCTTA	CTGTGCATTTATTCTTGTGATGCTAGG	CTTTCCTCATCATACTCTATGG	TCCTCATCATACACTATGG	0,0
Omy_117370-400	A	G	TGCAAACACAGAGGAAAGGGATTT	GGCTTATTTGTTCCGTACTTGCATT	CAACTCCAATGAATTAA	AACTCCAACGAATTAA	0,0
Omy_117540-259	T	G	GGCAGGTTAACACAGTCATCTACTATAAA	CAGCATGTTGCTTTAATCCTTCACA	TGTCACTTCAAAGTTTG	TGTCACTTCAACGTTTG	0,0
Omy_117815-81	C	T	CTGCTTTATGCACACCACATTGT	GCTCTTTCTGGAGAACAAGGTACTG	CTATACGGAGACCAGC	CTATACGGAAACCAGC	0,0
Omy_118175-396	T	A	AGGCTTCACACACACATGCA	GACGCGCAACCTCTAGATTATACTT	CTCTTGCAGACATACCCGTA	CTCTTGCAGACATTCCCGTA	0,0
Omy_118205-116	A	G	CTGCGGTGGGCTACACA	CGCAGCTGCGGATGAG	CTACTGAGGCTGAG	TACTGAGGCCGAGT	0,0

					TGCT	GCT	
Omy_118654-91	A	G	CAGCGTAGACCGTTTCCTCATTAT	GCGCCGATGAGCAGCTT	TCAGCTTGTCTTGCCGC	CAGCTTGTCCTGCCGC	0,0
Omy_120255-332	A	T	GGCTACAGGGACTTTACAATGGG	GCTAGCTAACATTGAAGGGTGGAAT	ACTATGCCATGAAGTTA	ACTATGCCAAGAAGTTA	0,0
Omy_128693-455	T	C	GCCTGCAGGAGAAGGTAGAGTTA	GAAATGGAATGGACCCCAATCCT	CACTCAACTGATACCC	CTCAGCTGATACCC	0,1.8
Omy_131460-646	C	T	GTGAAAAGGAATGGAGGAGTACAGT	TGCTAGGACAGGAAGATCATTTGTG	AATAAAGCAGAATTTGTTACTG	AAAGCAGAATTTATTACTG	0,0
Omy_187760-385	A	T	CGGCTATTCTCGCGTAAAAGCT	AAATGCAACCAGAAACGGAATGTC	TCCTTATCCAAAATTATTGTGC	CTTATCCAAAATAATTGTGC	0,0
Omy_96222-125	T	C	GTAAGGAACTAATTGGCGCAACATT	CAGTTTGTCTAACACCCAGGCATAT	AACTACAACTGTAGCTAATT	CAACTGTGGCTAATT	0,0
Omy_98683-165	A	C	GCCATTGCCAGAGAATTTGGTTAA	AACACACGCACCATCTTAAAGC	AGCCAGATACATATTTGT	CCAGATACAGATTTGT	0,0
Omy_BAMBI4.238	T	C	CATGATGAGGAGGACCAAGATGAG	AGGTGTGGTTCAGGGCAG	CACCGCAATCACCG	ACCGCGATCACCG	0,0
Omy_cyp17-153	C	T	GCCCTCCAAGTTCCAAGTGAAAA	CAGGTCATTGATGAAACGTCAGAAC	ATACCTGAGTGTCATCG	ATACCTGAGTATCATCG	0,0
Omy_ftzf1-217	A	T	ACAGGGATGGGCAACTTTGTT	GGATGACCCACGTGACACT	TCATGACGAGTTCTGATTT	TGACGAGTTCAGATTT	0,0
Omy_G3PD_2.246	C	T	TCATGTATCAATTAAGGCATTGTCTTGTCT	GTTAGACACAGTGACCACCTCTTT	AGTAAAGCCCATTGTTGAGT	AGTAAAGCCCATTATTGAGT	0,0
Omy_GH1P1_2	C	T	TGCATTTGATGGAAACAAACATATTTATAATGTGT	CAAAAACAAGGACAATTCTAAGTGACCTC	AAACTGTTGAACGGTAGTG	AAACTGTTGAACAGTAGTG	0,0
Omy_gsdf-291	T	C	GCATGGAACCAGTTCTCTACAAAAG	ATGGAGTGGAAAATCACAGCACATAT	CATAACCCAGAATTATTA	CATAACCCAGGTTTATTA	0,0
Omy_hsp90BA-193	C	T	GGAATCGATGACGACGAAGTGATC	TTCCTCCATGCGTGATGCA	CCTCCGCGCCTGC	CCTCCGCACCTGC	0,0
Omy_MYC_2	T	C	CGGTTGCAGAACTCTCATGTTTG	CACGCCATGTCTTAACTTGCATTA	CATAGACTTTTTGACCTTAT	CATAGACTTTTTGGCCTTAT	0,0
Omy_u09-61.043	A	T	TAGTCACATCCATAGTAATACTTCC	TGTTCAGAAGCAGAAAACCAATCTCT	CACTTGGTCCTTTTTCA	CTTGGTCCATTTTCA	0,0
Omy_UBA3b	A	T	GCCACTCAATGCATGTGTTTTCTAG	CAGCTAGCTTAAGTGGGATGCAA	TGGAGATAACGCTAACTATT	AGATAACGCAAACTATT	0,0
Omy_RAD17632-23	C	T	AAGCTCCTGCAGGTCATCTC	TCTGTGAACTGTCTTCTGCAAGT	CATGTGAGACCTTTGCA	CATGTGAGATCTTTGCA	0,0
Omy_RAD23577-43	T	C	AATAGGAACCAAGCCCCAGC	CAGAGCCTGAACCCATGGAG	TCTGGCTCTGTCGGTCT	TCTGGCTCCGTCGGTCT	0,0
Omy_RAD26080-69	G	A	TGTGGGACAGCACATACTCC	CCAGGACACCAGTGGAGAAG	ATTAGTAGCATCATCGAG	ATTAGTAACATCATCGAG	0,0
Omy_RAD29700-18	C	A	AATGGAATTGGCCCCAACCC	TCTCCATTGTGTGTAATCATGGT	ACAATTCAAATGATTTA	ACAATTAAAATGATTTA	0,0
Omy_RAD36848-7	G	A	CGAGGACGTTCATAGGGAGC	TCGATAAGTCCACCAGCTGG	TGCAGGGACACCACCCT	TGCAGGAACACCACCCT	0,0
Omy_RAD38269-10	C	A	AAGCCACACCGTTCAACTGA	TGGCTTATCGCGCTCTAGTG	TGCAGGCGCCCGGCCCGC	TGCAGGCGCACGGCCCGC	0.5,0
Omy_RAD43612-42	T	C	GTGGAGAGGGATTTTGGGGG	TGACAGGACAAACACAAGCCA	AAATGTGTATTTGTGTA	AAATGTGCATTTGTGTA	0,0
Omy_RAD45104-18	A	G	TGGTGCTTCAGTGCTGTCAA	AGAGTGAAAACTGTGTGCGG	CAAGACACCGCACACAG	CAAGACGCCGCACACAG	0,0
Omy_RAD47080-54	A	G	TCAAAACCTGCAGGACTTGGA	TGGTTATATCTACAGTACAGTTCGT	TGCAAGACTTAAAACGA	TGCAAGGCTTAAAACGA	0,0
Omy_RAD47444-53	C	T	GTCGTCTGGAGGAGCTGAAG	GGGTGACGTTTTCCTTCAGC	GGCGAGCTTGGCCCAAA	GGCGAGTTTGGCCCAAA	0,0
Omy_RAD48799-69	A	G	GCTGAGCCACCTACACACAG	GTCTAACACTCGCAGCAGGT	CATCCTAGAATAGAAGT	CATCCTGGAATAGAAGT	0,0
Omy_RAD5026-64	G	A	TCAGCGTTATACCTGCAGGA	CCCTCCTAACCTGTGGTGTT	TGTAACGCAACACCACA	TGTAACACAACACCACA	0,0
Omy_RAD52458-17	C	A	ACGTGTCCCTGAGGATGGTA	AGCTCTAGGTCTGGGTCCTG	ATGGCCCC[CT]AAGAACCC	ATGGCCCA[CT]AAGAACCC	0,0
Omy_RAD52812-28	C	G	AGGAGTCCTGTCCCATGTCA	GCTTAAGGCTGTGGTATGTGG	CAACCTC[TC]ATTCCACAT	CAACCTG[TC]ATTCCACAT	0,0
Omy_RAD58213-70	A	T	CCTGATGGGTGCTCTTCTCTC	AAACAGCATCATTATCCATAGTGTT	TTTTTT[TA]AAAATATACT	TTTTTT[AT]TAAATATACT	0,0
Omy_RAD58835-15	G	T	GTCTGCTAAGGTCCTGCAGG	GCCGACCATGAGAGACCTG	ATAGCTGCTGGGACCCA	ATAGCTTCTGGGACCCA	0,0
Omy_RAD62596-38	A	T	GCAGGACACTGGTTCCCAAA	CCTGAGATTTGAGATCACTGGCT	TTAAAAAATATATATTA	TTAAAATATATATATTA	0.5,0
Omy_RAD66834-17	C	T	CTCCTGCAGGTCATCTCTGG	CTGTCTTGTGCTCAATGCCTG	TCTGGCTGACACCTTTA	TCTGGTTGACACCTTTA	0,0
Omy_RAD69583-33	C	T	GACTCTAGACTGCTCCCTGC	GGCCTCAGTCTCCTTCCAGA	CCGGAGACATT[CT]CGCGT	CCGGAGATATT[CT]CGCGT	0,1
Omy_RAD7210-8	C	A	GCGCCTTGGTCTCCTTCATA	ACACCACACTCCACAAAGCA	TGCAGGACTTGCTTTGT	TGCAGGAATTGCTTTGT	0,0
Omy_RAD74691-49	A	G	CATCTCAGATCAGCCACCCG	AGCACAAATTCTTCTGTTGTGCA	ACTGTGGATTTGCACAA	ACTGTGGGTTTGCACAA	0,0
Omy_RAD77789-54	T	C	AGACAAAACCTGCAGGGGAC	AGCACGTTAAAACCAAACTGTCA	TAAATTATATTTGACAG	TAAATTACATTTGACAG	0,0
Omy_RAD88122-32	G	A	TCAGTGGATGGAGTGTCCCT	GGTCTTTGGCCTTGTTGCTG	GCTGTGGAGATCAT[CT]CG	GCTGTGGAAATCAT[CT]CG	0,0
*Omy_RAD16104-20	A	G	ATTCCAAAACCTGCAGGGGT	TCAGGATTTGGTAAGGTGGCC	AGGGCAAAG[AT]CAAAGG	AGGGCAAGG[AT]CAAAGG	0,0
*Omy_RAD35417-9	G	A	GCACTTGACCACATAGCTGG	ACTCCACACTCCACAAAGCA	TGCAGGACGTGCTTTGT	TGCAGGACATGCTTTGT	0,0
*Omy_RAD42793-59	T	C	CACGGCTAGTGGCATGTACC	CCACACCTGCATCAGTCTGT	CAGAGAATGCCAACAGA	CAGAGAACGCCAACAGA	0,0
*Omy_RAD47955-51	G	T	AGTGTGCTAGAATGGGCCTG	ACCATGGGCAGTTCATTTCA	TTGGAATAGAATCTATA	TTGGAATATAATCTATA	0,0
*Omy_RAD66218-58	T	C	CTGTGCAGGGAGACAGCTAG	GTGGTATCGTAGCATCGGGG	CTTGGAGTGTGTTGGTA	CTTGGAGCGTGTTGGTA	0,0
*Omy_RAD73204-63	G	C	CCTGGGCAATGACCTCCAC	AGCTCCCTTCTCTCTCCCTC	GTGCCCGCTCTCCACCG	GTGCCCCCTCTCCACCG	0,0

*Omy_RAD76882-63	A	T	GGTGGGGGCAAACTCAGTA	TGTCTCAGCTTAGAATGACAGATT	CAAATGAAAACTATGTA	CAAATGAAATCTATGTA	0,0
*Omy_RAD88028-7	G	A	TAGCCCAGTTCGGTTCCAAC	AGTGTCTTTGGTGCGTCCTC	TGCAGGGGCTGGGAAGG	TGCAGGAGCTGGGAAGG	0,0

Chapter 2: Genetic Baseline Expansion

Introduction

Distinct population aggregates of Chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*O. mykiss*), and the species *O. nerka* (Sockeye salmon and kokanee), have evolved through the cumulative effects of selection and genetic drift (Waples 1991; Nielsen et al. 2009). The homing behavior (philopatry) displayed by Pacific salmon means that fish typically return to spawn in their natal rearing sites or stream of origin. This distinctive life history attribute can significantly restrict gene flow, shape regional variation, and influence demographics among naturally reproducing populations (Hasler and Scholz 1983; McIssac and Quinn 1988; Quinn *et al.* 1991). Genetic differentiation is most easily resolved among populations that are geographically distant, where degree of gene flow is generally correlated with relative migration distances and adjacency in stream networks. However, local adaptations and the distribution of suitable spawning habitat within stream networks may influence finer (regional) scale genetic structure among watersheds in close proximity (Beacham *et al.* 2006; Matala et al. 2012). The natural phenomenon of immigration or straying (a homing miscue) buffers the loss of genetic diversity in salmon populations (Milner and Bailey 1989), but the rate of straying exhibited by wild fish is generally low (Quinn 1993; Heard *et al.* 1995) and genetic structure between populations may persist despite moderate gene flow from straying (e.g., Neville *et al.* 2007). Some evidence indicates that hatchery-origin fish exhibit a higher rate of straying which may be affected by changes is fish passage protocols, transport through the hydro system, artificial rearing practices, or inadequate acclimation (imprinting to natal waters by juvenile salmon). 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 2013).

In the Columbia River Basin, Chinook salmon have been studied in great detail (e.g., Narum et al. 2004; Waples et al. 2004; Beacham et al. 2006; Narum et al. 2008b; Matala et al. 2011), as have steelhead trout (Winans et al. 2004; Currens et al. 2009; Blankenship et al. 2012; Narum et al. 2011; Matala et al. 2016). The scope of Sockeye salmon genetic monitoring has been comparatively limited but has received greater attention in recent years (Gustafson et al. 1997; Iwamoto et al. 2012). Continued monitoring and evaluation of the genetic structure among salmon populations in the Columbia River Basin has guided managers in establishing and maintaining primary conservation units to protect fisheries resources. The delineation of such conservation units, including distinct population segment (DPS), evolutionarily significant unit (ESU), major population group (MPG), and viable salmonid population (VSP) is guided by a core set of criteria, including population ecology and viability, ancestry and descent, reproductive isolation, and genetic structure and local adaptation (Fraser and Bernatchez 2001; Fraser et al. 2011). Although an understanding of adaptive variation is critical to proper salmon management, the majority of genetic information available to managers is based on neutral genetic variation. Landscape genetics is an approach aimed at describing population differentiation relative to features in an organism's environment (Segelbacher 2010; Latch et al. 2011; Sepulveda-villet & Stepian 2012). Landscape genetics explores population differentiation relative to features in the environment such as migratory barriers (e.g., dams), or heterogeneous habitats such as variation in local climates or temperatures (Dionne et al. 2008; Narum et al. 2008). Although local adaptation may be inferred from landscape genetics (Olsen et al. 2011; Blankenship et al. 2012), inferences based primarily on neutral genetic differentiation risk

incorrectly identifying the underlying processes affecting population distinctions (Funk et al. 2012; Landguth & Balkenhol 2012). Techniques such as outlier detection methods, and genome wide association studies (GWAS) provide evidence of non-neutral population structure and allow a more resolved understanding of landscape differentiation beyond what can be concluded from neutral loci alone (Narum et al. 2010b; Matala et al. 2011; Ackerman et al. 2012a, Bourret et al. 2013). Putative non-neutral population differentiation can then be interpreted in the context of contemporary risks and vulnerabilities (e.g., climate change) for salmonid populations in the Columbia River Basin, revealing highly correlative relationships between genetic variation and the physical environment (see Limborg et al 2011). This additional information may ultimately influence conservation criteria for delineating populations across diverse landscapes.

Project objectives and higher level harvest management questions:
Objective two of project #2008-907-00 (Genetic Assessment of Columbia River Stocks) describes efforts to evaluate genetic diversity among populations that will inform managers in the areas of harvest monitoring, and conservation monitoring. Our approach involves the collection, analysis, interpretation and distribution of genotypic data. These data are being compiled as species-specific reference baselines for characterizing Chinook salmon, steelhead trout, and *O. nerka* population structure specific to the Columbia River Basin. Baselines were initially created from genotypes at single nucleotide polymorphism (SNP) loci, which are highly prolific in the genome and provide substantial coverage for linkage analyses (Moen et al. 2008). SNPs are amenable to superior high throughput capabilities and are relatively easily amplified and scored compared to other types of genetic markers, even with poor quality tissue (DNA) sources (Campbell and Narum 2008). Because SNPs are commonly found within or adjacent to coding and regulatory regions of a genome, corresponding allelic diversity and allele frequency variation are likely to be informative for understanding non-neutral influences (i.e. selection and local adaptation) on observed population structure. Large numbers of highly informative SNP loci have been discovered through our ongoing efforts using a next generation sequencing technology known as restriction-site associated DNA (RAD) sequencing (Miller et al. 2007; Baird et al. 2008; Hecht et al. 2013). Our two primary objectives for utilizing SNP baselines to monitor salmon species in the Columbia River are 1) genetic stock identification (GSI) of natural-origin stocks, and 2) parentage based tagging (PBT), a large-scale, non-lethal tagging technology for monitoring and evaluating hatchery stocks. The collaborative, inter-agency application of GSI continues to provide invaluable monitoring capabilities to understand relative stock proportions in sport, commercial and tribal harvests, as well as monitoring of stock specific run-timing at Bonneville Dam, Lower Granite Dam and other fish weirs in the basin. Moreover, GSI is being used in concert with PBT to monitor trends in hatchery production, harvest of hatchery fish, and population attributes of specific hatcheries (e.g., stray rates, survival/mortality, migratory behavior, hatchery/wild interactions). Additionally, our genetic baselines are being used to characterize populations in archival studies, in efforts to reintroduce fish into extirpated regions within historic ranges, and in domestication studies.

Time line for completion of objectives:
Objectives will be ongoing and our most recent results will be reported each year. As new genetic techniques are developed they will be applied to our objectives and data will be routinely uploaded to the FishGen.net database (http://www.fishgen.net/home.aspx) as a repository for data sharing and collaboration.

Methods
Baseline sampling and protocols:
Existing baselines comprised of putatively neutral SNPs (e.g. 180 loci for *O. myiss*) have been well characterized and are currently used extensively for genetic stock identification (GSI) as described in Hess et al. 2014 and Hess et al. 2015. Our most recent efforts focus on expanding genetic characterizations throughout the basin that will provide information about adaptive potentials and natural selective forces contributing to stock structure. Next generation sequencing technologies in genotyping (RAD) were initiated by the CRITFC genetics lab in order to expand SNP panels for Chinook salmon, Coho salmon, Steelhead and Pacific lamprey (Hess et al. 2016). We have begun employing the RAD sequencing approach for SNP discovery in Sockeyesalmon. Primer development and further screening are underway in order to finalize an expanded SNP panel for GSI of *O. nerka* in the Columbia River. Species or population specific details of laboratory methods are available in Hess et al. (2012) and in Monitoring Methods: https://www.monitoringmethods.org/Protocol/Details/230 (ID#230; owner Matthew Campbell). For methods using genotyping by sequencing, or RAD sequencing see Monitoring Methods #4144, owner: Nathan Campbell. SNP discovery using the RAD sequencing technique is described in Chapter-1 of this report. In 2016 we did not expand on the reference populations in our baseline which currently includes all extant and reintroduced Sockeye salmon stocks and the majority of extant native kokanee stocks in the Columbia River Basin.

Expansion and status of reference baselines:
Our three primary goals for Chinook salmon, Sockeye salmon and steelhead trout are: 1) 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) PBT broodstock sampling and genotyping of Columbia River Basin hatcheries for evaluating hatchery stock composition in various fisheries and to monitor hatchery impacts on wild populations, and 3) continued baseline maintenance and expansion using RAD-tag sequencing for application in various analyses, including population structure analyses, investigation of landscape genetics, and adaptive differentiation among populations. We collected RAD sequence data for 25 discrete collections or populations of Chinook salmon (n=1032 total; Table 1, Figure 1) and 56 discrete collections or populations of anadromous steelhead (Table 2, Figure 2) from throughout the Columbia Basin. RAD sequencing proceeded using the Sbf1 restriction enzyme protocol found at: https://www.monitoringmethods.org/Method/Details/4144. The program STACKS was used to identify and quality-filter SNPs from raw Illumina sequence data (Catchen et al. 2011). High-quality SNPs were then used to assess population structure based on principal component analyses (PCA) and pairwise genetic distance displayed in neighbor joining (NJ) trees using the 'adegenet' package in R (Jombart & Ahmed 2011). Reference baselines for GSI have not yet been established from expanded SNP panels and current GSI methods used for stock assessment are based on the panels described in Hess et al. (2016). For *O. nerka*, we initiated efforts to expand our current SNP panel from 92 SNPS to greater than 500 SNPs. SNP discovery for *O. nerka* was conducted using an ascertainment group of sampled individuals (Table 3, Figure 3) assembled from all major groups in the basin. This approach maximizes representation of existing diversity in the basin, while minimizes bias that may arise from subsequently evaluating genetic structure of non-represented groups. After the quality filtering phase of the analysis we have collected sequence genotypes for hundreds of informative RAD markers. Selection of SNPs

for the expanded baseline was based on highest absolute differences in mean allele frequency between major groups (e.g., kokanee vs. Sockeye salmon; Snake River vs. Columbia River origin) and based on observed minor allele frequencies (0.15<MAF<0.35).

Figure 1: Map displaying sampled Chinook salmon (O. tshawytscha) populations that have been RAD-sequenced. Collection IDs correspond with Table 1.

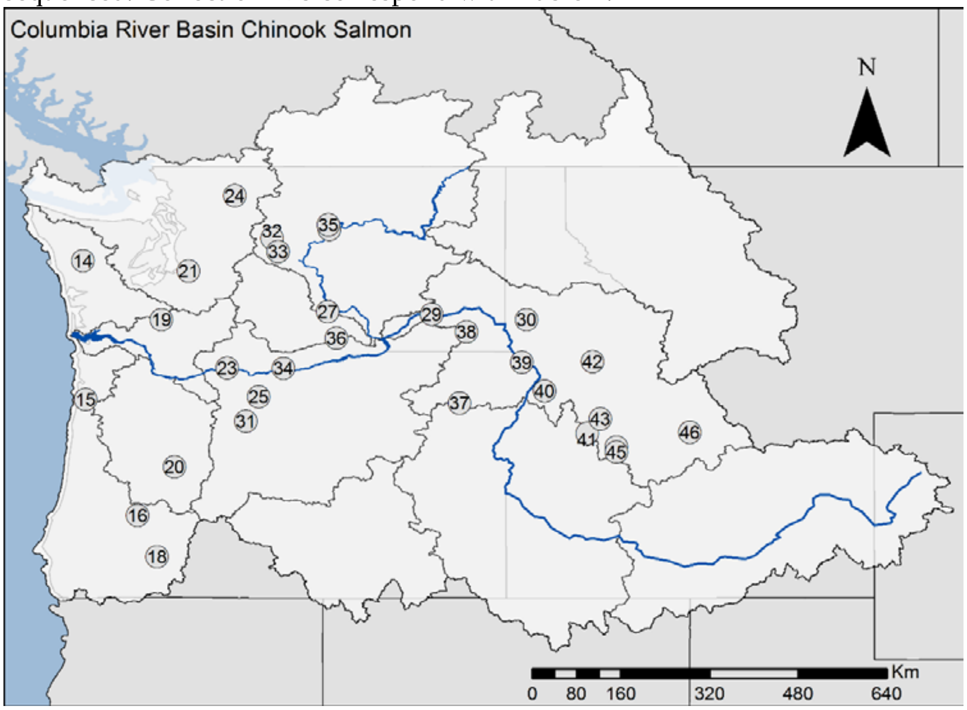


Figure 2: Map displaying sampled anadromous steelhead (O. mykiss) populations that have been RAD-sequenced. Collection IDs correspond with Table 2.

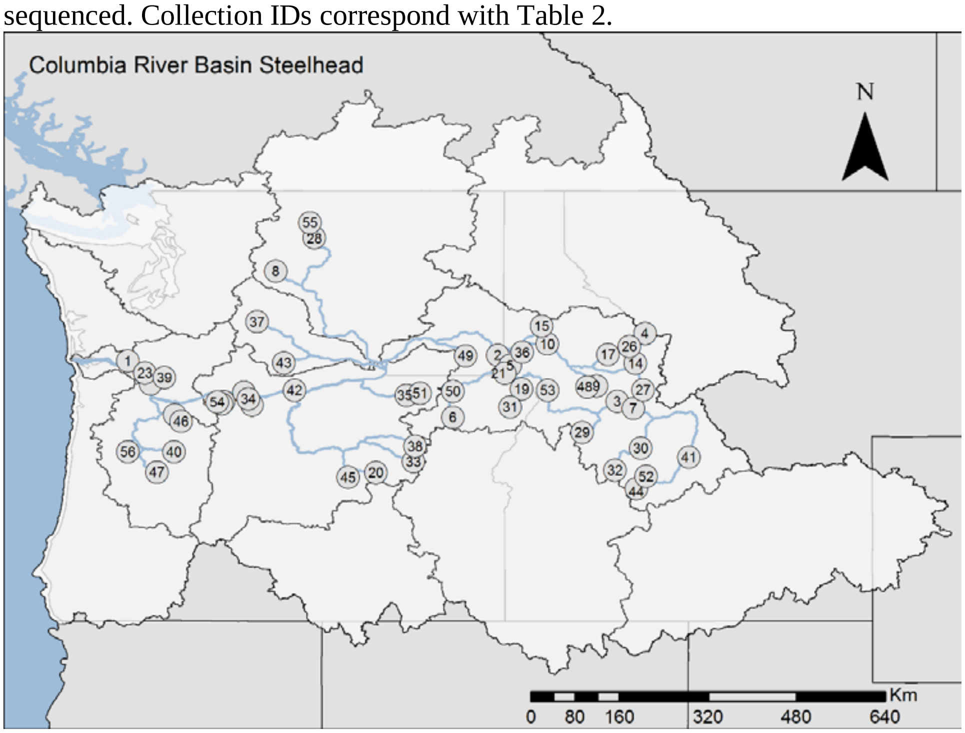


Table 1: Geographic distribution and locations of Chinook salmon RAD populations within the Columbia Basin. Populations are split between a southern coastal (SC) lineage, or inner Columbia River (ICRST) lineage.

ID#	Name	Population Abbr.	BPA Subbasin	Lineage	Run-type	Origin	lat	long	(n)
19	Cowlitz	COW	Cowlitz	SC	Fa	H	46.510	-122.615	43
20	McKenzie	MCK	Willamette	SC	Sp	H	44.123	-122.402	48
23	Spring	SPR	Columbia Gorge	SC	Fa	H	45.727	-121.546	39
25	Deschutes	DES	Deschutes	SC	Fa	N	45.257	-121.039	28
26	Wenatchee	WNC	Wenatchee	SC	Su	N	47.616	-120.723	45
27	Priest	PRH	Columbia L. Mid	SC	Fa	H	46.642	-119.914	46
28	Wells	WEL	Columbia U. Mid	SC	Su	H	47.973	-119.888	45
29	Lyons Ferry	LYO	Snake Lower	SC	Fa	H	46.595	-118.229	48
30	Clearwater	CLW	Clearwater	SC	Fa	N	46.513	-116.686	33
31	Warm Springs	WAR	Deschutes	ICRST	Sp	N	44.862	-121.245	47
32	White	WHI	Wenatchee	ICRST	Sp	N	47.834	-120.819	18
33	Nason/Chiwawa	WEN	Wenatchee	ICRST	Sp	N	47.616	-120.723	33
34	John Day	JDR	John Day	ICRST	Sp	N	45.721	-120.635	12
35	Methow	MET	Methow	ICRST	Sp	N	48.048	-119.905	47
36	Yakima	YAK	Yakima	ICRST	Sp	N	46.212	-119.772	47
37	Catherine	CAT	Grande Ronde	ICRST	Sp	N	45.158	-117.779	43
38	Tucannon	TUC	Tucannon	ICRST	Sp	N	46.315	-117.662	46
39	Imnaha	IMN	Imnaha	ICRST	Sp/Su	H	45.817	-116.765	48
40	Rapid	RAP	Salmon	ICRST	Sp	H	45.353	-116.395	46
41	McCall	MCH	Salmon	ICRST	Sp/Su	H	44.667	-115.705	43
42	Newsome	NEW	Clearwater	ICRST	Sp	N	45.828	-115.615	40
43	Johnson Creek	JOC	Salmon	ICRST	Sp/Su	N	44.899	-115.492	68
44	Marsh	MSH	Salmon	ICRST	Sp/Su	N	44.449	-115.231	39
45	Capehorn	CAP	Salmon	ICRST	Sp/Su	N	44.358	-115.228	37
46	Pahsimeroi	PAH	Salmon	ICRST	Sp/Su	H	44.682	-114.039	43

Table 2: Geographic distribution and locations of steelhead RAD populations in the Columbia Basin. Populations belong to three possible lineages, lower coastal (LC), upper snake (US), or middle upper Columbia / lower snake (MUCLS).

ID#	Name	Population Abbr	BPA Subbasin	Lineage	lat	long	(n)
1	Abernathy	ABER	Elochoman	LC	46.225	-123.148	21
2	Asotin	ASOT	Asotin	MUCLS	46.322	-117.137	45
3	Bargamin	BARG	Salmon	MUCLS	45.571	-115.192	24
4	Boulder	BOUL	Clearwater	US	46.675	-114.739	47
5	Captain John	CAPJ	Snake Hells Canyon	MUCLS	46.153	-116.934	43
6	Catherine	CATH	Grande Ronde	MUCLS	45.307	-117.866	36
7	Chamberlain	CHAM	Salmon	MUCLS	45.454	-114.931	48
8	Chiwaukum	CHIW	Wenatchee	MUCLS	47.687	-120.741	34
9	Crooked	CROO	Clearwater	US	45.821	-115.528	45
10	Dworshak Hatchery	DWOR	Clearwater	US	46.502	-116.330	27
11	Eagle	EAGL	Willamette	LC	45.351	-122.384	46
12	East Fork Hood	EFHJ	Hood	LC	45.563	-121.592	46
13	East Fork Lewis	ELEW	Lewis	LC	45.853	-122.780	38
14	East Fork Moose/Selway	EMOO	Clearwater	US	46.187	-114.899	40
15	East Fork Potlatch	EPOT	Clearwater	US	46.796	-116.421	34
16	Fifteen	FIFT	Fifteenmile Creek	MUCLS	45.506	-121.128	47
17	Fish/Lochsa	FISH	Clearwater	US	46.333	-115.347	47
18	Parkdale	PAHH	Hood	LC	45.523	-121.622	46
19	Cow	COW	Imnaha	MUCLS	45.768	-116.750	14
20	John Day Main Fork - Beech	JDMA	John Day	MUCLS	44.412	-119.116	30
21	Joseph	JOSE	Grande Ronde	MUCLS	46.027	-117.018	46
22	Kalama Summer	KALS	Kalama	LC	46.032	-122.870	46
23	Kalama Winter	KALW	Kalama	LC	46.032	-122.870	36
24	Klickitat - Summer run	KLIS	Klickitat	LC	45.716	-121.259	132
25	Klickitat - Winter run	KLIW	Klickitat	LC	45.716	-121.259	99
26	Lake/Lochsa	LAKE	Clearwater	US	46.463	-114.997	42
27	Little Clearwater/Selway	LCLW	Clearwater	US	45.753	-114.775	47
28	Methow	LIBB	Methow	MUCLS	48.228	-120.114	18
29	Lick Creek	LICK	Salmon	MUCLS	45.062	-115.760	43
30	Loon	LOON	Salmon	MUCLS	44.808	-114.811	45
31	LittleSheep	LSHE	Imnaha	MUCLS	45.477	-116.930	37

32	Marsh	MARS	Salmon	MUCLS	44.449	-115.230	45
33	Upper Middle Fork JD	NFJD	John Day	MUCLS	44.588	-118.506	46
34	Mill Creek	MILL	Columbia Gorge	MUCLS	45.606	-121.187	46
35	Minthorn	MINT	Umatilla	MUCLS	45.669	-118.620	47
36	Mission	MISS	Clearwater	MUCLS	46.367	-116.735	40
37	Naches -Nile Creek	NACH	Yakima	MUCLS	46.861	-121.048	46
38	Middle North Fork JD	MFJD	John Day	MUCLS	44.838	-118.477	47
39	North Fork Lewis	NLEW	Lewis	LC	45.956	-122.555	40
40	Little Rock/Mad	NSAN	Willamette	LC	44.746	-122.395	28
41	Pahsimeroi Hatchery	PAHH	Salmon	MUCLS	44.663	-114.027	29
42	Rock	ROCK	Lower Mid-Columbia	MUCLS	45.747	-120.436	40
43	Satus	SATU	Yakima	MUCLS	46.196	-120.612	47
44	Sawtooth	SAWN	Salmon	MUCLS	44.150	-114.883	41
45	South Fork John Day	SFJD	John Day	MUCLS	44.331	-119.565	80
46	Skamania Stock	SKAM	Willamette	LC	45.241	-122.281	47
47	South Fork Santiam/Wiley	SSAN	Willamette	LC	44.415	-122.673	31
48	Tenmile	TENM	Clearwater	US	45.805	-115.683	41
49	Tucannon	TUCN	Tucannon	MUCLS	46.309	-117.657	46
50	Upper Grande Ronde	UGRT	Grande Ronde	MUCLS	45.731	-117.864	43
51	Umatilla	UMAT	Umatilla	MUCLS	45.699	-118.396	46
52	West Fork Yankee Fork	WFYF	Salmon	MUCLS	44.349	-114.725	46
53	Whitebird	WHIT	Salmon	MUCLS	45.752	-116.322	44
54	West Fork Hood	WHOO	Hood	LC	45.559	-121.692	45
55	Winthrop	WNFH	Methow	MUCLS	48.475	-120.189	47
56	West Side Willamette	WWIL	Willamette	LC	44.747	-123.147	44

Figure 3: Map displaying sampled Sockeye salmon and Kokanee (*O. nerka*) populations. Ascertainment collections were genotyped using RAD sequencing for SNP discovery; numbers on the map correspond to collection descriptions (Table 3).

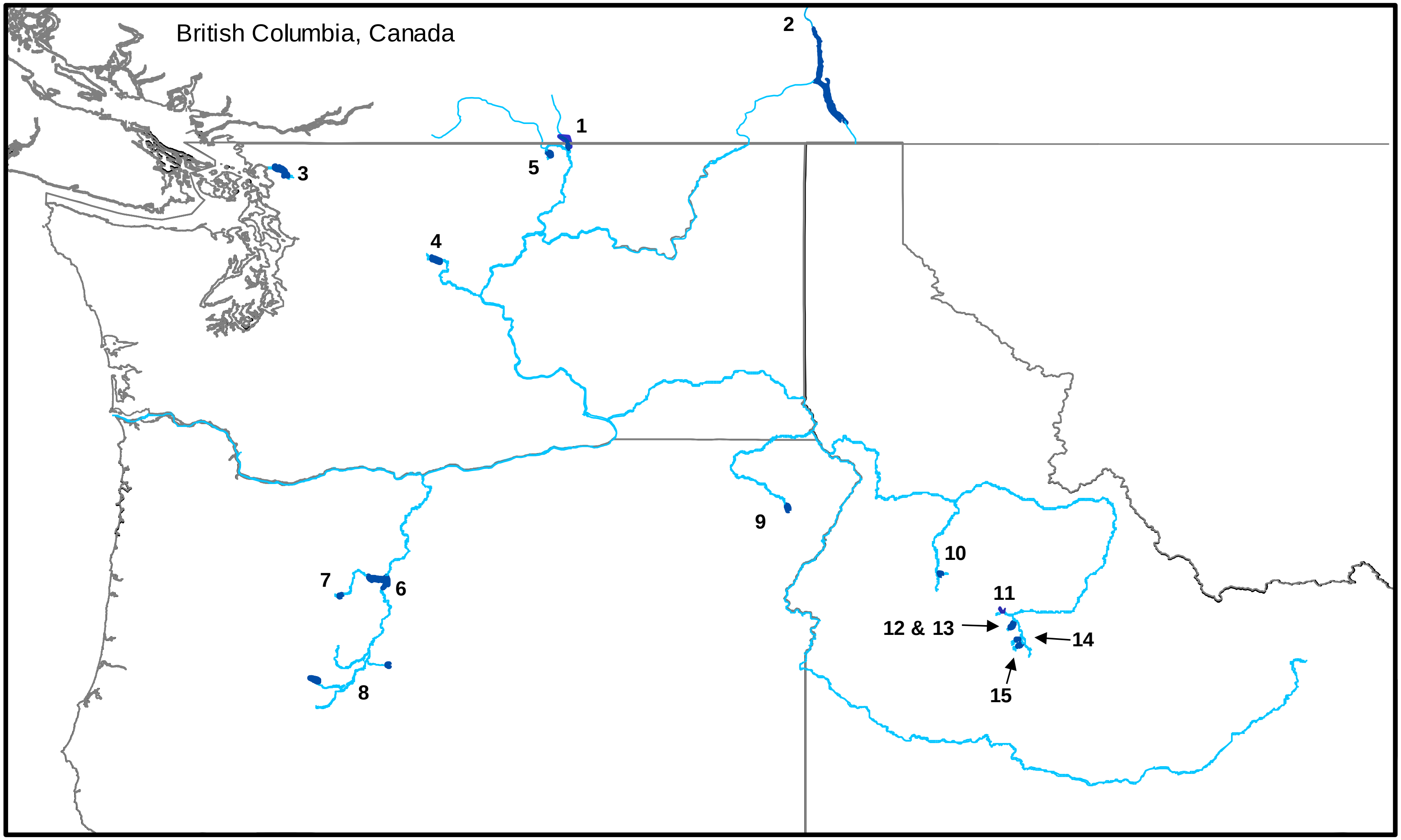
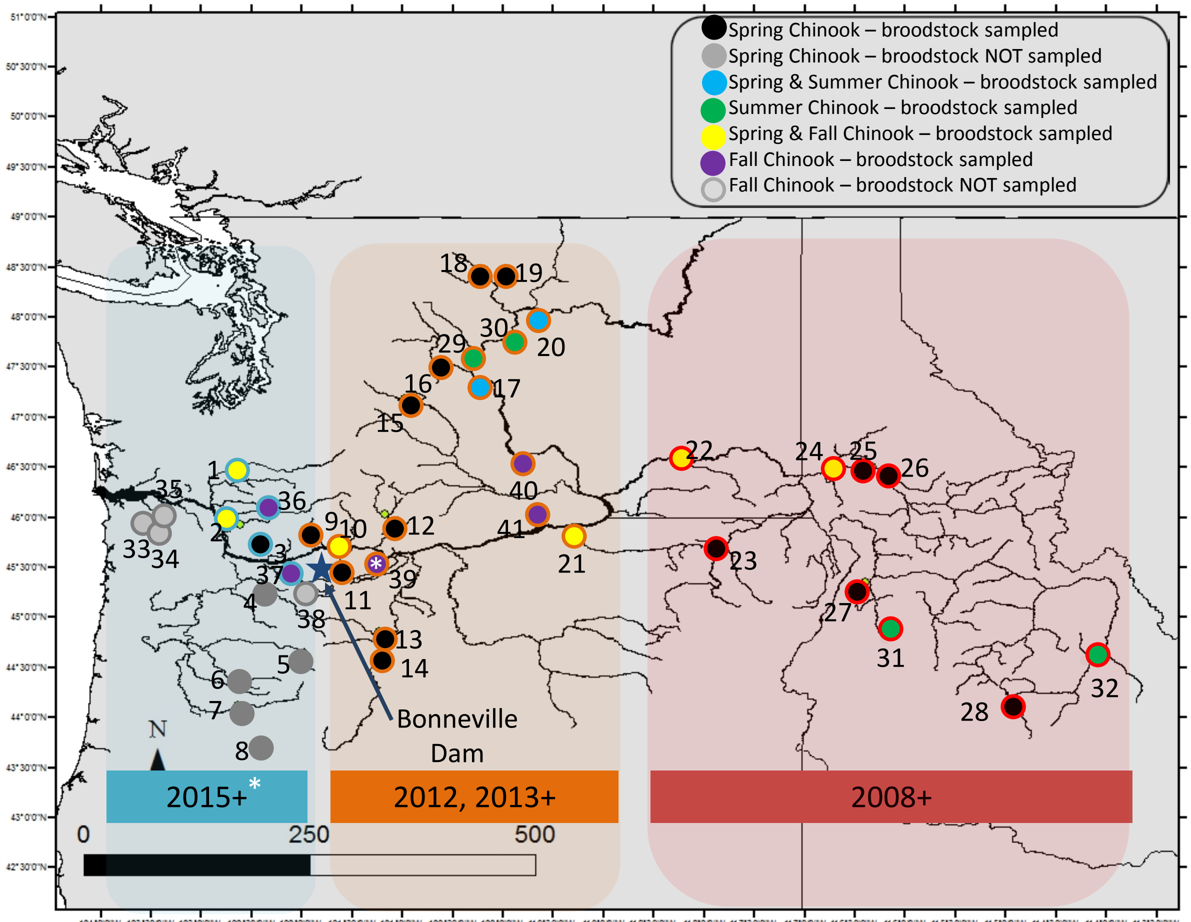


Table 3: Geographic distribution and locations of O. nerka reference collections in the ascertainment panel for SNP discovery.

	reference population					
ID#	name	state	region	sub-basin	life history	origin
1	Osoyoos Lake	Canada	Upper COL.	na	SOCK	NOR
2	Meadow Creek	Canada	Upper COL.	na	KOK	HAT
3	Lake Whatcom	WA	Puget Sound	na	KOK	HAT
4	Wenatchee Lake	WA	Upper COL.	Wenatchee	SOCK	NOR
5	Palmer Lake	WA	Upper COL.	Okanogan	KOK	stock
6	Lake Billy Chinook	OR	Middle Columbia	Deschutes	KOK	stock
7	Suttle Lake/ Link Creek	OR	Middle Columbia	Deschutes	KOK	stock
8	upper Deschutes	OR	Middle Columbia	Deschutes	KOK	stock
9	Wallowa Lake	OR	Snake	Grande Ronde	KOK	stock
10	Warm Lake	ID	Snake	Salmon (*S. F.*)	KOK	NOR
11	Stanley Lake	ID	Snake	Salmon (*Sawtooth*)	KOK	?
12	Redfish Lake	ID	Snake	Salmon (*Sawtooth*)	SOCK	NOR
13	Fishhook Creek	ID	Snake	Salmon (*Sawtooth*)	KOK	NOR
14	Pettit Lake	ID	Snake	Salmon (*Sawtooth*)	KOK	stock
15	Alturas Lake	ID	Snake	Salmon (*Sawtooth*)	KOK	stock

The PBT tagging technology has been implemented through annual hatchery broodstock sampling to create a temporally structured parental genotype baseline. PBT broodstock were non-lethally sampled by collected fin tissue from adult fish returning to hatcheries in the Columbia River basin (Figures 4 and 5, Tables 4 and 5). Required data for PBT sampling includes a hatchery record of phenotypic sex and spawn date. Additional and optional information was collected at some hatcheries when resources allowed, including fork length, and mated cross records of male and female broodstock individuals. The PBT baseline was expanded to include spawn years 2012 to 2015, including n=19,876 spring Chinook salmon, n=35,912 summer/fall Chinook salmon, and n=5357steelhead trout. DNA was extracted using modified Chelex extractions and Qiagen DNeasy 96 kits. Extracted genomic DNA was genotyped using the following protocols: 1) Fluidigm dynamic 96.96 array chips at 96 SNP loci (https://www.monitoringresources.org/Document/Method/Details/1332), and 2) GTseq at 298 SNP loci (https://www.monitoringresources.org/Document/Method/Details/5446).
Hatchery offspring that are subsequently sampled either as juveniles or adults (e.g., in a fishery) are then PBT assigned back to spawned parents which provides the individual age and specific hatchery of origin for each offspring.

Figure 4: Spring Chinook salmon and summer/fall Chinook Salmon PBT hatcheries. Star = Bonneville Dam, Gray = broodstock NOT sampled. The proportion (%) of released smolts that were tagged is estimated from the Fish Passage Center in migration year 2015.



PBT began with Chinook salmon and steelhead hatchery stocks in the Snake River basin of Idaho (2008-present; Steele et al., 2013; Steele et al., 2015). However, we have expanded PBT coverage to include Chinook salmon, steelhead, and Coho salmon broodstocks in all hatcheries above Bonneville Dam using expanded SNP panels of 261 loci for steelhead and 298 loci for Chinook salmon (see Chapter 1 in Hess et al. 2016). Each year the expansion effort is integrated with existing Snake River PBT baselines as data comes available. Adopting PBT to the broader Columbia River basin facilitates our ability to genetically track millions of salmonids and provide opportunities to address a variety of parentage-based research and management questions, including stock contributions to fisheries (Byrne et al., 2015), estimates of stock-specific abundance and run-timing at dams (Hess et al., 2016a; Vu et al. 2015), and use of thermal refugia during migration (Hess et al., 2016b).

Figure 5: Steelhead and coho salmon PBT hatcheries. Star = Bonneville Dam. The proportion (%) of released smolts that were tagged is estimated from the Fish Passage Center in migration year 2015.Only 77% of broodstock was sampled for PBT in 2015. Skamania released above Bonneville dam are PBT tagged (SY2015+, not sampling broodstock for below Bonneville releases).

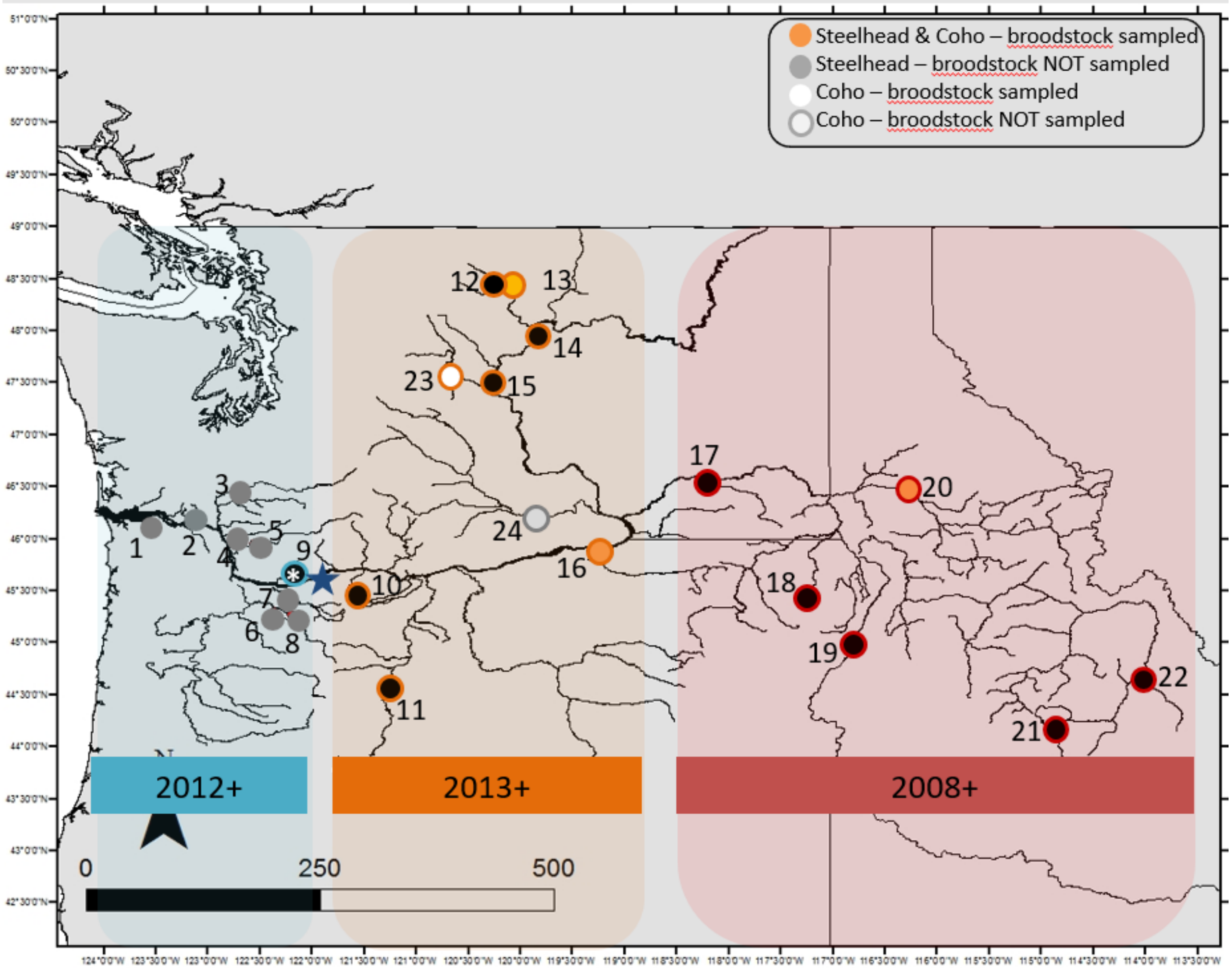


Table 4: Chinook salmon hatchery broodstock sampled for PBT baselines (see Figure 4).

Map 1 ID	lineage	spawning hatchery	Bonneville region	Initial PBT year	Genotyped
1	SPR	Cowlitz Salmon	below	2014	--- ---
2	SPR	Kalama Falls	below	2015	---
3	SPR	Lewis River	below	2015	---
4	SPR	Clackamas	below	na	---
5	SPR	Marion Forks	below	na	---
6	SPR	South Santiam	below	na	---
7	SPR	McKenzie	below	na	---
8	SPR	Willamette	below	na	---
9	SPR	Carson NFH	above	2012	2012-2015
10	SPR	Little White Salmon NFH	above	2013	2013-2015
11	SPR	Parkdale	above	2012	2012-2015
12	SPR	Klickitat	above	2008	2008-2015
13	SPR	Warm Springs NFH	above	2012	2012-2015
14	SPR	Round Butte	above	2012	2012-2015
15	SPR	Cle Elum SRF	above	2012	2012, 2014-2015
16	SPR	Leavenworth NFH	above	2013	2013
17	SPR	Eastbank	above	2012	tissues @ WDFW
18	SPR	Methow	above	2012	2012-2015
19	SPR	Winthrop NFH	above	2013	2013-2015
20	SPR	Chief Joseph	above	2014	2014-2015
21	SPR	Umatilla	above	2012	2012-2015
22	SPR	Lyons Ferry	Snake	2008	2008-2015
23	SPR	Lookingglass	Snake	2008	2008-2015
24	SPR	Nez Perce Tribal	Snake	2008	2008-2015
25	SPR	Dworshak NFH	Snake	2008	2008-2015
26	SPR	Clearwater	Snake	2008	2008-2015
27	SPR	Rapid River	Snake	2008	2008-2015
28	SPR	Sawtooth	Snake	2008	2008-2015
1 & 17	SUM	Eastbank	above	2012	2012
2 & 29	SUM	Entiat NFH	above	2013	2013-2014
3 & 30	SUM	Wells	above	2012	2012-2014
4 & 20	SUM	Chief Joseph	above	2013	2013-2014
5 & 31	SUM	SF Salmon, McCall	Snake	2008	2008-2015
6 & 32	SUM	Pahsimeroi	Snake	2008	2008-2015
1	FALL	NF Klaskanine	below	na	---
2	FALL	SF Klaskanine	below	na	---
3	FALL	Big Creek	below	2015 (~50%	---

				sampled)	
4	FALL	Cowlitz Salmon	below	2014	---
5	FALL	Toutle	below	2015	---
6	FALL	Kalama Falls	below	2015	---
7	FALL	Washougal	below	2015	---
8	FALL	Bonneville, Tanner Cr Tule	below	na	---
9	FALL	Little White Salmon NFH	above	2013	2013-2014
10	FALL	Spring Creek NFH	above	2015	---
11	FALL	Priest Rapids	above	2012	2012-2014
12	FALL	Prosser	above	2012 (missed 2014)	2012-2014
13	FALL	Umatilla	above	2012	2012-2014
14	FALL	Lyons Ferry	Snake	2011	2011-2015
15	FALL	Nez Perce Tribal	Snake	2011	2011-2015

Table 5: Steelhead and Coho salmon hatchery broodstock sampled for PBT baselines (see Figure 5).

Map 1 ID	species	spawning hatchery	Bonneville region	Initial PBT year	genotyped
1	steelhead	Big Creek	below	na	---
2	steelhead	Abernathy FTC	below	2012	---
3	steelhead	Cowlitz Trout	below	na	---
4	steelhead	Kalama Falls	below	na	---
5	steelhead	Merwin	below	na	---
6	steelhead	Clackamas	below	na	---
7	steelhead	Sandy	below	na	---
8	steelhead	Eagle Creek NFH	below	na	---
9	steelhead	Skamania	below	*	2013-2015
10	steelhead	Parkdale	above	2012	2012-2015
11	steelhead	Round Butte	above	2013	2013-2015
12	steelhead	Methow (Twisp)	above	2013	2013-2015
13	steelhead	Winthrop NFH	above	2012	2012-2015
14	steelhead	Wells	above	2013	2013-2015
15	steelhead	Eastbank	above	2012	tissues @ WDFW
16	steelhead	Umatilla	above	2012	2012-2015
17	steelhead	Lyons Ferry	Snake R.	2009	2009-2015
18	steelhead	Wallowa	Snake R.	2009	2009-2015
19	steelhead	Oxbow	Snake R.	2008	2008-2015
20	steelhead	Dworshak NFH	Snake R.	2008	2008-2015
21	steelhead	Sawtooth	Snake R.	2008	2008-2015

22	steelhead	Pahsimeroi	Snake R.	2008	2008-2015
1	coho	Winthrop NFH	above	2012	2012-2014
2	coho	Leavenworth NFH	above	2012	2012-2014
3	coho	Prosser	above	na	---
4	coho	Umatilla	above	2012	---
5	coho	Dworshak NFH	Snake	2012	---

* 2013-2015 (all brood), 2016+ (only above Bonneville dam releases)

Results

For application in GSI of Chinook salmon, we identified 19,703 high-quality SNPs with RAD sequencing (Hecht et al. 2015). A PCoA clustering plot (Figure 6) and the genetic distance topology displayed in a neighbor-joining tree (Figure 7) both show distinction between Columbia River populations belonging to the south coastal genetic lineage and populations belonging to the interior Columbian River lineage.

Figure 6: Principle component analysis (PCA) of Chinook salmon RAD populations within the Columbia Basin. When combined with range-wide samples, SNPs effectively distinguish two lineages within the Columbian basin: Interior Columbian River and South Coastal.

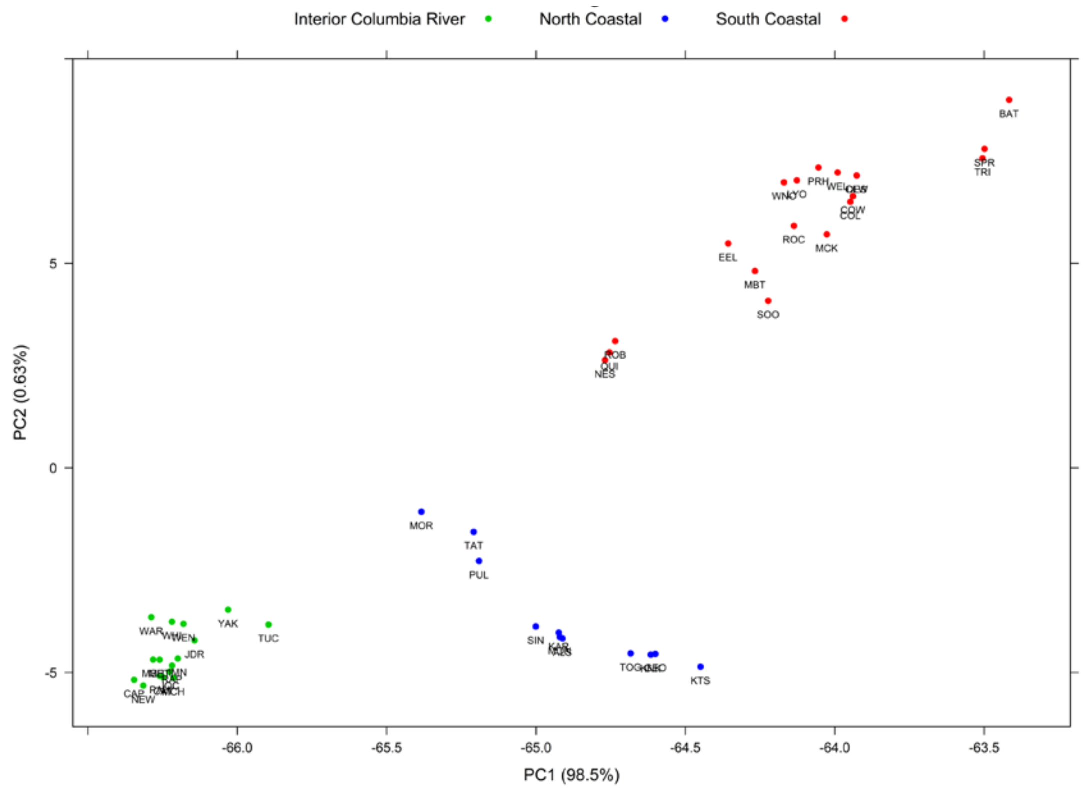
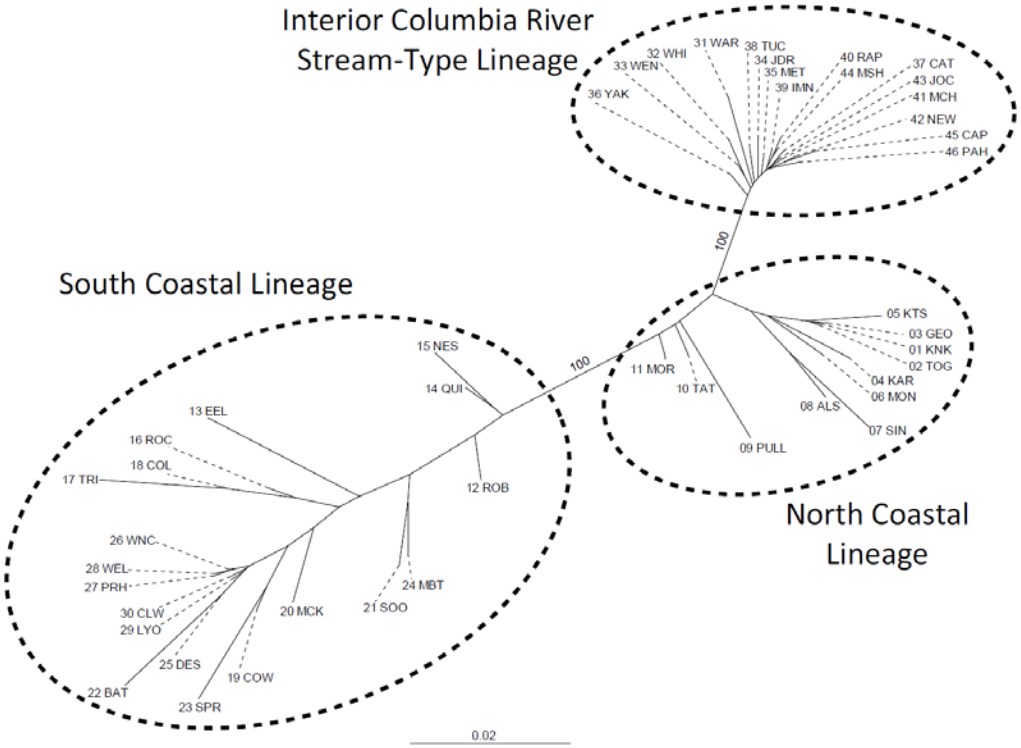


Figure 7: Chinook salmon neighbor-joining tree using RAD data. RAD SNPs distinguish lineages with high confidence using range-wide samples.



For anadromous steelhead, we identified 25,526 high-quality SNPs within the Columbia Basin with RAD sequencing. A PCoA clustering plot (Figure 8) and the genetic distance topology displayed in a neighbor-joining tree (Figure 9) both identify three distinct groups: the coastal genetic lineage, and an upper snake and middle upper Columbia / lower snake component of the inland genetic lineage.

Figure 8: Principle component analysis (PCA) of steelhead RAD populations in the Columbia Basin. Black represents the upper snake lineage, orange represents the coastal lineage, and green represented the upper middle Columbia lineage.

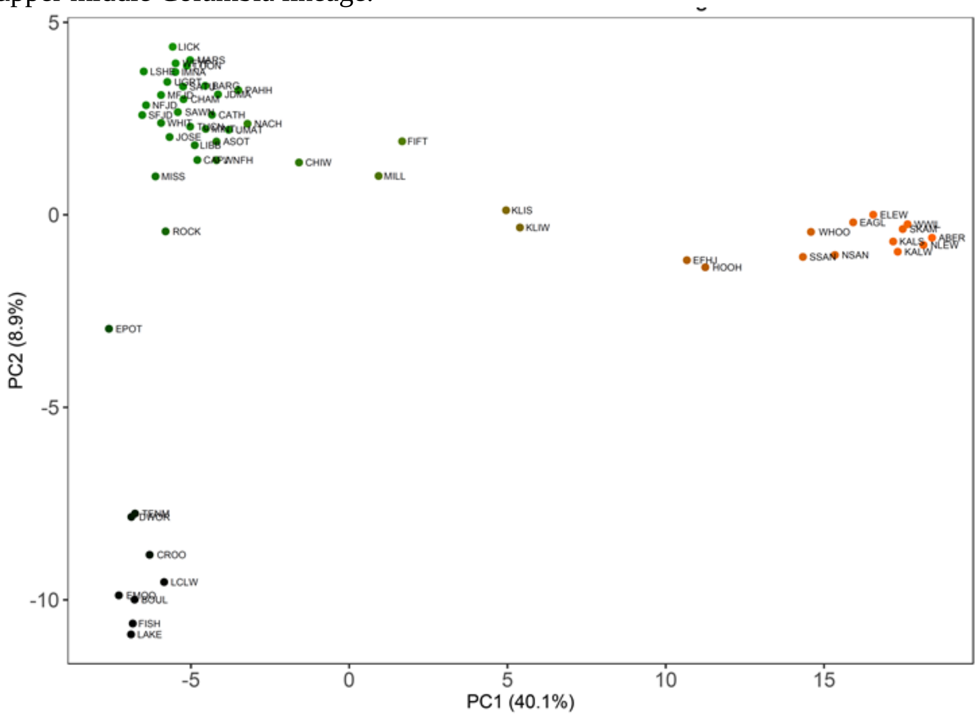
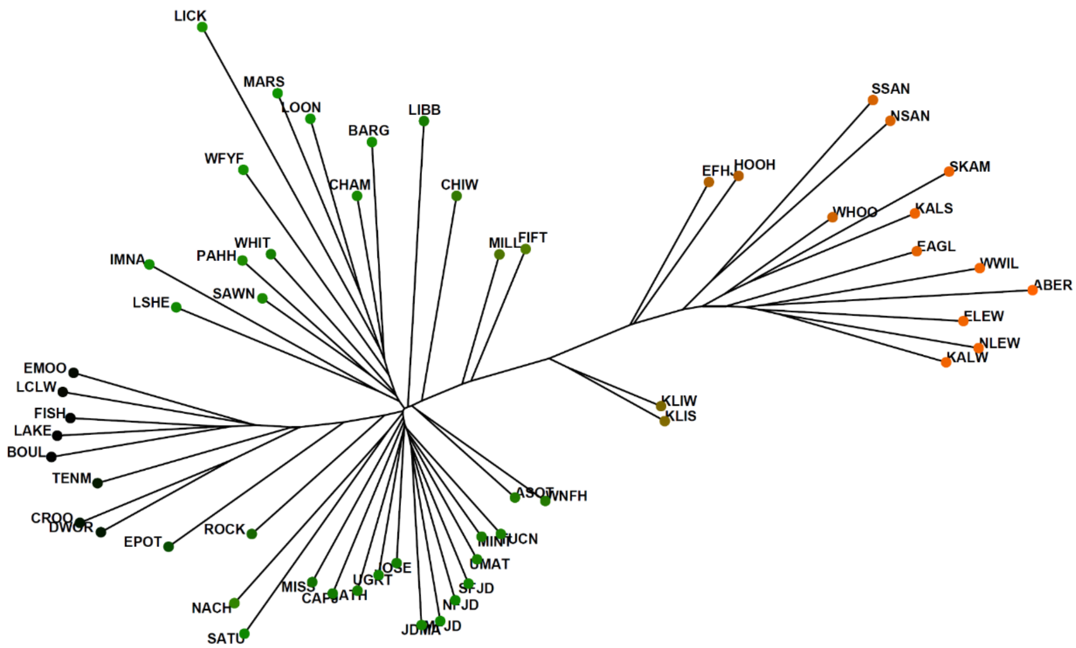
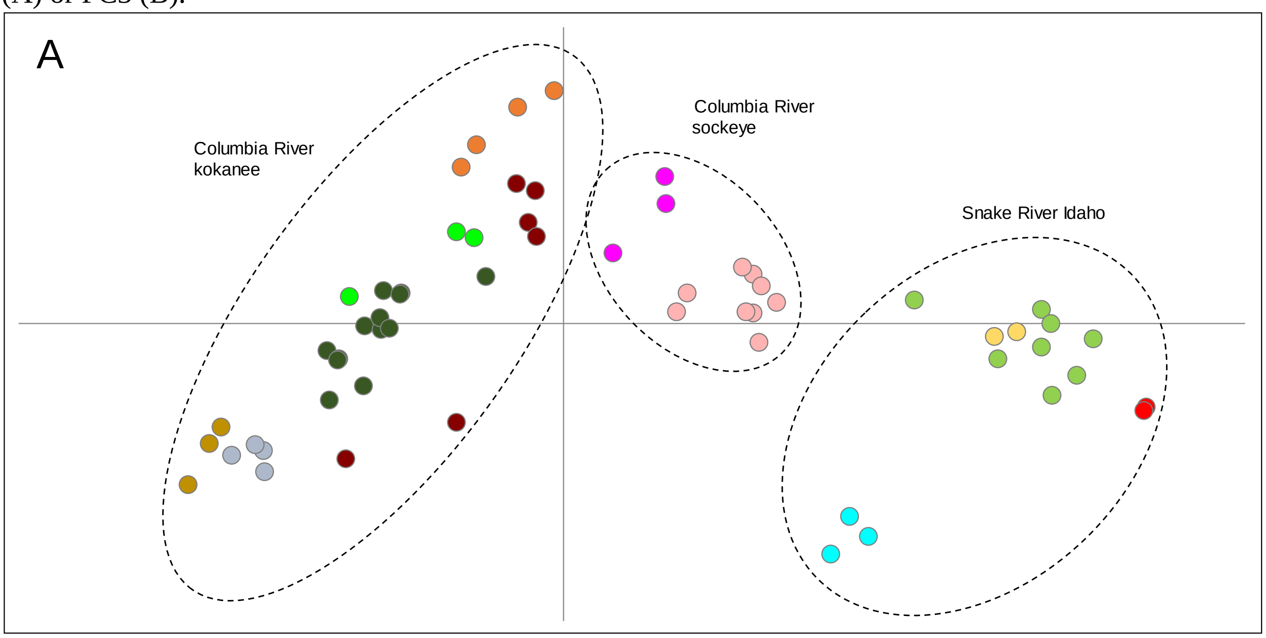


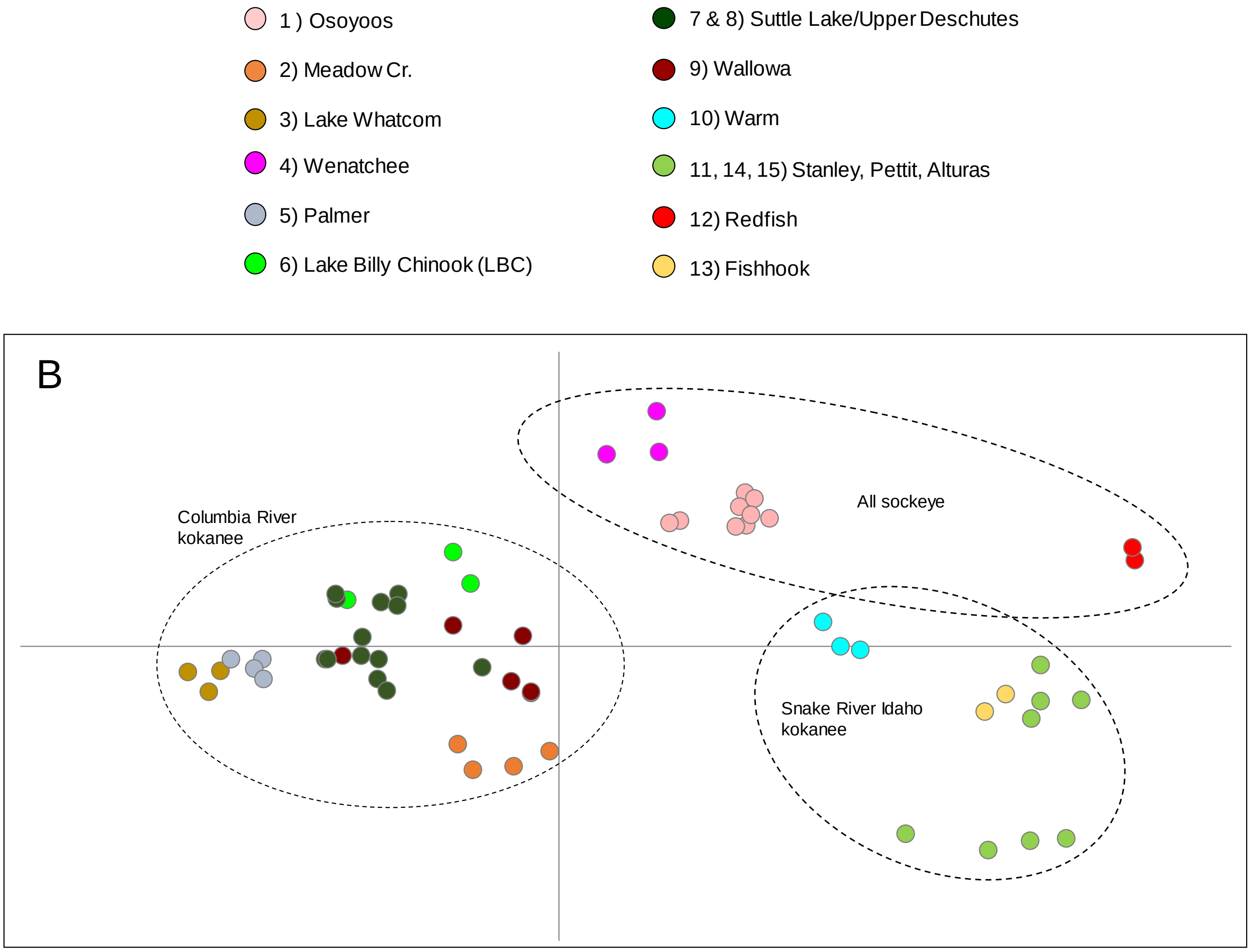
Figure 9: Columbia Basin steelhead RAD neighbor-joining tree showing differentiation between the coastal lineage (orange), middle upper Columbia group (green), and upper snake group (black).



For *O. nerka* SNP discovery, quality filtering of RAD sequencing data resulted in >6400 SNPs that were selected for further screening to maximize available information. After the quality filtering phase of the analysis we have collected genotypes for 484 informative RAD markers that exhibit a strong ability to differentiate major population groups of *O. nerka* within the basin as displayed in PCoA clustering plots (Figure 10). These include: 174 tags selected for high MAF among all ascertainment groups, 4 tags that are informative for differentiating between an out-group from Lake Ozette (see Hess et al. 2016) and all Columbia River Basin stocks, 8 tags that differentiate between sockeye salmon and kokanee life history types, 11 tags that differentiate kokanee hatchery stocks, 16 tags that differentiate Deschutes River stocks, 30 tags that differentiate between two upper Columbia sockeye salmon stocks, 104 tags that generally differentiate between Snake and Columbia river regions, and 137 tags that are informative for Redfish Lake sockeye salmon parentage. Primer design has been completed and testing of the new expanded SNP panel is scheduled to occur in 2017 using approximately 1000 samples from diverse locations.

Figure 10: PCoA plot showing clustering of O. nerka RAD collections used in SNP discovery. Ellipses group ascertainment samples by either life history type (kokanee or Sockeye) and by region (Columbia or Snake rivers). Note the exception in which Wallowa Lake kokanee from the Snake River in Oregon group with Columbia River kokanee. The X-axis in the plots represents PC1. The X-axis represents either PC2 (A) or PC3 (B).





The current expanded PBT baseline is comprised of spring Chinook salmon (n=18,797), summer/fall Chinook salmon (n=32,830), and steelhead (n=5,069) from the Columbia River basin that were successfully genotyped at a minimum of 90% of the loci in respective panels (Table 6).

Table 6: Chinook and steelhead PBT baselines throughout the Columbia River basin. Chinook and steelhead hatchery programs are shown, along with run type, lineage and years of availability. Chinook and steelhead were each genotyped using 96 SNPs (X), but Chinook were also genotyped at 298 SNPs (X), whereas steelhead were genotyped using 192 SNPs (X), and 269 SNPs (X) as shown. Chinook collections for which data are not yet available are denoted in red (i.e., X).

Hatchery	Species	Code	Run type	Lineage	Year							
					2008	2009	2010	2011	2012	2013	2014	2015
Clearwater Fish Hatchery	Chinook	OtsCLWH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Clearwater Fish Hatchery - Powell Facility	Chinook	OtsPOWP	Spring	Interior stream type	X	X	X	X	X	X	X	X
Dworshak National Fish Hatchery	Chinook	OtsDWOR	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Catherine Creek	Chinook	OtsCTHW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Grande Ronde	Chinook	OtsGRUW	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Imnaha River	Chinook	OtsIMNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Lookingglass Creek	Chinook	OtsLOOK	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Lostine River	Chinook	OtsLSTW	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery	Chinook	OtsLYON	Spring	Interior stream type	*	*	*	*	X	X	X	X
Lyons Ferry Fish Hatchery - Tucannon River	Chinook	OtsTUCW[a]	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery	Chinook	OtsLYON_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X
McCall Fish Hatchery - Johnson Creek	Chinook	OtsJHNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X
McCall Fish Hatchery - South Fork Salmon	Chinook	OtsMCCA	Spring	Interior stream type	X	X	X	X	X	X	X	X
Nez Perce Tribal Fish Hatchery (Fall)	Chinook	OtsNPFH_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X
Nez Perce Tribal Fish Hatchery (Spring)	Chinook	OtsNPFH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Pahsimeroi Fish Hatchery	Chinook	OtsPAHH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Rapid River Fish Hatchery	Chinook	OtsRAPH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery	Chinook	OtsSAWT	Spring	Interior stream type	X	X	X	X	X	X	X	X
Big Creek Hatchery	Chinook	OtsBIG	Fall	Interior ocean type	*	*	*	*	*	*	*	X
Carson National Fish Hatchery	Chinook	OtsCAR	Spring	Interior stream type	*	*	*	*	X	X	X	X
Chief Joseph Hatchery (Spring)	Chinook	OtsCJH_sp	Spring	Interior stream type	*	*	*	*	*	*	X	X
Chief Joseph Hatchery (Summer/Fall)	Chinook	OtsCJH_sufa	Summer	Interior ocean type	*	*	*	*		X	X	X
Cowlitz Salmon	Chinook	OtsCOW	Spring	Interior stream type	*	*	*	*	*	*	*	*
Eastbank Fish Hatchery	Chinook	OtsEASTBK	Summer	Interior ocean type	*	*	*	*	X	*	*	*
Entiat National Fish Hatchery	Chinook	OtsENFH	Summer	Interior ocean type	*	*	*	*	*	X	X	X
Kalama Falls	Chinook	OtsKAL	Spring	Interior stream type	*	*	*	*	*	*	*	*
Klickitat State Fish Hatchery	Chinook	OtsKH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Leavenworth National Fish Hatchery	Chinook	OtsLNFH	Spring	Interior stream type	*	*	*	*	*	X	X	X
Lewis River	Chinook	OtsLEW	Spring	Interior stream type	*	*	*	*	*	*	*	*
Little White Salmon National Fish Hatchery (Fall)	Chinook	OtsLWS_sufa	Fall	Interior ocean type	*	*	*	*	*	X	X	X
Little White Salmon National Fish Hatchery (Spring)	Chinook	OtsLWS_sp	Spring	Interior stream type	*	*	*	*	*	X	X	X
Methow State Fish Hatchery	Chinook	OtsMETH	Spring	Interior stream type	*	*	*	*	X	X	X	X
Parkdale Fish Facility	Chinook	OtsPFF	Spring	Interior stream type	*	*	*	*	X	X	X	X
Priest Rapids Hatchery	Chinook	OtsPRH	Fall	Interior ocean type	*	*	*	*	X	X	X	X
Round Butte Fish Hatchery	Chinook	OtsRB	Spring	Interior stream type	*	*	*	*	X	X	X	X
Spring Creek NFH	Chinook	OtsSPCR	Fall	Interior ocean type	*	*	*	*	*	*	*	X
Toutle	Chinook	OtsTOU	Fall	Interior ocean type	*	*	*	*	*	*	*	*
Umatilla Fish Hatchery (Fall)	Chinook	OtsUMA_sufa	Fall	Interior ocean type	*	*	*	*	X	X	X	
Umatilla Fish Hatchery (Spring)	Chinook	OtsUMA_sp	Spring	Interior stream type	*	*	*	*	X	X	X	X
Washougal	Chinook	OtsWAS	Fall	Interior ocean type	*	*	*	*	*	*	*	*
Warm Springs National Fish Hatchery	Chinook	OtsWSNFH	Spring	Interior stream type	*	*	*	*	X	X	X	X
Wells Fish Hatchery	Chinook	OtsWELLS	Summer	Interior ocean type	*	*	*	*	X	X	X	X
Winthrop National Fish Hatchery	Chinook	OtsWTP	Spring	Interior stream type	*	*	*	*	*	X	X	X
Yakima Nation Prosser Hatchery	Chinook	OtsPRO	Fall	Interior ocean type	*	*	*	*	X	X	*	X
Yakima River Roza Dam-Integrated	Chinook	OtsYRint	Spring	Interior stream type	*	*	*	*	X	*	X	X
Yakima River Roza Dam-Segregated	Chinook	OtsYRseg	Spring	Interior stream type	*	*	*	*	X	*	*	X
Dworshak National Fish Hatchery	Steelhead	OmyDWOR	Unknown	Interior	X	X	X	X	X	X	X	X
Little Sheep Creek Hatchery	Steelhead	OmyLSCR	Summer	Interior	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery- Touchet	Steelhead	OmyTOUW[b]	Summer	Interior	*	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery	Steelhead	OmyLYON[c]	Unknown	Interior	*	X	X	X	X	N/A	N/A	N/A
Lyons Ferry Fish Hatchery - Grande Ronde	Steelhead	OmyCGRW[b]	Summer	Interior	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery - Tucannon	Steelhead	OmyTUCW[b]	Summer	Interior	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery - Wallowa	Steelhead	OmyWALW	Summer	Interior	*	*	*	*	*	*	*	X
Oxbow	Steelhead	OmyOXBO	Summer	Interior	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery	Steelhead	OmySAWT	Summer	Interior	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery - East Fork Salmon	Steelhead	OmyEFSW[d]	Summer	Interior	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery - Squaw Creek	Steelhead	OmySQUW[e]	Summer	Interior	X	X	X	X	X	X	X	X
Pahsimeroi Fish Hatchery	Steelhead	OmyPAHH	Unknown	Interior	X	X	X	X	X	X	X	X
Wallowa	Steelhead	OmyWALL	Summer	Interior	*	X	X	X	X	X	X	X
Eastbank Hatchery	Steelhead	OmyEASTBK	Summer	Interior	*	*	*	*	*	*	*	*
Methow Hatchery (Twisp)	Steelhead	OmyTWP	Summer	Interior	*	*	*	*	*	X	X	X
Parkdale Fish Facility	Steelhead	OmyPFF	Winter	Coastal	*	*	*	*	X	X	X	X
Round Butte Fish Hatchery	Steelhead	OmyRB	Summer	Interior	*	*	*	*	*	X	X	X
Skamania Hatchery (Summer)	Steelhead	OmySKH_su[f]	Summer	Coastal	*	*	*	*	*	X	X	X
Skamania Hatchery (Winter)	Steelhead	OmySKH_wi[f]	Winter	Coastal	*	*	*	*	*	X	X	X
Umatilla Fish Hatchery	Steelhead	OmyUMA	Summer	Interior	*	*	*	*	X	X	X	X
Wells Hatchery	Steelhead	OmyWEL	Summer	Interior	*	*	*	*	*	X	X	X
Wells Hatchery - Omak stock	Steelhead	OmyWEL_OMA	Summer	Interior	*	*	*	*	*	*	*	X
Winthrop National Fish Hatchery	Steelhead	OmyWTP	Summer	Interior	*	*	*	*	X	X	X	X

[a]Chinook Lyons Ferry stock consolidated under 'OtsLYON' starting in 2012
[b]Steelhead Lyons Ferry stock consolidated under 'OmyLYON' starting in 2012
[c]Steelhead Lyons Ferry stock discontinued in 2013
[d]Steelhead Sawtooth stock consolidated under 'OmySAWT' from 2012-2013
[e]Sawtooth stock consolidated under 'OmySAWT' in 2012, renamed 'Upper Salmon B-run (YFLW) and consolidated under 'OmyPAHH' starting in 2013
[f]Steelhead Skamania stock is collected late in the calendar year, and is designated for the following broodyear (e.g., late 2012 collections are considered part of BY2013)
N/A – Stock discontinued/non-existent
*Broodstock not sampled

Discussion

Over the course of this project 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). Recently we have added coho salmon to our efforts. Our goal has been to construct SNP reference baselines that will be expanded or updated annually for continued evaluation of these species. This strategy assures the greatest likelihood of discerning reproductively distinct aggregations for each species through time (Waples 1991), while monitoring population viability 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) play a major role in how genetic divergence and differentiation is distributed geographically, and it will be important to evaluate these influential factors for potential effects on our ability to differentiate populations, both qualitatively (phenotypes; landscapes) and quantitatively (e.g., genetic stock identification). Our past results have been verified and substantiated through replication and comparisons of published data (Waples et al. 2004; Narum 2008b; Narum et al. 2010b). That work has led to the discovery of outlier loci for adaptive divergence. Our current efforts have expanded on our understanding of non-neutral genetic variation among populations for the species of interest (with the exception of coho salmon). The RAD datasets under construction will demonstrate the utility of SNPs to characterize adaptive variation, as was observed in a recent range-wide analysis of Chinook salmon (Hecht et al. 2015). Environmental and climate related variables that are likely to have significant influence on allele frequencies have been identified (e.g., precipitation, temperature maximums and minimums, elevation, etc.) and those data have been recorded for the purposes of in-depth testing of landscape associations.

The continued expansion of SNP panels and updating of baseline that include a more geographically broad set of collections/populations will help us achieve a greater level of resolution, which means greater statistical power to identify population distinctions among the major tributaries and sub-basins of the Columbia River Basin. The expansion efforts reported here complement previously reported results, and have provided improved ability to differentiate stocks on regional and local scales through application of GSI and PBT methods. The application of next generation sequencing techniques and resulting datasets demonstrate the ability to yield tens of thousands of potential SNPs, and has proven to be informative for identifying population differences distributed across landscapes (e.g., Narum et al. 2010a; Matala et al. 2011; Hecht et al. 2015). In our most recent studies, we have identified environmental and climate related variables (e.g., precipitation, temperature maximums and minimums, elevation, etc.) likely to have significant influence on allele frequencies among steelhead populations (Micheletti et al. *in preparation*), and those data have been recorded for the purposes of in-depth testing of landscape associations.

Collections of *O. tshawytscha*, *O. mykiss*, and *O. nerka* have been chosen for baseline expansion based on availability, novelty, and in accordance with our goal of reaching complete coverage of extant stocks within the Columbia River Basin. Priority collections for all three species have been identified as those important to basin-wide harvest and hatchery management, particularly in tribal fisheries. This includes major supplementation stocks for all three species, lower Columbia, ocean-type, and stream-type lineages of Chinook salmon, inland and coastal lineages

and summer-run and winter-run ecotypes of steelhead trout, and the anadromous (Sockeye salmon) and land-locked (kokanee) forms of *O. nerka*. Species-specific reference baselines may include life history variants such as potentially distinct populations of resident *O. mykiss* (Narum et al. 2008a; Narum et al. 2011). The application of GSI in fisheries continues to inform managers on several fronts, including: harvest management, abundance estimates, life history distinctions and conservation needs. Moreover, PBT is being used for multiple purposes including validation of assigned origins using GSI. In fact, PBT frequently reveals substantial numbers of unmarked hatchery-origin fish that are incorrectly identified as wild in the field due to mis-clipped adipose fins. Future efforts for baseline expansion include compiling marker "banks" that can be drawn from at any time should the need for more markers be necessary. An example of such need is basin-wide coverage to account for stock transfers or reintroductions throughout the basin (e.g., *O. nerka* in Cle Elum Lake).

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Section 3: Genetic Stock Identification of Chinook Salmon, Sockeye 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, Hess et al. 2014). Within the CRB, Chinook salmon consist of three major genetic lineages and steelhead consist of two major genetic lineages that can be further divided 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 used separate groups of SNP markers to discriminate 19 reporting groups for Chinook salmon, 14 reporting groups for steelhead, and four reporting groups for sockeye salmon.

Despite continuous improvements of the power of our Chinook salmon and steelhead baselines in GSI applications (Hess et al. 2014), 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 (i.e., 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, and was recently (i.e., 2012-present) expanded beyond Snake River hatcheries (Steele et al. 2011) to tag all Chinook salmon and steelhead hatchery broodstock from hatcheries in the CRB above Bonneville Dam. This application has effectively tagged all Snake River hatchery Chinook salmon and steelhead starting with the 2008 brood years, and elsewhere in the CRB above Bonneville Dam beginning with the 2012 brood year. When parent pairs of 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 harvest study to identify hatchery-origin fish, and then use GSI to estimate stock-of-origin of all other hatchery fish that were not assigned with PBT and for all natural origin fish. For sockeye salmon, we rely solely on GSI to determine stock of origin since PBT is not necessary to identify stocks. For the 2015 Chinook harvest, all age classes (3-, 4-, and 5-year old fish) can be identified from Snake River stocks using PBT. However, because our PBT baseline continues to expand for Columbia River stocks, only 3- and 4-year old fish can currently be identified for certain Columbia River stocks.

We continue to employ the genotyping-in-thousands by sequencing (GT-seq) approach that has been developed in our laboratory (Campbell et al. 2015). This approach has increased

the cost-effectiveness for genotyping moderate numbers of SNP loci (100s) for relatively large numbers of individuals (1000s), which allows us to run all SNP loci regardless of whether we intend to use primarily PBT analyses or a combination of PBT and GSI. Therefore, we avoid the difficult decisions we have had to make in past years (Hess et al. 2015), in which we needed to consider the primary goal of the project before selecting which panels of SNP markers were required. Not only is the genotyping decision process simplified, but our projects now benefit from the additional data that comes from genotyping with all available markers.

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 support sustainable fisheries, PBT and GSI can be used to address two primary questions: 1) how are Chinook salmon stocks temporally and spatially distributed in the mainstem Columbia River; and 2) how are these stocks temporally and spatially distributed in the harvests of fisheries.

Project objectives and higher level harvest management questions

Our study had two primary objectives: 1) utilize a combination of PBT and GSI analyses to determine stock composition of Chinook salmon harvested in sport, commercial, and tribal fisheries in the mainstem Columbia River, and 2) utilize GSI to estimate stock composition of sockeye salmon harvested above and below Bonneville Dam in commercial, sport, and tribal fisheries. Results from these objectives were used to address:

Harvest RM&E: F&W Program Management Question: What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?

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. For 2012-2014, we examined the stock composition of the Wind River sport harvest and provided context by comparing stock proportions among the various samples from other fisheries and Bonneville Dam that were analyzed that same year. We repeated this analysis for fish harvested in 2015. We include in this report the 3rd year of analysis of sockeye salmon fisheries in the Columbia River mainstem. Differences in relative abundance of the three main stocks (Okanagan, Wenatchee, and Snake) present challenges to managing lower river harvest, because of the desire to harvest the highly abundant Okanagan stock around the much less abundant Snake River stock and moderately abundant Wenatchee River stock. Stock composition estimates are expected to help determine how harvest is impacting these various stocks.

Time line for completion of objectives

Objectives will be ongoing and PBT/GSI results updated each year for harvest analyses of salmonids throughout the accords-funding. As new genetic techniques are developed they will be applied to this project and results will be compared between years to determine the extent of improvements.

Our study was not designed to address the following question:

Harvest RM&E: F&W Program Management Question: Can selective fisheries targeting hatchery fish or healthy populations reduce impacts on ESA-listed populations?

Accuracy testing of PBT and GSI baselines

Prior to conducting analyses for fisheries harvest collections and mixture samples encountered at Bonneville Dam (Section 4), we assessed the accuracy of our PBT and GSI baselines in assigning fish to their hatchery brood (PBT: Chinook salmon, steelhead) or reporting group (GSI: Chinook salmon, sockeye salmon, steelhead) of origin (see Results section).

Methods

Methods for estimating stock composition are available at (https://www.monitoringmethods.org/Protocol/Details/229). The Monitoring Methods Protocol is entitled Snake River steelhead and Chinook salmon stock composition estimates (2010-026-00) v1.0.

Tissue collection of Chinook salmon, Sockeye salmon, and steelhead

Tissues were sampled from Chinook salmon in 2015 from a total of ten different mixture sources: 1) Bonneville Dam (see Section 4), and the spring-run seasons of the following fisheries: 2) lower river commercial, 3) lower river sport, 4) lower river test, 5) Wind R. sport, 6) tribal spring, and 7) tribal ceremonial, the summer management period harvests of the following fisheries: 8) lower river commercial and 9) lower river sport, and the fall-run harvest from 10) the lower river mark-selective sport fishery of 2015. Drano Lake samples from the spring sport fishery were not provided to us in 2015. While fisheries generally harvest jack sized Chinook salmon at low rates and do not have specific harvest limits on jacks, jacks do comprise part of the harvest and may be sampled if encountered. Jacks are sampled at the Bonneville AFF trap in the proportion that they are encountered in the sampling. Sampling restrictions at the AFF can result in biases in the size of fish sampled compared to the run at large. A portion of the spring season Zone 6 tribal harvest was sampled by Megan Begay as part of the Yakama Nation fishery program. 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) and the Yakama Nation. The spring management period Chinook salmon fisheries were sampled below Bonneville Dam in the sport and commercial fishery and tribal bank fishery immediately downstream of Bonneville Dam (regions A and B), and sampled above Bonneville Dam in Zone 6as part of the Wind River sport fishery and Yakama Nation Zone 6

fishery (Figure 1; Table 1). The summer management period fisheries were sampled below Bonneville Dam in the sport and commercial fisheries, and above Bonneville Dam in Zone 6 in the tribal commercial fishery. Due to limited funds, we analyzed a subset of samples (approximately 50 fish per weekly strata) obtained from the spring and summer Chinook salmon sport and commercial fisheries below Bonneville Dam and the spring season Zone 6 tribal fishery. A subset of samples was analyzed from the spring season sport mark-selective Wind River fishery, due to budgetary constraints. For fisheries in which we had to subsample the harvest, we selected fish randomly and with a balanced design across spatial regions.

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). Here, we do not discriminate between Region 01 and Region 02 in the Zone 6 fishery, because that information did not accompany the samples we received. 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.

Non-tribal fisheries during the spring management period for 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. Fish with intact adipose fins that are caught in these fisheries are released, but mortality rates are unknown from these releases. In addition to sampling hatchery-origin fish from the mark selective commercial and sport fisheries, we were able to obtain samples from non-clipped hatchery and natural origin fish from Bonneville Dam and the tribal Zone 6 fishery above Bonneville Dam.

Tissues were sampled from sockeye salmon in 2015 from four fishery mixture sources: 1) lower river commercial, 2) lower river sport, 3) Bonneville Dam (see Section 4), and 4) the tribal fishery in Zone 6. All samples obtained from these fisheries were analyzed.

Molecular data

Methods for DNA extraction, DNA amplification, and genotyping of SNP assays using genotyping-in-thousands by sequencing (GT-seq) are available at (https://www.monitoringresources.org/Document/Method/Details/5446). Additional details regarding how 192 SNPs were reduced to 186 SNPs can be found in Hess et al. (2012, 2013). Subsequently, we have reduced our Chinook salmon GSI baseline from 186 SNPs to 179 SNPs, because we were unable to transition the full set of 186 SNPs to GT-seq protocols. These 179 SNP markers were used for GSI, and for PBT analyses, we used 95 of the SNPs. We used 93 SNP markers for GSI of sockeye mixtures.

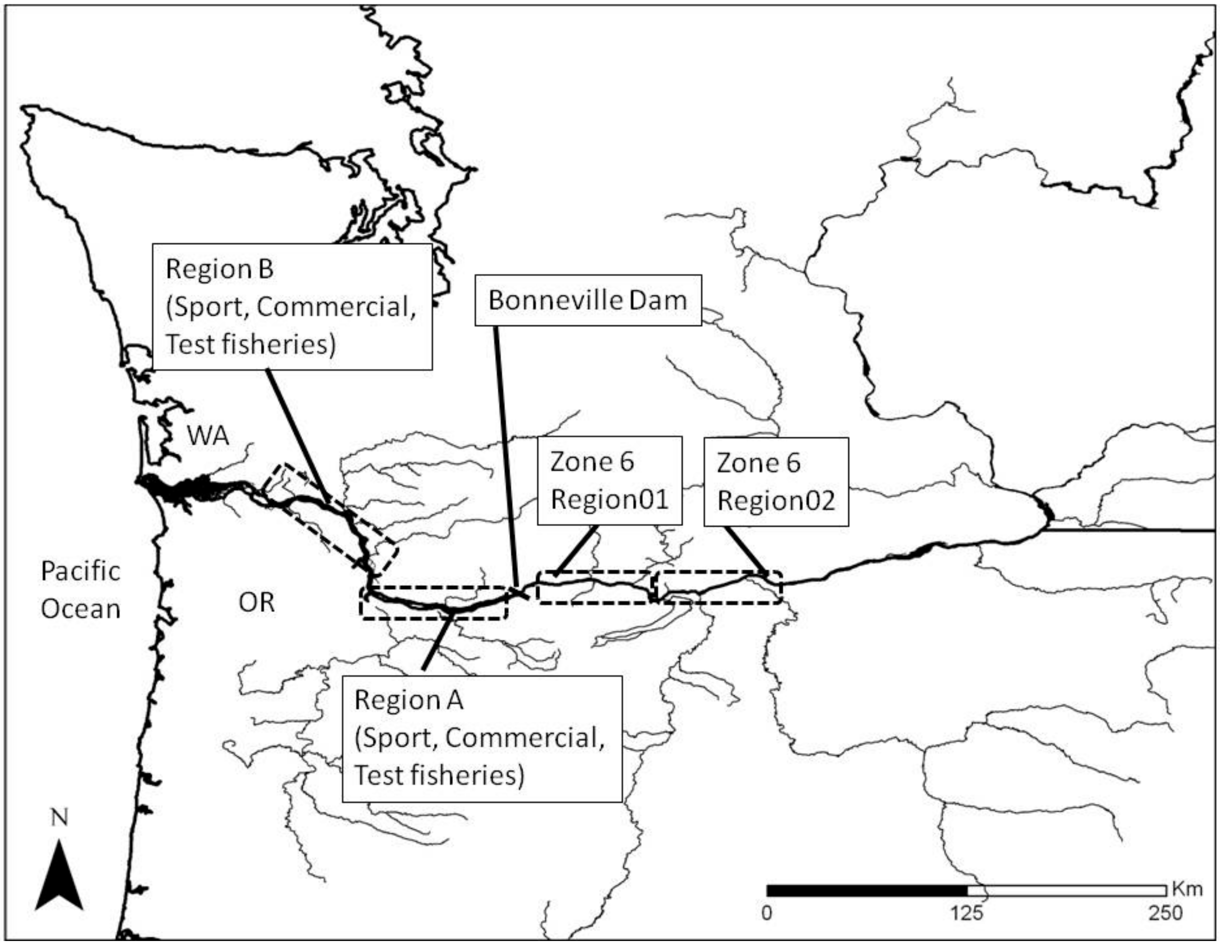


Figure 1: Project scope showing sources of Chinook salmon and sockeye salmon harvest mixtures that were analyzed using PBT/GSI.

Table 1: Characteristics of Chinook salmon harvest samples by fishery, region, life history stage, and origin by weekly strata.

Run Type	Region	Harvest Region	Stage	Origin	Sampled (N)	Genotyped (N)	Statistical week																																	
							Spring Management period														Summer Management period							Fall Management period												
							11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Spring	Tribal Ceremonial	Zone 6	Adult	Hatchery	299	82						41	41																											
				Wild	67	18						9	9																											
			Jack	Wild	1	0																																		
	Tribal	Zone 6	Adult	Hatchery	1910	300										34	32	33	31	23	24	23	16	29	38	17														
				Wild	1473	250										16	18	17	19	27	26	27	34	21	12	33														
			Jack	Hatchery	41	41										3	8	10			3	3	8	2	4															
				Wild	17	17											3	1			1	3	1	3	2	3														
	Lower River Commercial	Region A	Adult	Hatchery	699	349				50	49				50	50		50	50	50																				
				Wild	2	1					1																													
			Jack	Hatchery	121	73					1				49	23																								
		Region B	Adult	Hatchery	841	348				50	50				50	50		50	50	48																				
				Wild	2	1														1																				
			Jack	Hatchery	91	79									50	29																								
	Lower River Test Fishery	Region B	Adult	Hatchery	278	208			21	33	47	42	47	4	14																									
				Wild	37	29			1	2	3	8	3	6	6																									
			Jack	Hatchery	14	13					1	1	5	1	3	1	1																							
				Wild	3	3							1		2																									
			Unknown	Wild	2	0																																		
	Lower River Sport	Region A	Adult	Hatchery	850	460		22	41	50	50	50		50	50	15	38	30	27	37																				
			Jack	Hatchery	86	69								13	22	16	18																							
		Region B	Adult	Hatchery	1188	433	41	38	50	50	50	50		48	50	7	14	8	14	13																				
			Jack	Hatchery	56	27								12	15																									
	Wind River Sport	Zone 6	Adult	Hatchery	209	69						2	19	38	10																									
			Jack	Hatchery	5	0																																		
Summer	Lower River Commercial	Region A	Adult	Hatchery	143	55															24			17		14														
				Wild	244	95															26			33		36														
			Jack	Wild	4	4															1			1		2														
		Region B	Adult	Hatchery	232	62															28			22		12														
				Wild	189	64															22			28		14														
			Jack	Hatchery	6	6															4			2																
				Wild	5	5															3			2																
	Sport	Region A	Adult	Hatchery	72	71															22	32	10	2	5															
				Wild	16	12																		5	7															
			Jack	Hatchery	9	9															3	4	1		1															
				Wild	3	3																			1		2													
		Region B	Adult	Hatchery	145	110															18	29	31	2	30															
				Wild	41	27																		7	20															
			Jack	Hatchery	11	11															3		3		3	2														
				Wild	1	1																			1															
			Mini-jack	Hatchery	4	0																																		
		Zone 6	Adult	Hatchery	1	0																																		
Fall	Commercial	Region A	Adult	Hatchery	815	94																							11	11	14	14		14	17	9	4			
				Wild	663	100																							13	13	11	10		10	8	15	20			
			Jack	Hatchery	36	36																							3	4	21	2			5	1				
				Wild	14	14																							3		1				3	3	4			
			Mini-jack	Hatchery	3	0																																		
		Region B	Adult	Hatchery	135	22																														9	4	4	5	
				Wild	590	74																														15	20	20	19	
			Jack	Hatchery	52	52																														18	21	3	10	
				Wild	174	174																														27	68	16	63	
			Mini-jack	Hatchery	3	0																																		
				Wild	8	0																																		
	Sport	Region A	Adult	Hatchery	239	77																										15	15	16	16	15				
				Wild	867	0																																		
			Jack	Hatchery	30	30																									1	2	1	6	5	7	2	1	3	2
				Wild	75	75																									1	5	2	10	12	10	21	7	3	4
			Mini-jack	Hatchery	1	0																																		
				Wild	6	0																																		
		Region B	Adult	Hatchery	410	77																								16	16	16	16	13						
				Wild	823	0																																		
			Jack	Hatchery	21	21																						1	1	1	5	4	8				1			
				Wild	20	20																							1		3	6	1			1	4	4		
			Mini-jack	Hatchery	3	0																																		

Note: Mini- jack (<399mm TL); Jack (400-620 mm TL); Adult (≥ 621 mm TL)

GSI baselines for Chinook salmon, sockeye salmon, and steelhead

Chinook salmon GSI analyses were performed using the updated baseline referred to as "Columbia River Basin Chinook salmon GSI baseline version 3.1" and is available on the FishGen website (https://www.fishgen.net). This baseline consists of 61 collections that are delineated into the following 19 reporting groups: Columbia Rogue "01_YOUNGS", West Cascade spring-run "02_WCASSP", West Cascade fall-run "03_WCASFA", Willamette River spring-run "04_WILLAM", Spring Creek Group Tule fall-run "05_SPCRTU", Klickitat River spring-run "06_KLICKR", Deschutes River spring-run "07_DESCSP", John Day River spring-run "08_JOHNDR", Yakima River spring-run "09_YAKIMA", upper Columbia River spring-run "10_UCOLSP", Tucannon River spring-run "11_TUCANO", Hells Canyon spring-run "12_HELLSC", South Fork Salmon River spring-run "13_SFSALM", Chamberlain Creek spring-run "14_CHMBLN", Middle Fork Salmon River spring-run "15_MFSALM", upper Salmon River spring-run "16_UPSALM", Deschutes River fall-run "17_DESCFA", upper Columbia River summer-/fall-run "18_UCOLSF", and Snake River fall-run "19_SRFALL" (Table 2; Figure 2). Reporting groups were primarily determined by the relative genetic similarity among populations according to a phylogenetic analysis, and our previous results demonstrate sufficient power to discern three reporting groups (17_DESCFA, 18_UCOLSF, and 19_SRFALL) among the interior ocean-type collections. In one year, we had grouped all interior ocean-type collections into a single reporting group "Interior_Columbia_R_su/fa" (Hess et al. 2013). 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 3).

The 10_UCOLSP reporting group includes the following Bonneville pool hatchery stocks: Carson stock (Ots22), and Little White Salmon R. (Ots23) because they are genetically indistinguishable from Upper Columbia R. spring Chinook salmon (includes Walla Walla and Umatilla River stocks). 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 10_UCOLSP reporting group. The 01_YOUNGS 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 group data is available on FishGen.

Sockeye salmon GSI analyses utilized the baseline described in Hess et al. (2013), and has previously been shown to accurately discriminate among the three major stocks in the Columbia River: Wenatchee, Okanagan, and Snake River sockeye salmon. Here, we refer to this as "Sockeye GSI baseline v1.0". The transition to GT-seq required omission of several loci due to poor genotyping quality with the new protocols. A total of 90 SNPs was used for these analyses.

For steelhead, we used GSI baseline version 3.3 that comprises 116 collections from throughout the Columbia River basin that are partitioned into the following 14 reporting groups: 01_WCOAST (Quinault River), 02_LOWCOL (lower Columbia River), 03_SKAMAN (Skamania hatchery releases at three sites in lower Columbia River, Willamette River, and Klickitat River), 04_ WILLAM (Willamette River), 05_BWSALM (Big White Salmon River), 06_KLICKR (Klickitat River), 07_MGILCS (middle Columbia River, Grande Ronde River, Imnaha River, lower Snake River, lower Clearwater River, and lower Salmon River), 08_YAKIMA (Yakima River), 09_UPPCOL (upper Columbia River), 10_SFCLWR (South Fork Clearwater River), 11_UPCLWR (upper Clearwater River), 12_SFSALM (South Fork Salmon River), 13_MFSALM (Middle Fork Salmon River), and 14_UPSALM (upper Salmon River) (see Table 11; Figure 4). 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 5).

Combined application of PBT and GSI

We combined PBT and GSI results together by first accepting all confident PBT assignments (i.e., LOD≥14 & FDR<0.01) to hatchery broodstock for Chinook salmon (See methods for Parentage assignments using SNPPIT software v1.0, ID: 1341). For the remaining individuals, we used the best estimate of GSI assignments (probability of assignment ≥0.80) provided by the program ONCOR to determine likely reporting group of origin (Method: Assigning individual samples using Individual Assignment (IA) genetic methods v1.0, ID: 1334). For the assignment of sockeye, GSI via ONCOR was used. For Chinook salmon, all age classes (3-, 4-, and 5-year old fish) can be identified from Snake River stocks using PBT. However, because our PBT baseline continues to expand for Columbia River stocks, only 3- and 4-year old fish can currently be identified for certain Columbia River stocks.

Results

We first present results from our assessment of PBT and GSI baseline accuracy, and then address results from the assignment of the 2015 harvest samples.

Accuracy testing of the PBT baseline

Chinook (94 SNPs)

To test the accuracy of the Chinook salmon PBT baseline (94 SNPs) in assigning known samples to their hatchery brood of origin, we selected six hatchery collections from 2015 and converted them to unknown samples. These hatchery collections represented four interior stream type lineages (i.e., Klickitat Hatchery, McCall Hatchery (South Fork Salmon stock), Carson National Fish Hatchery, and Round Butte Hatchery) and two interior ocean type lineages (i.e., Lyons Ferry Hatchery and Nez Perce Tribal Hatchery). These collections were chosen because of

Table 2: Sample sizes and reporting groups of Chinook salmon baseline populations**.** Lineages: ST (stream type), OT (ocean type), LC (Lower Columbia).

ID	Collection	(n)	Lineage	Reporting Groups	Reporting Group description
OTS01	Youngs Bay fall-run	91	Rogue	01_YOUNGS	Youngs Bay- Columbia Rogue stock
OTS02	Cowlitz R spring-run	90	LC	02_WCASSP	West Cascade spring-run
OTS03	Kalama R spring-run	83	LC	02_WCASSP	West Cascade spring-run
OTS04	Cowlitz R fall-run	82	LC	03_WCASFA	West Cascade fall-run
OTS05	Elochoman R fall-run	86	LC	03_WCASFA	West Cascade fall-run
OTS06	Lewis R fall-run	93	LC	03_WCASFA	West Cascade fall-run
OTS07	NF Lewis fall-run	178	LC	03_WCASFA	West Cascade fall-run
OTS08	Sandy R fall-run	83	LC	03_WCASFA	West Cascade fall-run
OTS09	McKenzie R spring-run	78	LC	04_WILLAM	Willamette River spring-run
OTS10	N Santiam R spring-run	79	LC	04_WILLAM	Willamette River spring-run
OTS11	Sandy R spring-run	48	LC	04_WILLAM	Willamette River spring-run
OTS12	White Salmon fall-run	77	LC	05_SPCRTU	Spring Creek tule fall-run
OTS13	Spring Creek NFH tule fall-run	49	LC	05_SPCRTU	Spring Creek tule fall-run
OTS14	Klickitat R spring-run	84	ST	06_KLICKR	Klickitat River spring-run
OTS15	Shitike R spring-run	93	ST	07_DESCSP	Deschutes River spring-run
OTS16	Warm Springs R spring-run	90	ST	07_DESCSP	Deschutes River spring-run
OTS17	John Day R spring-run	78	ST	08_JOHNDR	John Day River spring-run
OTS18	Middle Fork John Day R spring-run	47	ST	08_JOHNDR	John Day River spring-run
OTS19	North Fork John Day R spring-run	42	ST	08_JOHNDR	John Day River spring-run
OTS20	American R spring-run	76	ST	09_YAKIMA	Yakima River spring-run
OTS21	Cle-Elum spring-run	88	ST	09_YAKIMA	Yakima River spring-run
OTS22	Winthrop NFH spring-run	82	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS23	little White Salmon R spring-run	93	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS24	Wenatchee R spring-run	109	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS25	Entiat R spring-run	98	ST	10_UCOLSP	upper Columbia River spring-run/ Carson Hatchery spring-run
OTS26	Tucannon R spring-run	81	ST	11_TUCANO	Tucannon River spring-run
OTS27	Wenaha R spring-run	179	ST	12_HELLSC	Hells Canyon spring-run
OTS28	Lostine R spring-run	212	ST	12_HELLSC	Hells Canyon spring-run
OTS29	Grande Ronde R spring-run	314	ST	12_HELLSC	Hells Canyon spring-run
OTS30	Imnaha R spring-run	96	ST	12_HELLSC	Hells Canyon spring-run
OTS31	Lolo Cr spring-run	89	ST	12_HELLSC	Hells Canyon spring-run
OTS32	Red R spring-run	221	ST	12_HELLSC	Hells Canyon spring-run
OTS33	Powell R spring-run	56	ST	12_HELLSC	Hells Canyon spring-run
OTS34	Red R weir spring-run	91	ST	12_HELLSC	Hells Canyon spring-run
OTS35	South Forth Salmon R spring-run	139	ST	13_SFSALM	South Fork Salmon River spring/summer-run
OTS36	Johnson Cr spring-run	137	ST	13_SFSALM	South Fork Salmon River spring/summer-run
OTS37	Secesh R spring-run	252	ST	13_SFSALM	South Fork Salmon River spring/summer-run
OTS38	Chamberlain Cr spring-run	219	ST	14_CHMBLN	Chamberlain Creek spring/summer-run
OTS39	Big Cr spring-run	139	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS40	Camas Cr spring-run	55	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS41	Loon Cr spring-run	107	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS42	Sulphur Cr spring-run	94	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS43	Bear Valley Cr spring-run	135	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS44	Capehorn Cr spring-run	214	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS45	Marsh Cr spring-run	228	ST	15_MFSALM	Middle Fork Salmon River spring/summer-run
OTS46	North Fork Salmon R spring-run	55	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS47	Lemhi R spring-run	96	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS48	Pahsimeroi R spring-run	92	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS49	East Fork Salmon R spring-run	286	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS50	Salmon R spring-run	83	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS51	West Fork Yankee Fork spring-run	75	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS52	Valley Cr spring-run	100	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS53	Sawtooth Hatchery weir spring-run	186	ST	16_UPSALM	upper Salmon River spring/summer-run
OTS54	upper Deschutes R fall-run	252	OT	17_DESCFA	Deschutes River fall-run
OTS55	lower Yakima R fall-run	62	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS56	Hanford Reach fall-run	93	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS57	Wenatchee R summer-run	92	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS58	Entiat R summer-run	51	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS59	Methow R summer-run	87	OT	18_UCOLSF	upper Columbia River summer/fall-run
OTS60	Lyons Ferry weir fall-run	90	OT	19_SRFALL	Snake River fall-run
OTS61	Clearwater R fall-run	228	OT	19_SRFALL	Snake River fall-run

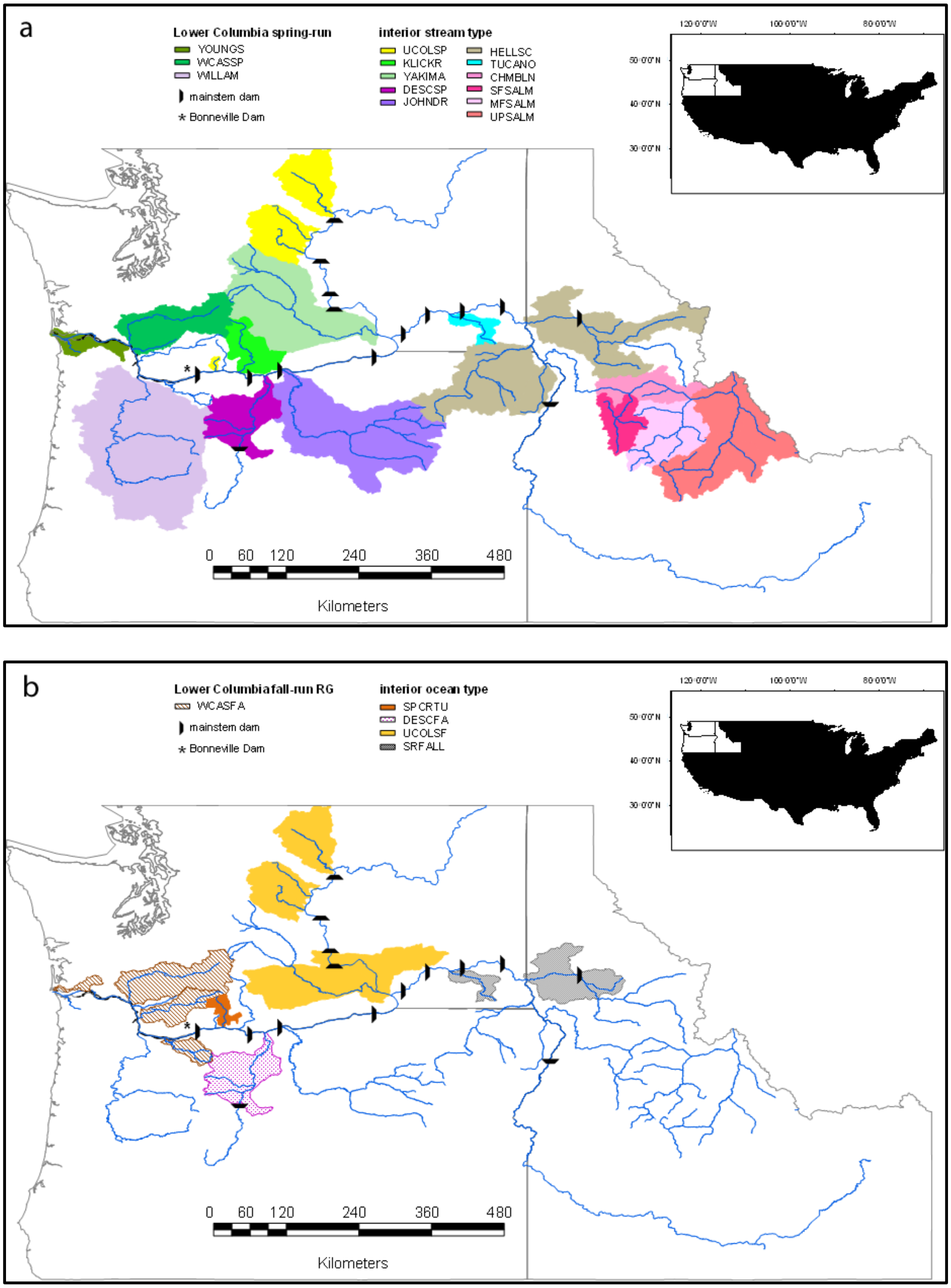


Figure 2: Map of Chinook salmon GSI reporting groups for a) Lower Columbia (LC) and interior stream type (ST) lineage, and b) interior ocean type (OT) lineage.

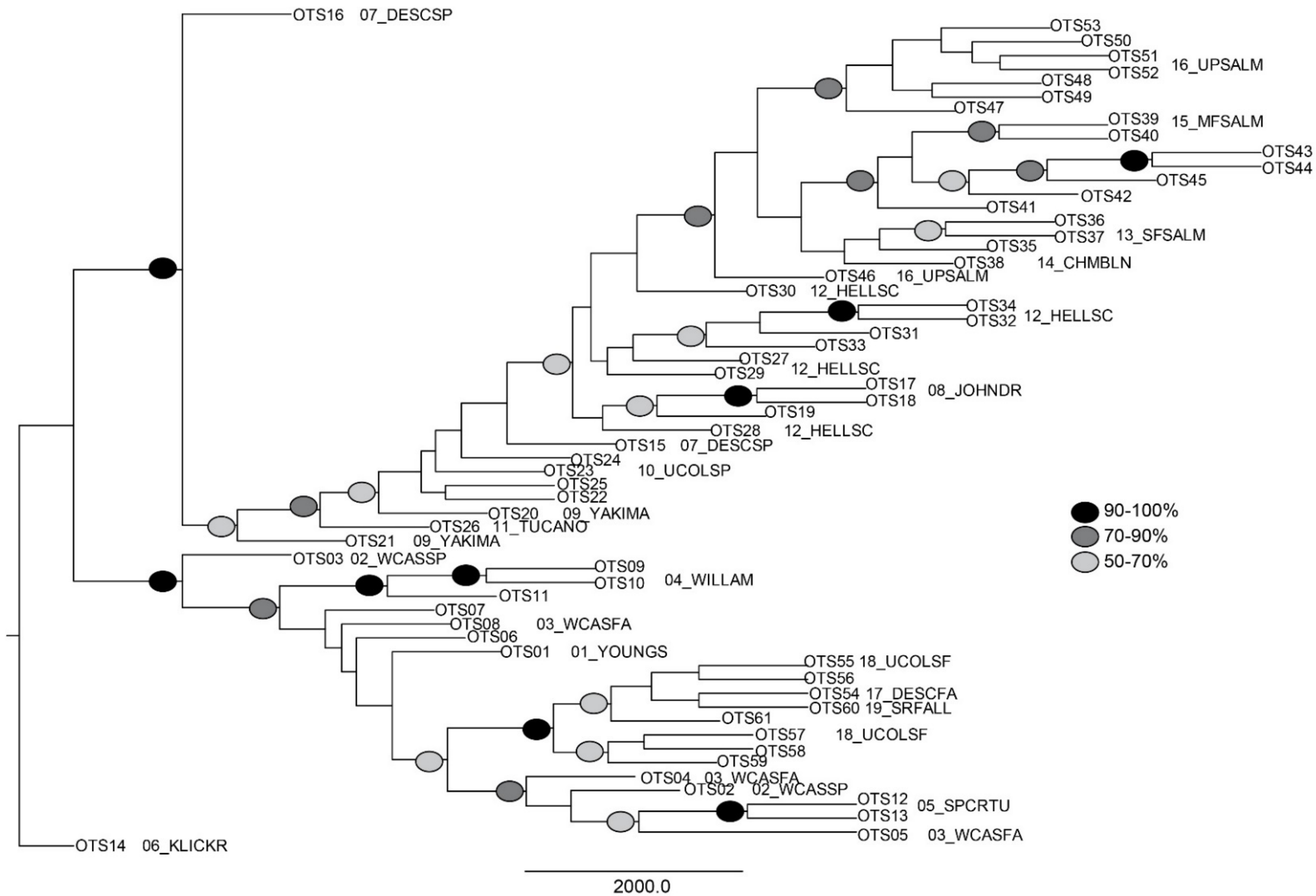


Figure 3: Neighbor-joining tree of Chinook salmon baseline populations using Nei's 1972 genetic distance of 179 SNP loci. The clusters are labeled with names of reporting groups used to aggregate the collections based on a combination of factors including genetic similarity, life history, and geographic proximity. Bootstrap support is shown with shaded ovals (Source: Hess et al. 2015).

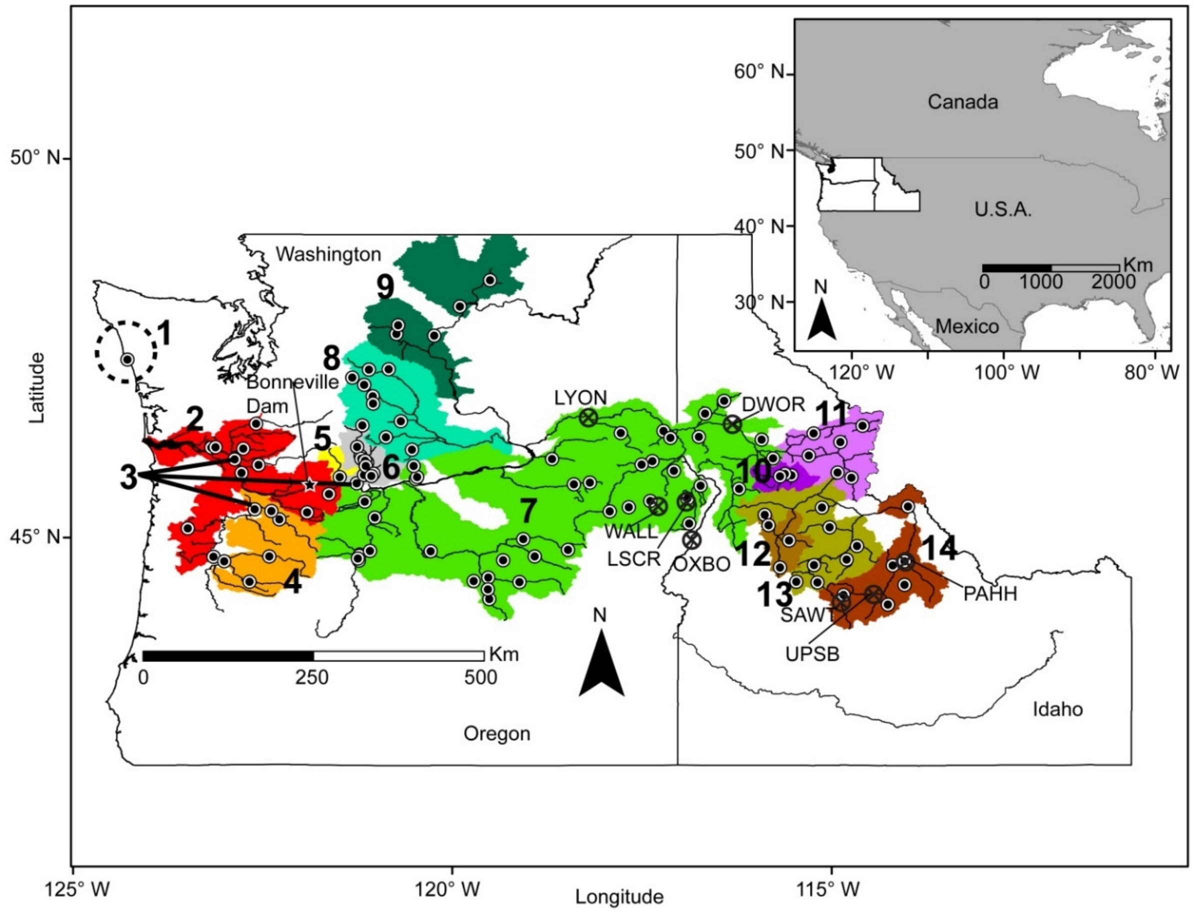


Figure 4: Geographic distribution of collections represented in the Columbia River steelhead GSI and PBT genetic baselines. The shape overlay represents the geographic extent of the following 14 reporting groups in the GSI baseline: 1) Quinault (WCOAST), 2) lower Columbia River (LOWCOL), 3) Skamania hatchery releases at three sites in lower Columbia River, Willamette River, and Klickitat River (SKAMAN), 4) Willamette River (WILLAM), 5) Big White Salmon River (BWSALM), 6) Klickitat River (KLICKR), 7) middle Columbia River, Grande Ronde River, Imnaha River, lower Snake River, lower Clearwater River, and lower Salmon River (MGILCS), 8) Yakima River (YAKIMA), 9) upper Columbia River (UPPCOL), 10) South Fork Clearwater River (SFCLWR), 11) upper Clearwater River (UPCLWR), 12) South Fork Salmon River (SFSALM), 13) Middle Fork Salmon River (MFSALM), and 14) upper Salmon River (UPSALM). There are 116 collections (filled circles) categorized into reporting groups. The PBT baseline is indicated as 8 stocks (crossed circles) corresponding to the following sites where fish are collected and spawned for broodstock: Lyons Ferry Hatchery (LYON), Wallowa (WALL), Little Sheep Creek (LSCR), Oxbow Hatchery (OXBO), Dworshak Hatchery (DWOR), upper Salmon River B-run (UPSB), Sawtooth Hatchery (SAWT), and Pahsimeroi Hatchery (PAHH). Bonneville Dam (star) is the site where fish were non-lethally sampled for the mixed-stock analysis.

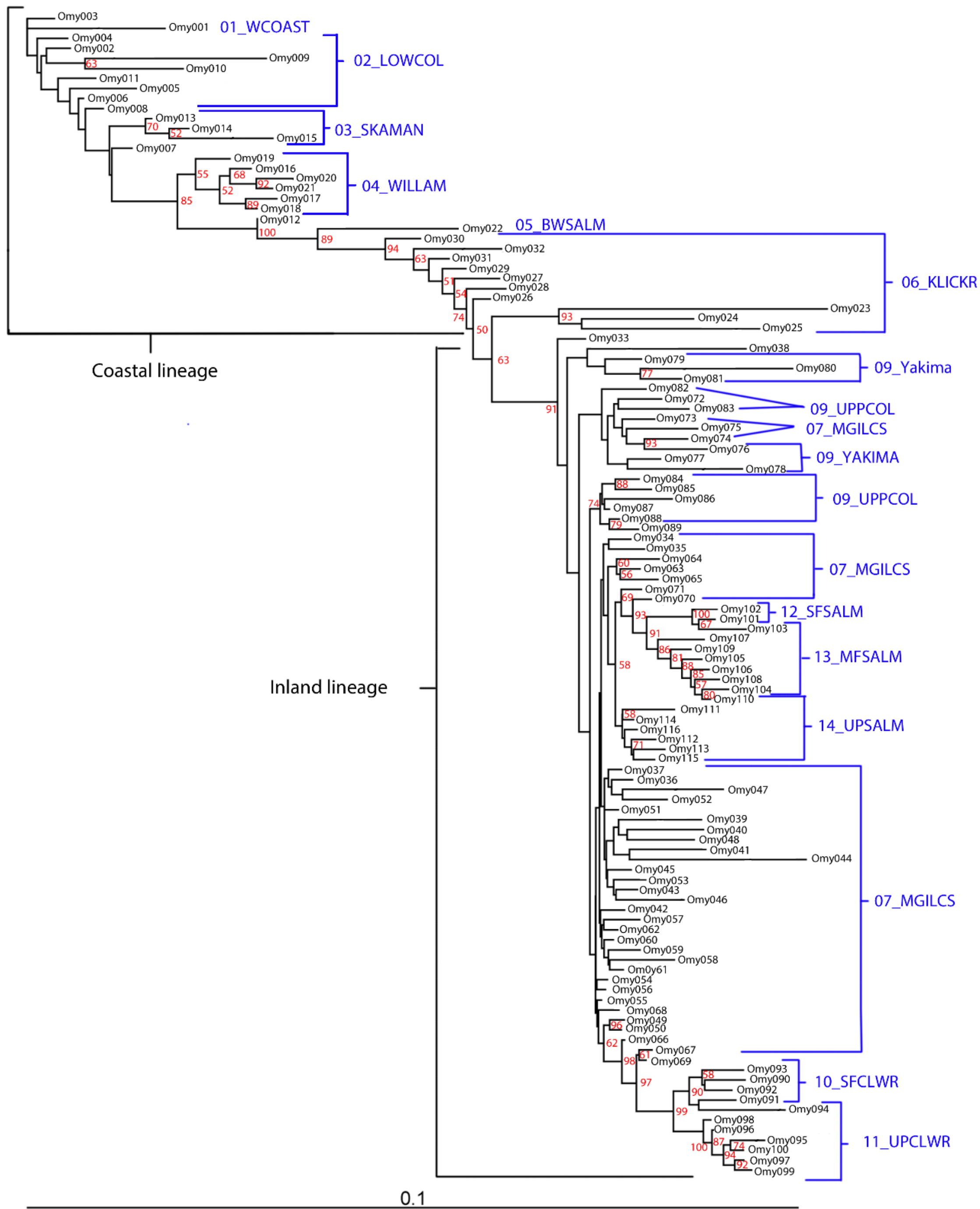


Figure 5: Neighbor-joining tree of steelhead baseline populations in GSI baseline v.3.3 (186 SNPs) using Nei's 1972 genetic distance. Bootstrap values (in red) ≥50% (based on 1000 bootstraps) are shown. Reporting group names (in blue) are provided and are clustered by lineage (i.e, coastal or inland).

the availability of PBT baselines in previous years that these samples could be assigned to parents (Table 3). For the interior stream type lineage from the Klickitat State Fish Hatchery and the McCall Fish Hatchery, we have PBT baselines that extend as far back as 2008 that allow us to assign fish from age classes 3, 4 and 5. However, for the interior stream type lineage from the Carson National Fish Hatchery and the Round Butte Fish Hatchery, our baselines extend to 2012 and only permit the assignment of jacks (i.e., age class 3). For the interior ocean type lineage from the Lyons Ferry Fish Hatchery and Nez Perce Tribal Fish Hatchery, our baselines extend to 2011 and permit the assignment of fish from age classes 3- and 4-, but not 5-year-old fish. After converting 2015 hatchery returns at each of these facilities to unknown samples, we conducted PBT assignments using brood years 2010-2012 for all hatcheries throughout the Columbia River basin that are represented in our PBT baseline with SNPPIT (See methods for Parentage assignments using SNPPIT software v1.0, ID: 1341). The expectation was that hatchery origin fish returning to each facility should assign to parent broodstock in the previous generation from the same hatchery. As described above, not all age classes were represented in PBT baselines for each program so we expected missing assignments for certain stocks. Even when all age classes are available, tagging rates may be lower than 100% that would also result in unassigned offspring. Further, some hatchery programs integrate natural origin fish, so these fish would also not be expected to assign to parent broodstock in the previous generation. Given that some offspring did not have true parents in the PBT baseline, this approach allowed us to test for false positive assignments in addition to broodstock assignment accuracy. Only those individuals that had a LOD-score ≥14 and a False Discovery Rate (FDR) ≤0.01 were considered to be successfully ‘assigned’, while all others were considered ‘unassigned’.

Carson National Fish Hatchery & Round Butte Hatchery:

Since the PBT baseline for these hatcheries only extended to BY2012 and offspring from 2015 were tested, we only expected to assign 3-year old fish to parents. Of the offspring tested, only 4% of the Carson samples from 2015 and 12% of the Round Butte samples from 2015 were successfully assigned. This reflects the limited baseline currently available for these hatcheries programs (i.e., 2012-present) but will improve in the future as more age classes are represented. Of those jacks that were assigned, 100% of the Carson fish and 73% of the Round Butte fish assigned to their expected broodstock (i.e. OtsCAR12S and OtsRB12S, respectively). Unexpected broodstock origins for Round Butte included assignments to multiple hatcheries from the Snake River (Table 4). Chinook from the Snake River are known to stray into the Deschutes River, and it is likely that some of these strays have been incorporated into the Round Butte Hatchery program (i.e., these are not mis-assignments). This will be tested further with additional years of returns, but initial data suggests that there were little to no false positive assignments.

Table 3: Chinook PBT baselines throughout the Columbia River basin. Chinook hatchery programs are shown, along with run type, lineage and years of availability. Tissues genotyped using 96 SNPs (X) and 298 SNPs (X) are shown, and collections for which data are not yet available are denoted in red (i.e., X).

Hatchery	Code	Run type	Lineage	Year							
				2008	2009	2010	2011	2012	2013	2014	2015
Clearwater Fish Hatchery	OtsCLWH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Clearwater Fish Hatchery - Powell Facility	OtsPOWP	Spring	Interior stream type	X	X	X	X	X	X	X	X
Dworshak National Fish Hatchery	OtsDWOR	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Catherine Creek	OtsCTHW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Grande Ronde	OtsGRUW	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Imnaha River	OtsIMNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Lookingglass Creek	OtsLOOK	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lookingglass Fish Hatchery - Lostine River	OtsLSTW	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery	OtsLYON	Spring	Interior stream type	*	*	*	*	X	X	X	X
Lyons Ferry Fish Hatchery - Tucannon River	OtsTUCW[a]	Spring	Interior stream type	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery	OtsLYON_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X
McCall Fish Hatchery - Johnson Creek	OtsJHNW	Spring/Summer	Interior stream type	X	X	X	X	X	X	X	X
McCall Fish Hatchery - South Fork Salmon	OtsMCCA	Spring	Interior stream type	X	X	X	X	X	X	X	X
Nez Perce Tribal Fish Hatchery (Fall)	OtsNPFH_1	Fall	Interior ocean type	*	*	*	X	X	X	X	X
Nez Perce Tribal Fish Hatchery (Spring)	OtsNPFH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Pahsimeroi Fish Hatchery	OtsPAHH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Rapid River Fish Hatchery	OtsRAPH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery	OtsSAWT	Spring	Interior stream type	X	X	X	X	X	X	X	X
Big Creek Hatchery	OtsBIG	Fall	Interior ocean type	*	*	*	*	*	*	*	X
Carson National Fish Hatchery	OtsCAR	Spring	Interior stream type	*	*	*	*	X	X	X	X
Chief Joseph Hatchery (Spring)	OtsCJH_sp	Spring	Interior stream type	*	*	*	*	*	*	X	X
Chief Joseph Hatchery (Summer/Fall)	OtsCJH_sufa	Summer	Interior ocean type	*	*	*	*		X	X	X
Cowlitz Salmon	OtsCOW	Spring	Interior stream type	*	*	*	*	*	*	*	*
Eastbank Fish Hatchery	OtsEASTBK	Summer	Interior ocean type	*	*	*	*	X	*	*	*
Entiat National Fish Hatchery	OtsENFH	Summer	Interior ocean type	*	*	*	*	*	X	X	X
Kalama Falls	OtsKAL	Spring	Interior stream type	*	*	*	*	*	*	*	*
Klickitat State Fish Hatchery	OtsKH	Spring	Interior stream type	X	X	X	X	X	X	X	X
Leavenworth National Fish Hatchery	OtsLNFH	Spring	Interior stream type	*	*	*	*	*	X	X	X
Lewis River	OtsLEW	Spring	Interior stream type	*	*	*	*	*	*	*	*
Little White Salmon National Fish Hatchery (Fall)	OtsLWS_sufa	Fall	Interior ocean type	*	*	*	*	*	X	X	X
Little White Salmon National Fish Hatchery (Spring)	OtsLWS_sp	Spring	Interior stream type	*	*	*	*	*	X	X	X
Methow State Fish Hatchery	OtsMETH	Spring	Interior stream type	*	*	*	*	X	X	X	X
Parkdale Fish Facility	OtsPFF	Spring	Interior stream type	*	*	*	*	X	X	X	X
Priest Rapids Hatchery	OtsPRH	Fall	Interior ocean type	*	*	*	*	X	X	X	X
Round Butte Fish Hatchery	OtsRB	Spring	Interior stream type	*	*	*	*	X	X	X	X
Spring Creek NFH	OtsSPCR	Fall	Interior ocean type	*	*	*	*	*	*	*	X
Toutle	OtsTOU	Fall	Interior ocean type	*	*	*	*	*	*	*	*
Umatilla Fish Hatchery (Fall)	OtsUMA_sufa	Fall	Interior ocean type	*	*	*	*	X	X	X	
Umatilla Fish Hatchery (Spring)	OtsUMA_sp	Spring	Interior stream type	*	*	*	*	X	X	X	X
Washougal	OtsWAS	Fall	Interior ocean type	*	*	*	*	*	*	*	*
Warm Springs National Fish Hatchery	OtsWSNFH	Spring	Interior stream type	*	*	*	*	X	X	X	X
Wells Fish Hatchery	OtsWELLS	Summer	Interior ocean type	*	*	*	*	X	X	X	X
Winthrop National Fish Hatchery	OtsWTP	Spring	Interior stream type	*	*	*	*	*	X	X	X
Yakima Nation Prosser Hatchery	OtsPRO	Fall	Interior ocean type	*	*	*	*	X	X	*	X
Yakima River Roza Dam-Integrated	OtsYRint	Spring	Interior stream type	*	*	*	*	X	*	X	X
Yakima River Roza Dam-Segregated	OtsYRseg	Spring	Interior stream type	*	*	*	*	X	*	*	X

[a]Chinook Lyons Ferry stock consolidated under 'OtsLYON' starting in 2012

*Broodstock not sampled

Table 4: Results of the accuracy testing for the Chinook salmon PBT baseline. Collections and their respective lineage are shown along with the PBT baselines available for their assignment. The total number and percentage of successful assignments at two assignment criteria (i.e., LOD≥14 & FDR≤3.0; LOD≥14 & FDR≤0.010) are shown. The number that assigned to their expected brood stock are displayed, as well as the number of unexpected assignments and their sources.

		PBT Baselines available				LOD≥14, FDR≤ 3.0		LOD≥14, FDR≤ 0.010					
										Assigned to Expected Broodstock		Assigned to Unexpected Broodstock	
Collection	**Lineage**	**2010**	**2011**	**2012**	**Samples**	**Total Assignments**	**Percent assigned**	**Total Assignments**	**Percent assigned**	**N**	**Proportion**	**N**	**Unexpected Source(s)**
Carson 2015	Interior stream type			X	986	40	4.1%	35	3.5%	35	1.0000	0	
Round Butte 2015	Interior stream type			X	888	129	15%	110	12%	80	0.7273	30	OtsCLWH11S (1), OtsIMNW10S (12), OtsLSTW11S (4), OtsRAPH11S (3), OtsMCCA11S (5), OtsMCCA12S (1), OtsPAHH11S (2), OtsSAWT11S (1), OtsSAWT12S (1)
Klickitat 2015	Interior stream type	X	X	X	427	299	70%	297	70%	297	1.0000	0	
McCall 2015	Interior stream type	X	X	X	1180	1120	95%	1115	94%	1100	0.9865	15	OtsGRUW11S (8), OtsLOOK11S (4), OtsNPFH11S (2), OtsPOWP11S (1)
Lyons Ferry 2015	Interior ocean type		X	X	1773	993	56%	643	36%	641	0.9969	2	OtsPAHH11S (1), OtsUMA12 (1)
Nez Perce 2015	Interior ocean type		X	X	822	445	54%	279	34%	279	1.0000	0	

Klickitat Hatchery and McCall Hatchery:

The Klickitat and McCall samples from 2015 had the greatest percentage of successful assignments (i.e., 70% and 94%, respectively). This reflects more complete parental baselines available for assignment of all the age classes that are anticipated to be encountered in these samples. Unassigned fish for Klickitat Hatchery were due to incomplete broodstock sampling (lower tagging rates) and natural origin fish in the samples from 2015. Of the offspring that were successfully assigned to parents, 100% of the Klickitat samples from 2015 and 99% of the McCall samples from 2015 assigned to their expected broodstock (Table 4). Unexpected broodstock origins for the McCall samples from 2015 included other Snake River hatcheries, and likely represent a small number of strays.

Lyons Ferry Hatchery and Nez Perce Tribal Hatchery:

For interior ocean type lineages (fall Chinook), 36% of the Lyons Ferry samples from 2015 and 34% of the Nez Perce samples from 2015 were successfully assigned. This comparatively low level of successful assignment may partially reflect the absence of PBT baselines for these hatchery programs from 2010, but it is unlikely that the remaining ~65% of missing assignments could be attributable to age class 5 alone. Offspring samples for both of these programs also included natural origin fish which would also not be expected to assign to parent broodstock in the previous generation, but another source of missing assignments is possible (see below). Of those fish that were successfully assigned, over 99% of Lyons Ferry 2015 and 100% of the Nez Perce 2015 were assigned to their expected broodstock (Table 4). Unexpected broodstock origins for the Lyons Ferry 2015 samples included one individual from the Umatilla 2012 (fall) hatchery program that was likely a stray fish, and one individual from the Pahsimeroi 2011(spring) hatchery program. It is possible that a late migrating spring Chinook could have been inadvertently integrated into the Lyons Ferry hatchery program in 2015. However, spawn date for the assigned parents is not available to either support or refute this possibility.

A possible additional source of missing assignments for fall Chinook may have resulted from increased false negative assignments (Type II error) stemming from the combined use of LOD-score $\geq$14 with FDR $\leq$0.01in determining which fish were considered 'assigned'. Recent simulations with interior ocean type Chinook suggest that combining a LOD-score $\geq$14 and a FDR $\leq$0.01 may result in elevated levels of Type II error (IDFG, unpublished data). However, our empirical data suggests that considering LOD-score $\geq$14 alone results in elevated Type I error (false positive assignments), and that jointly considering LOD and some FDR threshold is preferable for controlling both Type I and Type II error. For demonstration purposes, we set our LOD-score $\geq$14 with FDR $\leq$ 3.0 and adjusted assignments based on these thresholds. While we observed a modest increase in the percentage of interior stream type lineage samples that assigned as a result of this adjustment (i.e., 0.6-3.0%), a far greater number of interior ocean type

Chinook from the Lyons Ferry and Nez Perce Tribal Hatchery were assigned (i.e., 20%) (Table 4). Although the specific FDR threshold to consider for the assignment of fall Chinook has yet to be determined, we will be in a position to assess this in the near future.

Steelhead (95 SNPs)

To test the accuracy of the steelhead PBT baseline (95 SNPs) in assigning known samples to their hatchery brood of origin, we selected five hatchery collections from 2015 and converted them to unknown samples. These hatchery collections were represented by two inland lineages from the from Snake River (i.e., Dworshak Hatchery and Wallowa Hatchery), two inland lineages from the Columbia River (i.e., Umatilla Hatchery and Winthrop Hatchery), and one coastal lineage from the Columbia River (i.e., Parkdale Fish Facility). These collections were chosen because of the availability of PBT baselines in previous years that these samples could be assigned to (Table 5). For the interior lineage from the Snake River (i.e., Dworshak Hatchery and Wallowa Hatchery), PBT baselines extend as far back as 2008 and 2009, respectively, that allow us to assign fish from age classes 3, 4 and 5. However, for the interior and coastal lineage from the Columbia River (i.e., Umatilla Hatchery, Winthrop Hatchery, and Parkdale Fish Facility) our baselines extend to 2012 and only permit the assignment of age class 3. After converting 2015 hatchery returns at each of these facilities to unknown samples, we conducted PBT assignments using brood years 2010-2013 for all hatcheries throughout the Columbia River basin that are represented in our PBT baseline with SNPPIT (See methods for Parentage assignments using SNPPIT software v1.0, ID: 1341). The expectation was that hatchery origin fish returning to each facility should assign to parent broodstock in the previous generation from the same hatchery. As described above, not all age classes were represented in PBT baselines for each program so we expected missing assignments for certain stocks. Even when all age classes are available, tagging rates may be lower than 100% that would also result in unassigned offspring. Further, some hatchery programs integrate natural origin fish so these fish would also not be expected to assign to parent broodstock in the previous generation. Given that some offspring did not have true parents in the PBT baseline, this approach allowed us to test for false positive assignments in addition to broodstock assignment accuracy. Only those individuals that had a LOD-score ≥14 and a False Discovery Rate (FDR) ≤0.01 were considered to be successfully 'assigned', while all others were considered 'unassigned'.

Dworshak Hatchery & Wallowa Hatchery:

The Dworshak and Wallowa samples from 2015 had the greatest percentage of successful assignments (i.e., 93% and 94%, respectively). This reflects the availability of parental baselines for assignment of all the age classes that are anticipated to be encountered in these samples. Of the offspring that were successfully assigned to parents, 100% of the Wallowa samples from 2015 and 99% of the Dworshak samples from 2015 assigned to their expected broodstock (Table

6). The origin for the single Dworshak sample that was assigned to an unexpected broodstock came from another Snake River hatchery, and likely represents a stray individual.

Umatilla Hatchery, Winthrop Hatchery & Parkdale Facility:

Since the PBT baseline for these facilities only extended to BY2012 and offspring from 2015 were tested, we only expected to assign 3-year old (i.e. 1-ocean fish from BY2012) fish to parents. Of the offspring tested, only 19% of the Umatilla samples from 2015 were successfully assigned, while none of the Winthrop samples or Parkdale samples from 2015 were successfully assigned. For the Umatilla, this reflects the limited baseline currently available for these hatchery programs (i.e., 2012-present) but will improve in the future as more age classes are represented. For Winthrop, only 19 of the 70 samples from the Winthrop 2015 collection were of hatchery origin and could potentially be assigned to broodstock parents. For Parkdale, this program typically has largely natural origin fish so we would did not expect assignments to broodstock parents. (The exact proportion of hatchery origin fish for the Umatilla and Parkdale collections from 2015 was unknown since it was not recorded at time of sampling.) Of the 3-year old (1-ocean) Umatilla fish that were successfully assigned, 94% were assigned to their expected broodstock. The single Umatilla sample that was assigned to an unexpected broodstock came from the Wallowa Hatchery in 2012, and likely represents a stray individual. For all stocks, false-positive assignments (i.e., assignments to the wrong parents) were highly unlikely but it is possible that the few stray fish observed were actually mis-assigned.

Accuracy testing of the GSI baseline

We used two complementary approaches to test the accuracy of the GSI baselines for sockeye salmon, Chinook salmon and steelhead in assigning known samples to their reporting group of origin. We first used the ‘leave-one-out’ procedure implemented in ONCOR v1.0 (Kalinowski et al. 2007) to evaluate how well individuals could be assigned to their population and reporting group of origin. Additionally, we conducted a ‘mixture analysis’ and randomly selected and removed ~10% of the samples from each population represented in the GSI baseline for each species, and assigned them to reporting groups using the remaining samples in the GSI baseline. For this second approach, we used the individual assignment method implemented in ONCOR, and only accepted assignments to reporting groups with a probability $\geq$0.80; individuals with assignment probability <0.80 were considered ‘unassigned’.

Table 5: Steelhead PBT baselines throughout the Columbia River basin. Steelhead hatchery programs are shown, along with run type, lineage and years of availability. Tissues genotyped using 96 SNPs (X), 192 SNPs (X), and 269 SNPs (X) are shown.

Hatchery	Code	Run type	Lineage	Year							
				2008	2009	2010	2011	2012	2013	2014	2015
Dworshak National Fish Hatchery	OmyDWOR	Unknown	Interior	X	X	X	X	X	X	X	X
Little Sheep Creek Hatchery	OmyLSCR	Summer	Interior	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery- Touchet	OmyTOUW[a]	Summer	Interior	*	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery	OmyLYON[b]	Unknown	Interior	*	X	X	X	X	N/A	N/A	N/A
Lyons Ferry Fish Hatchery - Grande Ronde	OmyCGRW[a]	Summer	Interior	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery - Tucannon	OmyTUCW[a]	Summer	Interior	X	X	X	X	X	X	X	X
Lyons Ferry Fish Hatchery - Wallowa	OmyWALW	Summer	Interior	*	*	*	*	*	*	*	X
Oxbow	OmyOXBO	Summer	Interior	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery	OmySAWT	Summer	Interior	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery - East Fork Salmon	OmyEFSW[c]	Summer	Interior	X	X	X	X	X	X	X	X
Sawtooth Fish Hatchery - Squaw Creek	OmySQUW[d]	Summer	Interior	X	X	X	X	X	X	X	X
Pahsimeroi Fish Hatchery	OmyPAHH	Unknown	Interior	X	X	X	X	X	X	X	X
Wallowa	OmyWALL	Summer	Interior	*	X	X	X	X	X	X	X
Eastbank Hatchery	OmyEASTBK	Summer	Interior	*	*	*	*	*	*	*	*
Methow Hatchery (Twisp)	OmyTWP	Summer	Interior	*	*	*	*	*	X	X	X
Parkdale Fish Facility	OmyPFF	Winter	Coastal	*	*	*	*	X	X	X	X
Round Butte Fish Hatchery	OmyRB	Summer	Interior	*	*	*	*	*	X	X	X
Skamania Hatchery (Summer)	OmySKH_su[e]	Summer	Coastal	*	*	*	*	*	X	X	X
Skamania Hatchery (Winter)	OmySKH_wi[e]	Winter	Coastal	*	*	*	*	*	X	X	X
Umatilla Fish Hatchery	OmyUMA	Summer	Interior	*	*	*	*	X	X	X	X
Wells Hatchery	OmyWEL	Summer	Interior	*	*	*	*	*	X	X	X
Wells Hatchery - Omak stock	OmyWEL_OMA	Summer	Interior	*	*	*	*	*	*	*	X
Winthrop National Fish Hatchery	OmyWTP	Summer	Interior	*	*	*	*	X	X	X	X

[a]Lyons Ferry stock consolidated under 'OmyLYON' starting in 2012
[b]Lyons Ferry stock discontinued in 2013
[c]Sawtooth stock consolidated under 'OmySAWT' from 2012-2013
[d]Sawtooth stock consolidated under 'OmySAWT' in 2012, renamed 'Upper Salmon B-run (YFLW) and consolidated under 'OmyPAHH' starting in 2013
[e]Skamania stock is collected late in the calendar year, and is designated for the following broodyear (e.g., late 2012 collections are considered part of BY2013)
N/A – Stock discontinued/non-existent
*Broodstock not sampled

Table 6: Results of the accuracy testing for the steelhead PBT baseline. Collections and their respective lineage/run type are shown along with the PBT baselines available for their assignment. The total number of successful assignments, and the number that assigned to their expected brood stock are displayed, as well as the number of unexpected assignments and their sources.

		PBT Baselines available						Assigned to Expected Broodstock		Assigned to Unexpected Broodstock	
Collections	**Lineage/Run type**	**2010**	**2011**	**2012**	**Samples**	**Total Assignments**	**Percent assigned**	**N**	**Proportion**	**N**	**Source(s)**
Dworshak 2015	Interior/Unknown	X	X	X	1490	1383	93%	1382	0.9993	1	OmyLYON11S
Wallowa 2015	Interior/Unknown	X	X	X	468	439	94%	439	1.0000	0	
Umatilla 2015	Interior/Summer			X	88	17	19%	16	0.9412	1	OmyWALL12S
Winthrop 2015	Interior/Summer			X	70	0	0%	0	0.0000	0	
Parkdale 2015	Coastal/Winter			X	46	0	0%	0	0.0000	0	

Sockeye (87 SNPs)

Leave-one-out analysis

For sockeye salmon, we used GSI baseline v1.0 that comprises four reporting groups (i.e., Wenatchee, Okanogan, Redfish Lake, and Lake Whatcom; N=694). Missing data across loci resulted in the exclusion of 121 of these samples for the leave-one-out analysis. Of the remaining 573 samples, only 15 individuals were assigned incorrectly from the Okanogan reporting group to the Wenatchee reporting group. All remaining samples assigned correctly to their reporting group of origin (Table 7).

10 % Mixture analysis

For the 'mixture analysis', we randomly selected and removed 71 individuals from the baseline and assigned them to the remaining samples in the baseline. Every sample was successfully assigned to one of the reporting groups, and all of the samples from the Wenatchee, Redfish Lake and lake Whatcom collections assigned back to their reporting group of origin. There was only a single individual from the Okanogan collection that assigned to an alternate reporting group (i.e., Wenatchee) (Table 8).

The results of these tests are broadly concordant, and indicate that GSI baseline v1.0 is suitable for the assignment of sockeye mixture samples to these reporting groups. Expansion of the baseline to include other collections that may represent additional reporting groups within the Columbia River basin may be possible and could refine the spatial scale of assignments. This will be explored in 2017.

Chinook (179 SNPs)

Leave-one-out analysis

For Chinook salmon, we used GSI baseline v3.1 that comprises 61 collections from throughout the Columbia River basin that are partitioned into 19 reporting groups (N=7083) (Figure 2). Missing data across loci resulted in the exclusion of 2824 of these samples for the leave-one-out analysis. Of the remaining 4259 samples, 3507 (77%) assigned correctly to their reporting group of origin (Table 7). Across all reporting groups, the proportion of correct assignments ranged from 0.63 (08_JOHNDR) to 1.0 (01_YOUNGS, 04_WILLAM and 09_YAKIMA).

The highest proportion of correct assignments was observed for Lower Columbia (LC) lineage reporting groups, and ranged from 0.82 (02_WCASSP) to 1.0 (01_YOUNGS and 04_WILLAM) (Table 8; Figure 6). Incorrect assignments for LC lineage populations were typically to other reporting groups within this lineage with the most common incorrect assignments to reporting groups that included collections from the same river (i.e., Cowlitz spring and fall run). However, three individuals were incorrectly assigned to the Interior Ocean

Table 7: Results of the leave-one-out analysis for the sockeye GSI baseline v1.0. Reporting group of origin, the number of samples available to be assigned, and their assignment by reporting group are shown, as well as the proportion of correct assignments.

Reporting Group	N	Number assigned to reporting group				
		Wenatchee	Okanogan	Redfish	Whatcom	Proportion correct
Wenatchee	280	280	0	0	0	1.000
Okanogan	182	15	167	0	0	0.918
Redfish Lake	76	0	0	76	0	1.000
lake Whatcom	35	0	0	0	35	1.000

Table 8: Results of accuracy testing for the sockeye salmon GSI baseline v1.0. Reporting group of origin, the number of samples successfully assigned and their assignment by reporting group are shown, as well as the proportion of correct assignments.

Reporting Group	N	N assigned (p≥0.80	Number assigned to reporting group					
			Wenatchee	Okanogan	Redfish	Whatcom	Unassigned (p<0.80)	Proportion correct
Wenatchee	33	33	33	0	0	0	0	1.000
Okanogan	24	24	1	23	0	0	0	0.958
Redfish Lake	9	9	0	0	9	0	0	1.000
Lake Whatcom	5	5	0	0	0	5	0	1.000

Type (OT) lineage (18_UCOLSF; 19_SRFALL), and seven individuals were assigned incorrectly to the Interior Stream Type (ST) lineage (06_KLICKR); likely due introgression that has occurred between lineages in the Klickitat sub-basin (Hess et al. 2014).

The proportion of correct assignments for OT lineage reporting groups ranged from 0.68 (19_SRFALL) to 0.92 (17_DESCFA) (Table 9; Figure 6). Incorrect assignments for OT lineage populations were typically to other reporting groups within this lineage, or to LC lineage reporting groups (i.e., OTS54 – Upper Deschutes River fall run and OTS55 Lower Yakima River fall run to 02_WCASSP and 03_WCASFA). There was 1 individual from OTS57 – Wenatchee River summer run that incorrectly assigned to 06_KLICKR (Table 7). These types of incorrect assignments were not as common as those to reporting group of the same run type.

The proportion of correct assignments for ST lineage reporting groups ranged from 0.63 (08_JOHNDR) to 1.0 (09_YAKIMA) (Table 9; Figure 6). Incorrect assignments were typically to other reporting groups within this lineage; although, we did observe a single instance where a ST lineage sample was assigned incorrectly to a LC lineage reporting group (OTS17 – John Day River spring run to 02_WCASSP) (Table 9).

10% Mixture analysis

We randomly selected and removed 711 samples from these collections and assigned them to the remaining samples in the baseline. Of these randomly selected samples, 154 did not have an assignment probability $\geq$0.80 and were 'unassigned'. Nearly all fish assigned correctly to lineage, with only one incorrectly assigned fish from LC to ST (i.e., OTS03 Kalama River spring run to 06_KLICKR) (Table 10). The proportion of fish that assigned correctly to reporting group for each of the 61 collections varied widely (0.40-1.00), and could reflect limited samples sizes in some instances (Table 10). When taken in aggregate (i.e., by reporting group of origin), the proportion of fish that assigned correctly to reporting groups ranged from 0.69 (10_UCOLSP) to 1.0 (several reporting groups) (Table 10). For the LC lineage, the proportion of fish that assigned correctly to reporting groups ranged from 0.88 (02_WCASSP) to 1.0 (01_YOUNGS, 04_WILLAM, and 05_SPCRTU) (Table 10). For the OT lineage, the proportion of fish that assigned correctly to reporting group ranged from 0.86 (18_UCOLSF) to 1.0 (19_SRFALL) (Table 8). For the ST lineage, the proportion of fish that assigned correctly to reporting group ranged from 0.69 (10_UCOLSP) to 1.0 (06_KLICKR) (Table 10). These results are broadly consistent with the results of the 'leave-one-out' analysis, and reflect the population genetic structure of Chinook salmon lineages within the Columbia River basin (Figure 3).

Table 9: Results of the leave-one-out analysis for the Chinook salmon GSI v3.1 baseline. Lineage, run type, reporting group, population ID, population name and samples size used in analysis are provided for each collection in the baseline. The number of correct assignments to reporting group for each population in the baseline are reported (gray shading) and are tallied to provide the number of correct assignments for the reporting group overall (yellow shading). The proportion of correct assignments to reporting group for each population and reporting group (yellow shading) in the baseline is provided.

Lineage	Run type	Reporting Group	Pop ID	Pop Name	N	Number assigned to reporting group: Spring 01_YOUNGS	Spring 02_WCASSP	Fall 03_WCASFA	Spring 04_WILLAM	Fall 05_SPCRTU	Spring 06_KLICKR	Spring 07_DESCSP	Spring 08_JOHNDR	Spring 09_YAKIMA	Spring 10_UCOLSP	Spring 11_TUCANO	Spring 12_HELLSC	Spring 13_SFSALM	Spring 14_CHMBLN	Spring 15_MFSALM	Spring/Summer 16_UPSALM	Fall 17_DESCFA	Summer/Fall 18_UCOLSF	Fall 19_SRFALL	Proportion correct
Lower Columbia	Spring	01_YOUNGS	OTS01	Youngs Bay fall-run	44	44																			1.0000
				Reporting Group total	**44**	**44**																			**1.0000**
Lower Columbia	Spring	02_WCASSP	OTS02	Cowlitz R spring-run	66		57	8		1															0.8636
			OTS03	Kalama R spring-run	39		29		3		7														0.7436
				Reporting Group total	**105**		**86**																		**0.8190**
Lower Columbia	Fall	03_WCASFA	OTS04	Cowlitz R fall-run	41		4	37																	0.9024
			OTS05	Elochoman R fall-run	36	2	1	28		5															0.7778
			OTS06	Lewis R fall-run	49			49																	1.0000
			OTS07	NF Lewis fall-run	89			89																	1.0000
			OTS08	Sandy R fall-run	50			49															1		0.9800
				Reporting Group total	**265**			**252**																	**0.9509**
Lower Columbia	Spring	04_WILLAM	OTS09	McKenzie R spring-run	13				13																1.0000
			OTS10	N Santiam R spring-run	21				21																1.0000
			OTS11	Sandy R spring-run	18				18																1.0000
				Reporting Group total	**52**				**52**																**1.0000**
Lower Columbia	Fall	05_SPCRTU	OTS12	White Salmon fall-run	21			1		20															0.9524
			OTS13	Spring Creek NFH tule fall-run	22					20														2	0.9091
				Reporting Group total	**43**					**40**															**0.9302**
Interior stream type	Spring	06_KLICKR	OTS14	Klickitat R spring-run	35						34				1										0.9714
				Reporting Group total	**35**						**34**														**0.9714**
Interior stream type	Spring	07_DESCSP	OTS15	Shitike R spring-run	69						1	58	1		6			2		1					0.8406
			OTS16	Warm Springs R spring-run	56							55					1								0.9821
				Reporting Group total	**125**							**113**													**0.9040**
Interior stream type	Spring	08_JOHNDR	OTS17	John Day R spring-run	50		1						29		3		15	1			1				0.5800
			OTS18	Middle Fork John Day R spring-run	25								20		1		3	1							0.8000
			OTS19	North Fork John Day R spring-run	22								12	1	2		5	2							0.5455
				Reporting Group total	**97**								**61**												**0.6289**
Interior stream type	Spring	09_YAKIMA	OTS20	American R spring-run	35									35											1.0000
			OTS21	Cle-Elum spring-run	0																				
				Reporting Group total	**35**									**35**											**1.0000**
Interior stream type	Spring	10_UCOLSP	OTS22	Winthrop NFH spring-run	54								2		36	1	9	2		1	3				0.6667
			OTS23	little White Salmon R spring-run	70								1		57		6	3			3				0.8143
			OTS24	Wenatchee R spring-run	46								1		38		3	1		1	2				0.8261
			OTS25	Entiat R spring-run	65						1	1	3	1	39	1	13	1		1	4				0.6000
				Reporting Group total	**235**										**170**										**0.7234**
Interior stream type	Spring	11_TUCANO	OTS26	Tucannon R spring-run	37									2	2	28	4			1					0.7568
				Reporting Group total	**37**											**28**									**0.7568**
Interior stream type	Spring	12_HELLSC	OTS27	Wenaha R spring-run	122						4		9		13	2	89	3			2				0.7295
			OTS28	Lostine R spring-run	135								10	1	3		114			2	5				0.8444
			OTS29	Grande Ronde R spring-run	111						1		13	1	14	1	65	4		4	8				0.5856
			OTS30	Imnaha R spring-run	77							1	1		3		53	3		6	10				0.6883
			OTS31	Lolo Cr spring-run	62								1		5		46	3	1	2	4				0.7419
			OTS32	Red R spring-run	186								6		20		140	3		5	12				0.7527
			OTS33	Powell R spring-run	41								1		8		25			4	3				0.6098
			OTS34	Red R weir spring-run	81								1		1		72	1		2	4				0.8889
				Reporting Group total	**815**												**604**								**0.7411**
Interior stream type	Spring	13_SFSALM	OTS35	South Forth Salmon R spring-run	118								3		2		17	54	3	19	20				0.4576
			OTS36	Johnson Cr spring-run	117							1					4	93	1	10	8				0.7949
			OTS37	Secesh R spring-run	98								1		1		6	83		5	2				0.8469
				Reporting Group total	**333**													**230**							**0.6907**
Interior stream type	Spring	14_CHMBLN	OTS38	Chamberlain Cr spring-run	166												8	6	143	5	4				0.8614
				Reporting Group total	**166**														**143**						**0.8614**
Interior stream type	Spring	15_MFSALM	OTS39	Big Cr spring-run	102								2				12	4	1	75	8				0.7353
			OTS40	Camas Cr spring-run	47												1		2	41	3				0.8723
			OTS41	Loon Cr spring-run	89							1	1				3	3	2	77	2				0.8652
			OTS42	Sulphur Cr spring-run	78								1		2		5	3		66	1				0.8462
			OTS43	Bear Valley Cr spring-run	111															110	1				0.9910
			OTS44	Capehorn Cr spring-run	139										1		5	5	1	126	1				0.9065
			OTS45	Marsh Cr spring-run	150								1		1		1	9		128	10				0.8533
				Reporting Group total	**716**															**623**					**0.8701**
Interior stream type	Spring/Summer	16_UPSALM	OTS46	North Fork Salmon R spring-run	18										1		2	1		1	13				0.7222
			OTS47	Lemhi R spring-run	73								1				6	3		6	57				0.7808
			OTS48	Pahsimeroi R spring-run	73										1		2	3		1	66				0.9041
			OTS49	East Fork Salmon R spring-run	220												8	4		8	200				0.9091
			OTS50	Salmon R spring-run	62										1			1		4	56				0.9032
			OTS51	West Fork Yankee Fork spring-run	68								1				1	2			64				0.9412
			OTS52	Valley Cr spring-run	87								1				5	2		2	77				0.8851
			OTS53	Sawtooth Hatchery weir spring-run	135								1				7	9	1	12	105				0.7778
				Reporting Group total	**736**																**638**				**0.8668**
Interior ocean type	Fall	17_DESCFA	OTS54	upper Deschutes R fall-run	112		1	1														103	4	3	0.9196
				Reporting Group total	**112**																	**103**			**0.9196**
Interior ocean type	Summer/Fall	18_UCOLSF	OTS55	lower Yakima R fall-run	36		1	1															26	8	0.7222
			OTS56	Hanford Reach fall-run	53																		41	12	0.7736
			OTS57	Wenatchee R summer-run	54						1												52	1	0.9630
			OTS58	Entiat R summer-run	18																		17	1	0.9444
			OTS59	Methow R summer-run	52																		50	2	0.9615
				Reporting Group total	**213**																		**186**		**0.8732**
Interior ocean type	Fall	19_SRFALL	OTS60	Lyons Ferry weir fall-run	26																		4	22	0.8462
			OTS61	Clearwater R fall-run	69																	3	23	43	0.6232
				Reporting Group total	**95**																			**65**	**0.6842**

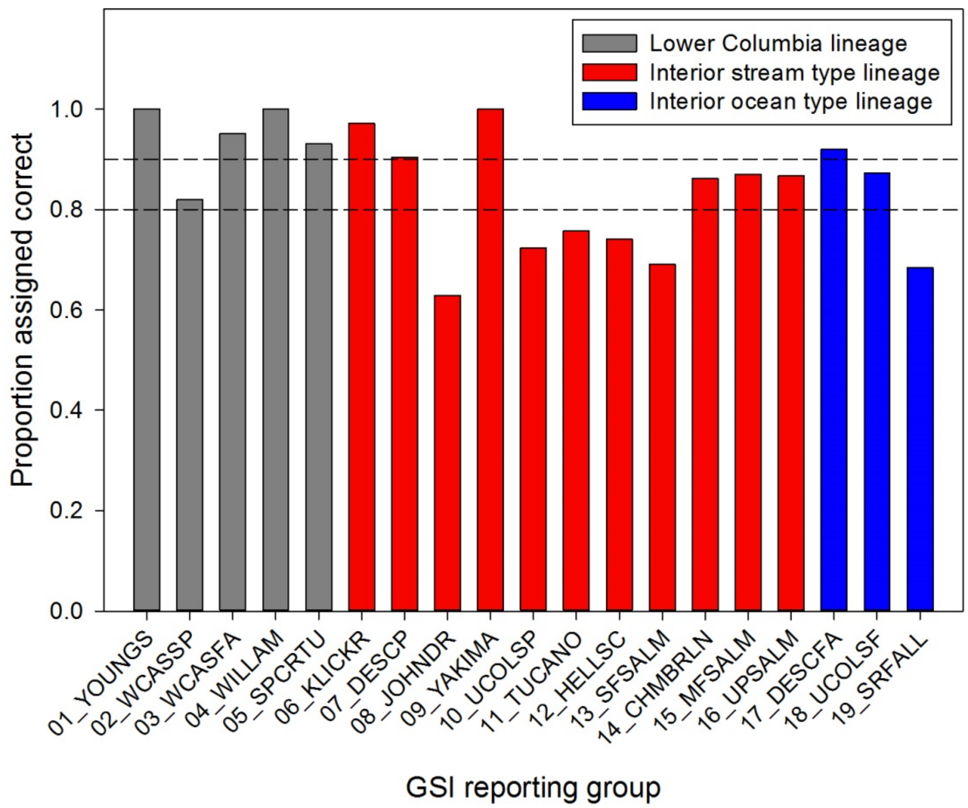


Figure 6: Proportion of Chinook salmon in leave-one-out tests that assigned correctly for each reporting group by lineage. The dashed lines indicate 80% and 90% thresholds for correct assignment.

Table 10: Results of the 10% mixture analysis for the Chinook salmon GSI v3.1 baseline. Lineage, run type, reporting group, population ID, population name and samples size used in analysis are provided for each collection in the baseline. The number of fish that had a probability of assignment ≥0.80 is provided. The number of correct assignments to reporting group for each population in the baseline are reported (gray shading) and are tallied to provide the number of correct assignments for the reporting group overall (yellow shading). The number and proportion of unassigned (p<0.80) fish is shown. The proportion of correct assignments to reporting group for each population and reporting group (yellow shading) in the baseline is provided.

						N assigned	Number assigned to reporting group																			Unassigned (p<0.80)		Proportion assigned
Lineage	Run type	Reporting Group	Pop ID	Pop Name	N	(p≥0.80	01_YOUNGS	02_WCASSP	03_WCASFA	04_WILLAM	05_SPCRTU	06_KLICKR	07_DESCSP	08_JOHNDR	09_YAKIMA	10_UCOLSP	11_TUCANO	12_HELLSC	13_SFSALM	14_CHMBLN	15_MFSALM	16_UPSALM	17_DESCFA	18_UCOLSF	19_SRFALL	N	Proportion	correct
Lower Columbia	Spring	01_YOUNGS	OTS01	Youngs Bay fall-run	9	9	9																			0	0.0000	1.0000
			Reporting Group total		**9**	**9**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0000**	**1.0000**
Lower Columbia	Spring	02_WCASSP	OTS02	Cowlitz R spring-run	9	9		9																		0	0.0000	1.0000
			OTS03	Kalama R spring-run	8	7		5		1		1														1	0.1429	0.7143
			Reporting Group total		**17**	**16**	**0**	**14**	**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.0625**	**0.8750**
Lower Columbia	Fall	03_WCASFA	OTS04	Cowlitz R fall-run	8	7			7																	1	0.1429	1.0000
			OTS05	Elochoman R fall-run	9	9	1		7		1															0	0.0000	0.7778
			OTS06	Lewis R fall-run	9	9			9																	0	0.0000	1.0000
			OTS07	NF Lewis fall-run	18	18			18																	0	0.0000	1.0000
			OTS08	Sandy R fall-run	8	7			7																	1	0.1429	1.0000
			Reporting Group total		**52**	**50**	**1**	**0**	**48**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0.0400**	**0.9600**
Lower Columbia	Spring	04_WILLAM	OTS09	McKenzie R spring-run	8	8				8																0	0.0000	1.0000
			OTS10	N Santiam R spring-run	8	8				8																0	0.0000	1.0000
			OTS11	Sandy R spring-run	5	4				4																1	0.2500	1.0000
			Reporting Group total		**21**	**20**	**0**	**0**	**0**	**20**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.0500**	**1.0000**
Lower Columbia	Fall	05_SPCRTU	OTS12	White Salmon fall-run	8	8					8															0	0.0000	1.0000
			OTS13	Spring Creek NFH tule fall-run	5	5					5															0	0.0000	1.0000
			Reporting Group total		**13**	**13**	**0**	**0**	**0**	**0**	**13**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0000**	**1.0000**
Interior stream type	Spring	06_KLICKR	OTS14	Klickitat R spring-run	8	7						7														1	0.1429	1.0000
			Reporting Group total		**8**	**7**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.1429**	**1.0000**
Interior stream type	Spring	07_DESCSP	OTS15	Shitike R spring-run	9	9							8			1										0	0.0000	0.8889
			OTS16	Warm Springs R spring-run	9	8							8													1	0.1250	1.0000
			Reporting Group total		**18**	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**16**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.0588**	**0.9412**
Interior stream type	Spring	08_JOHNDR	OTS17	John Day R spring-run	8	2								2												6	3.0000	1.0000
			OTS18	Middle Fork John Day R spring-run	5	3								2				1								2	0.6667	0.6667
			OTS19	North Fork John Day R spring-run	4	1								1												3	3.0000	1.0000
			Reporting Group total		**17**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**1.8333**	**0.8333**
Interior stream type	Spring	09_YAKIMA	OTS20	American R spring-run	8	8									8											0	0.0000	1.0000
			OTS21	Cle-Elum spring-run	9	7									6			1								2	0.2857	0.8571
			Reporting Group total		**17**	**15**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0.1333**	**0.9333**
Interior stream type	Spring	10_UCOLSP	OTS22	Winthrop NFH spring-run	8	5								1		4										3	0.6000	0.8000
			OTS23	little White Salmon R spring-run	9	6										5		1								3	0.5000	0.8333
			OTS24	Wenatchee R spring-run	11	8										6		1				1				3	0.3750	0.7500
			OTS25	Entiat R spring-run	10	7								1	1	3		2								3	0.4286	0.4286
			Reporting Group total		**38**	**26**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**1**	**18**	**0**	**4**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**12**	**0.4615**	**0.6923**
Interior stream type	Spring	11_TUCANO	OTS26	Tucannon R spring-run	8	5											4	1								3	0.6000	0.8000
			Reporting Group total		**8**	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0.6000**	**0.8000**
Interior stream type	Spring	12_HELLSC	OTS27	Wenaha R spring-run	18	10												10								8	0.8000	1.0000
			OTS28	Lostine R spring-run	21	15										2		13								6	0.4000	0.8667
			OTS29	Grande Ronde R spring-run	31	23										1		21			1					8	0.3478	0.9130
			OTS30	Imnaha R spring-run	10	4												2			1	1				6	1.5000	0.5000
			OTS31	Lolo Cr spring-run	9	6										1		5								3	0.5000	0.8333
			OTS32	Red R spring-run	22	16										1		14				1				6	0.3750	0.8750
			OTS33	Powell R spring-run	6	3										1		2								3	1.0000	0.6667
			OTS34	Red R weir spring-run	9	8												7	1							1	0.1250	0.8750
			Reporting Group total		**126**	**85**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**74**	**1**	**0**	**2**	**2**	**0**	**0**	**0**	**41**	**0.4824**	**0.8706**
Interior stream type	Spring	13_SFSALM	OTS35	South Forth Salmon R spring-run	14	7													7							7	1.0000	1.0000
			OTS36	Johnson Cr spring-run	14	10													10							4	0.4000	1.0000
			OTS37	Secesh R spring-run	25	17													16	1						8	0.4706	0.9412
			Reporting Group total		**53**	**34**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**33**	**1**	**0**	**0**	**0**	**0**	**0**	**19**	**0.5588**	**0.9706**
Interior stream type	Spring	14_CHMBLN	OTS38	Chamberlain Cr spring-run	22	17												1		16						5	0.2941	0.9412
			Reporting Group total		**22**	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**16**	**0**	**0**	**0**	**0**	**0**	**5**	**0.2941**	**0.9412**
Interior stream type	Spring	15_MFSALM	OTS39	Big Cr spring-run	14	11												2			7	2				3	0.2727	0.6364
			OTS40	Camas Cr spring-run	6	5															5					1	0.2000	1.0000
			OTS41	Loon Cr spring-run	11	10												1			9					1	0.1000	0.9000
			OTS42	Sulphur Cr spring-run	9	8															8					1	0.1250	1.0000
			OTS43	Bear Valley Cr spring-run	14	13															13					1	0.0769	1.0000
			OTS44	Capehorn Cr spring-run	21	21													1	1	19					0	0.0000	0.9048
			OTS45	Marsh Cr	23	17															17					6	0.3529	1.0000

				spring-run																								
			Reporting Group total		**98**	**85**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**1**	**1**	**78**	**2**	**0**	**0**	**0**	**13**	**0.1529**	**0.9176**
Interior stream type	Spring/Summer	16_UPSALM	OTS46	North Fork Salmon R spring-run	6	3												1				2				3	1.0000	0.6667
			OTS47	Lemhi R spring-run	10	6															1	5				4	0.6667	0.8333
			OTS48	Pahsimeroi R spring-run	9	8																8				1	0.1250	1.0000
			OTS49	East Fork Salmon R spring-run	29	20													1			19				9	0.4500	0.9500
			OTS50	Salmon R spring-run	8	6																6				2	0.3333	1.0000
			OTS51	West Fork Yankee Fork spring-run	8	8																8				0	0.0000	1.0000
			OTS52	Valley Cr spring-run	10	5																5				5	1.0000	1.0000
			OTS53	Sawtooth Hatchery weir spring-run	19	11																11				8	0.7273	1.0000
			Reporting Group total		**99**	**67**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**64**	**0**	**0**	**0**	**32**	**0.4776**	**0.9552**
Interior ocean type	Fall	17_DESCFA	OTS54	upper Deschutes R fall-run	25	22																	21		1	3	0.1364	0.9545
			Reporting Group total		**25**	**22**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**1**	**3**	**0.1364**	**0.9545**
Interior ocean type	Summer'Fall	18_UCOLSF	OTS55	lower Yakima R fall-run	6	5																		2	3	1	0.2000	0.4000
			OTS56	Hanford Reach fall-run	9	8																		7	1	1	0.1250	0.8750
			OTS57	Wenatchee R summer-run	9	9																		9		0	0.0000	1.0000
			OTS58	Entiat R summer-run	5	4																		4		1	0.2500	1.0000
			OTS59	Methow R summer-run	9	9																		8	1	0	0.0000	0.8889
			Reporting Group total		**38**	**35**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**30**	**5**	**3**	**0.0857**	**0.8571**
Interior ocean type	Fall	19_SRFALL	OTS60	Lyons Ferry weir fall-run	9	9																			9	0	0.0000	1.0000
			OTS61	Clearwater R fall-run	23	19																			19	4	0.2105	1.0000
			Reporting Group total		**32**	**28**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**28**	**4**	**0.1429**	**1.0000**

Steelhead (180 SNPs)

Leave-one-out analysis

For steelhead, we used GSI baseline v3.3 that comprises 116 collections from throughout the Columbia River basin that are partitioned into 14 reporting groups (N= 9991) (Figure 4). Missing data across loci resulted in the exclusion of 4746 samples for the leave-one-out analysis. Of the remaining 5245 samples, 84% assigned correctly to lineage. The highest proportion of correct assignments to was observed for the coastal lineage reporting groups and ranged from 0.83 (06_KLICKR) to 1.0 (01_WCOAST) (Table 11). The proportion of correct assignments for inland lineage reporting groups ranged from 0.57 (09_UPPCOL) to 0.94 (11_UPCLWR) (Table 11). Few samples from the coastal lineage (i.e., 01_WCOAST – 06_KLICKR reporting groups) were incorrectly assigned to reporting groups of the inland lineage (i.e., 07_MGILCS – 14_UPSALM reporting groups) (Table 11). The converse situation was also true – few samples from reporting groups from the inland lineage were incorrectly assigned to coastal lineage reporting groups (Table 11).

Across all reporting groups, the proportion of correct assignments ranged from 0.57 (09_UPPCOL) to 1.0 (01_WCOAST) (Table 11; Figure 7). The proportion of correct assignments for most reporting groups was >0.80; the two exceptions being 09_UPPCOL (0.57) and 14_UPSALM (0.65) (Table 11; Figure 7) with the majority of incorrect assignments to the 07_MGILCS reporting group. Incorrect assignments were typically distributed across multiple reporting groups, but the largest proportion of incorrect assignments were consistently to three reporting groups (07_MGILCS, 09_UPPCOL, 14_UPSALM). Samples from the geographically large 07_MGILCS reporting group were incorrectly assigned to every reporting group upstream (i.e, 08_YAKIMA – 14_UPSALM) (Table 11). Furthermore, the 07_MGILCS reporting group comprised the greatest proportion of incorrectly assigned samples from each reporting group from 08_YAKIMA – 14_UPSALM (Table 11).

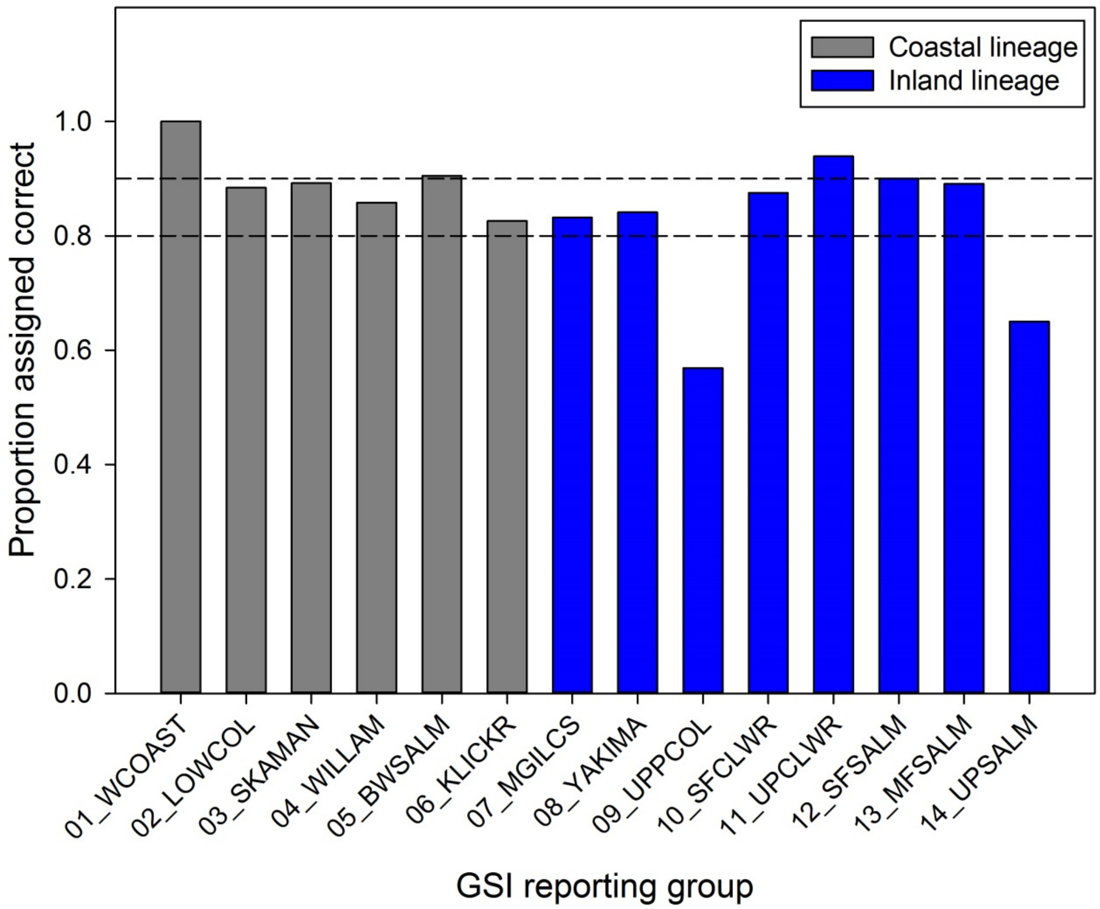
10% mixture analysis

We randomly selected and removed 1008 samples from these collections and assigned them to the remaining samples in the baseline. Of these randomly selected samples, 196 did not have an assignment probability ≥0.80 and were 'unassigned'. The proportion of fish that assigned correctly to reporting group for each of the 116 collections varied widely (0.00-1.00), and could reflect limited samples sizes in some instances (Table 12). When taken in aggregate (i.e., by reporting group of origin), the proportion of fish that assigned correctly to reporting groups ranged from 0.32 (09_UPPCOL) to 1.0 (01_WCOAST and 05_BWSALM, and 12_SFSALM). The proportion of fish that assigned correctly for most other reporting groups was ≥0.80, with a couple of exceptions (i.e., 08_YAKIMA and 14_UPSALM) (Table 12). These results are broadly concordant with those from those of the 'leave-one-out analysis', and reflect the population genetic structure of steelhead within the Columbia River Basin (Figure 5).

Table 11: Results of the leave-one-out analysis for the steelhead GSI v3.3 baseline. Reporting group, population ID, population name and samples size used in analysis are provided for each collection in the baseline. The number of correct assignments to reporting group for each population in the baseline are reported (gray shading) and are tallied to provide the number of correct assignments for the reporting group overall (yellow shading). The proportion of correct assignments to reporting group for each population and reporting group (yellow shading) in the baseline is provided.

Lineage	Reporting Group	PopID	Pop Name	N	Number assigned to reporting group: 01_WCOAST	02_LOWCOL	03_SKAMAN	04_WILLAM	05_BWSALM	06_KLICKR	07_MGILCS	08_YAKIMA	09_UPPCOL	10_SFCLWR	11_UPCLWR	12_SFSALM	13_MFSALM	14_UPSALM	Proportion assigned correct
Coastal	01_WCOAST	OMY001	Quinault River	56	56														1.000
			Reporting Group total	**56**	56														1.000
Coastal	02_LOWCOL	OMY002	Mill Creek	28	2	26													0.929
		OMY003	Germany Creek	22	1	19		2											0.864
		OMY004	Coweeman River	21		20	1												0.952
		OMY005	Cowlitz River	46		46													1.000
		OMY006	Kalama River - winter run	25		24	1												0.960
		OMY007	East Fork Lewis River	44		31	8	4						1					0.705
		OMY008	North Fork Lewis River	51		48	1	2											0.941
		OMY009	Luckiamute River	9		9													1.000
		OMY010	Willamina Creek	6		6													1.000
		OMY011	Still Creek	13		9	2	2											0.692
		OMY012	East Fork Hood River	20		14		2		4									0.700
			Reporting Group total	**285**		252													0.884
Coastal	03_SKAMAN	OMY013	Kalama River - summer run	49	1	13	35												0.714
		OMY014	Clackamas River - summer run	19		2	17												0.895
		OMY015	Klickitat-Skamania - summer run	80			80												1.000
			Reporting Group total	**148**			132												0.892
Coastal	04_WILLAM	OMY016	Clackamas River - winter run	44		2	2	39			1								0.886
		OMY017	North Fork Eagle River	28		7		21											0.750
		OMY018	Eagle River	19		2		17											0.895
		OMY019	Little Rock/Mad River	40			4	35	1										0.875
		OMY020	North Fork Santiam River	18		1		17											0.944
		OMY021	South Fork Santiam River/Wiley	48		1	7	40											0.833
			Reporting Group total	**197**				169											0.858
Coastal	05_BWSALM	OMY022	Big White Salmon River	21					19	2									0.905
			Reporting Group total	**21**					19										0.905
Coastal	06_KLICKR	OMY023	Upper Trout Creek	20						19				1					0.950
		OMY024	Suveyors Creek	12						12									1.000
		OMY025	Snyder Creek	13						12				1					0.923
		OMY026	Lower Summit Creek	17		1	1			13						1		1	0.765
		OMY027	Lower Trout Creek	9			1			8									0.889
		OMY028	Lower White Creek	6						5			1						0.833
		OMY029	Little Klickitat River	8		1				6	1								0.750
		OMY030	Dead Canyon Creek	6			2			3	1								0.500
		OMY031	Bowman Creek	12		2				6	2							2	0.500
		OMY032	Swale Creek	12				1		11									0.917
			Reporting Group total	**115**						95									0.826
Inland	07_MGILCS	OMY033	Fifteenmile Creek	37						1	32	2	1					1	0.865
		OMY034	Pelton	22							13	2				2	1	4	0.591
		OMY035	Shitike Creek	9							7		1					1	0.778
		OMY036	Buck Hollow Creek	21							19		1					1	0.905
		OMY037	Deschutes River-Trout Creek	20							15	1	2				1	1	0.750
		OMY038	Deschutes River-upper mainstem	17							17								1.000
		OMY039	Beech	7							6							1	0.857
		OMY040	John Day River - upper mainstem	8							8								1.000
		OMY041	Baldy	13							13								1.000
		OMY042	John Day River - lower mainstem	11							11								1.000
		OMY043	John Day River - upper middle fork	50							48		1				1		0.960
		OMY044	Granite	6							6								1.000
		OMY045	North Fork John Day River	32							30		1			1			0.938
		OMY046	Big Wall	14							12		1			1			0.857
		OMY047	Deer Creek	10							10								1.000
		OMY048	Murderers Creek	17							17								1.000
		OMY049	Rock Creek	83							68	2	2	1	3			7	0.819
		OMY050	Squaw Creek	95							72	2	6	3	2		2	8	0.758
		OMY051	Iskuulpa Creek	105						1	87	6	5					6	0.829
		OMY052	Umatilla River	18							15		1		1		1		0.833
		OMY053	Touchet River	51							51								1.000
		OMY054	Alpowa Creek	65							43	3	11	4	1			3	0.662
		OMY055	Asotin Creek	119							85	6	17	2	1	1	2	5	0.714
		OMY056	Tucannon River	66							49	5	7				2	3	0.742
		OMY057	Joseph Creek	49							46		1				1	1	0.939
		OMY058	Little Minam River	23							20	2	1						0.870
		OMY059	Menatchee Creek	17							15	1	1						0.882
		OMY060	Upper Grand Ronde River	86							76	1	4				2	3	0.884
		OMY061	Wallowa River	58							49	3	3					3	0.845
		OMY062	Wenaha River	101							87		9				1	4	0.861
		OMY063	Big Sheep Creek	79							73	1	2				1	2	0.924
		OMY065	Lightning Creek	9							7	1						1	0.778
		OMY066	Upper Imnaha River	25							21		2				2		0.840
		OMY067	Big Bear Creek	142							125	1	6	2	3			5	0.880
		OMY068	East Fork Potlatch River	103						1	87		4	5	3	2		1	0.845
		OMY069	Lapwai Creek	58							48	2	4					4	0.828
		OMY072	West Fork Potlatch River	57							46		3	7				1	0.807
		OMY073	Little Salmon River	87							62	2		2		1	5	15	0.713
		OMY074	Slate Creek	50							34		2			2	1	11	0.680
			Reporting Group total	**1840**							1530								0.832
Inland	08_YAKIMA	OMY075	Naches River - Rattlesnake Creek	13							2	10	1						0.769
		OMY076	Naches River - Nile Creek	30							2	25	2				1		0.833
		OMY077	Naches River - Pileup Creek	8								7						1	0.875
		OMY078	Naches River - Quartz Creek	11							2	9							0.818
		OMY079	North Fork Little Naches River	5							1	3	1						0.600
		OMY080	Satus Creek	30							2	26				1		1	0.867

		OMY081	Toppenish Creek	16								16							1.000
		OMY081.2	Ahtanum Creek	4							1	3							0.750
		OMY081.3	Yakima River - Big Creek	6								6							1.000
		OMY081.4	Cowiche River - Crow Creek	4								4							1.000
		OMY081.5	Teanaway River	13						1	2	10							0.769
		OMY081.6	Little Rattlesnake Creek	11							2	8	1						0.727
			Reporting Group total	**151**								127							0.841
		OMY082	Chiwaukum Creek	16							3	1	12						0.750
		OMY083	Upper Chiwaukum Creek	12							3		6				2	1	0.500
		OMY084	Nason River	11							4		7						0.636
Inland	09_UPPCOL	OMY085	Entiat River	122							49	5	60	1	2	1	1	3	0.492
		OMY086	Methow River	48							19	1	25		1			2	0.521
		OMY088	Omak River	44							6	1	34					3	0.773
			Reporting Group total	**253**									144						0.569
		OMY070	Lolo Creek	59							2			53	4				0.898
		OMY090	Clear Creek	28						1	2			23	2				0.821
Inland	10_SFCLWR	OMY091	Crooked River	60							4			54	2				0.900
		OMY092	Newsome Creek	62										58	4				0.935
		OMY093	Tenmile Creek	31							3			22	6				0.710
			Reporting Group total	**240**										210					0.875
		OMY094	Bear Creek	49											48			1	0.980
		OMY095	Lower Selway River	136							6		1	12	117				0.860
		OMY096	Middle Lochsa River	87							2			1	83			1	0.954
Inland	11_UPCLWR	OMY097	Middle Lochsa River	88							2		1	2	83				0.943
		OMY098	Upper Lochsa River	74										4	70				0.946
		OMY099	Upper Lochsa River	153							1		1	1	150				0.980
			Reporting Group total	**587**											551				0.939
		OMY100	East Fork South Fork Salmon River	77							5		1			68	3		0.883
Inland	12_SFSALM	OMY101	Secesh River	136							9		1			122	1	3	0.897
		OMY102	South Fork Salmon River	18												18			1.000
			Reporting Group total	**231**												208			0.900
		OMY103	Bear Valley Creek	124							4					1	119		0.960
		OMY104	Big Creek	131							7			1			119	4	0.908
		OMY105	Camas Creek	45							2	1					42		0.933
		OMY106	Chamberlain Creek	106							9		1			1	92	3	0.868
Inland	13_MFSALM	OMY107	Loon Creek	71							1				1		68	1	0.958
		OMY108	Marsh Creek	120							9	2	5				93	11	0.775
		OMY109	Middle Fork Salmon River	112							8					1	99	4	0.884
			Reporting Group total	**709**													632		0.891
		OMY110	Herd Creek	38						1	8		1					28	0.737
		OMY111	Lemhi River	18							3						1	14	0.778
		OMY112	Morgan Creek	26					1		4		1					20	0.769
Inland	14_UPSALM	OMY113	North Fork Salmon River	73							34		3		1	1	1	33	0.452
		OMY114	Pahsimeroi River	55					2		14						1	38	0.691
		OMY115	Sawtooth Hatchery	202							63		2				2	135	0.668
			Reporting Group total	**412**														268	0.650



856

857 Figure 7: Proportion of steelhead in leave-one-out tests that assigned correctly for each reporting
858 group by lineage. The dashed lines indicate 80% and 90% thresholds for correct assignment.

Table 12: Results of the 10% mixture analysis for the steelhead GSI v3.3 baseline. Reporting group, population ID, population name and samples size used in analysis are provided for each collection in the baseline. The number of fish that had a probability of assignment ≥0.80 is provided. The number of correct assignments to reporting group for each population in the baseline are reported (gray shading) and are tallied to provide the number of correct assignments for the reporting group overall (yellow shading). The number and proportion of unassigned (p<0.80) fish is shown. The proportion of correct assignments to reporting group for each population and reporting group (yellow shading) in the baseline is provided.

					Number assigned to reporting group															Unassigned (p<0.80)		
Lineage	Reporting Group	PopID	Pop Name	N	N assigned (p>0.80)	01_WCOAST	02_LOWCOL	03_SKAMAN	04_WILLAM	05_BWSALM	06_KLICKR	07_MGILCS	08_YAKIMA	09_UPPCOL	10_SFCLWR	11_UPCLWR	12_SFSALM	13_MFSALM	14_UPSALM	N	Proportion	Proportion assigned correct
Coastal	01_WCOAST	OMY001	Quinault River	9	9	9														0	0.0000	1.000
			Reporting Group total	**9**	**9**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0000**	**1.000**
Coastal	02_LOWCOL	OMY002	Mill Creek	4	4		4													0	0.0000	1.000
		OMY003	Germany Creek	5	5	1	4													0	0.0000	0.800
		OMY004	Coweeman River	5	4		4													1	0.2000	1.000
		OMY005	Cowlitz River	9	9		9													0	0.0000	1.000
		OMY006	Kalama River - winter run	9	8		8													1	0.1111	1.000
		OMY007	East Fork Lewis River	8	7		7													1	0.1250	1.000
		OMY008	North Fork Lewis River	9	9		9													0	0.0000	1.000
		OMY009	Luckiamute River	3	3		3													0	0.0000	1.000
		OMY010	Willamina Creek	3	3		3													0	0.0000	1.000
		OMY011	Still Creek	3	1		1													2	0.6667	1.000
		OMY012	East Fork Hood River	5	4		0		1		3									1	0.2000	0.000
			Reporting Group total	**63**	**57**	**1**	**52**	**0**	**1**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0.0952**	**0.912**
Coastal	03_SKAMAN	OMY013	Kalama River - summer run	9	7		3	4												2	0.2222	0.571
		OMY014	Clackamas River - summer run	6	6			6												0	0.0000	1.000
		OMY015	Klickitat-Skamania - summer run	25	25			25												0	0.0000	1.000
			Reporting Group total	**40**	**38**	**0**	**3**	**35**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0.0500**	**0.921**
Coastal	04_WILLAM	OMY016	Clackamas River - winter run	9	8				8											1	0.1111	1.000
		OMY017	North Fork Eagle River	4	4		1		3											0	0.0000	0.750
		OMY018	Eagle River	5	4				4											1	0.2000	1.000
		OMY019	Little Rock/Mad River	5	4				4											1	0.2000	1.000
		OMY020	North Fork Santiam River	4	4				4											0	0.0000	1.000
		OMY021	South Fork Santiam River/Wiley	9	8			2	6											1	0.1111	0.750
			Reporting Group total	**36**	**32**	**0**	**1**	**2**	**29**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0.1111**	**0.906**
Coastal	05_BWSALM	OMY022	Big White Salmon River	8	8					8										0	0.0000	1.000
			Reporting Group total	**8**	**8**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0000**	**1.000**
Inland	06_KLICKR	OMY023	Upper Trout Creek	3	3						3									0	0.0000	1.000
		OMY024	Suveyors Creek	3	3						3									0	0.0000	1.000
		OMY025	Snyder Creek	3	3						3									0	0.0000	1.000
		OMY026	Lower Summit Creek	4	4						4									0	0.0000	1.000
		OMY027	Lower Trout Creek	2	2						2									0	0.0000	1.000
		OMY028	Lower White Creek	3	3						2	1								0	0.0000	0.667
		OMY029	Little Klickitat River	3	3						3									0	0.0000	1.000
		OMY030	Dead Canyon Creek	2	1						1									1	0.5000	1.000
		OMY031	Bowman Creek	4	4						3								1	0	0.0000	0.750
		OMY032	Swale Creek	2	2						2									0	0.0000	1.000
			Reporting Group total	**29**	**28**	**0**	**0**	**0**	**0**	**0**	**26**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0.0345**	**0.929**
Inland	07_MGILCS	OMY033	Fifteenmile Creek	9	8							7	1							1	0.1111	0.875
		OMY034	Pelton	5	3							2							1	2	0.4000	0.667
		OMY035	Shitike Creek	3	3							3								0	0.0000	1.000
		OMY036	Buck Hollow Creek	6	4							4								2	0.3333	1.000
		OMY037	Deschutes River-Trout Creek	6	4							4								2	0.3333	1.000
		OMY038	Deschutes River-upper mainstem	6	5							5								1	0.1667	1.000
		OMY039	Beech	2	1							1								1	0.5000	1.000
		OMY040	John Day River - upper mainstem	3	3							3								0	0.0000	1.000
		OMY041	Baldy	3	3							3								0	0.0000	1.000
		OMY042	John Day River - lower mainstem	4	3							3								1	0.2500	1.000
		OMY043	John Day River - upper middle fork	11	9							9								2	0.1818	1.000
		OMY044	Granite	2	2							2								0	0.0000	1.000
		OMY045	North Fork John Day River	6	6							6								0	0.0000	1.000
		OMY046	Big Wall	2	2							2								0	0.0000	1.000
		OMY047	Deer Creek	2	1							1								1	0.5000	1.000
		OMY048	Murderers Creek	2	2							2								0	0.0000	1.000
		OMY049	Rock Creek	13	10							7	1		1	1				3	0.2308	0.700
		OMY050	Squaw Creek	14	11							11								3	0.2143	1.000
		OMY051	Iskuulpa Creek	15	13							13								2	0.1333	1.000
		OMY052	Umatilla River	3	1							1								2	0.6667	1.000
		OMY053	Touchet River	9	9							9								0	0.0000	1.000
		OMY054	Alpowa Creek	10	9							7			2					1	0.1000	0.778
		OMY055	Asotin Creek	19	10							10								9	0.4737	1.000
		OMY056	Tucannon River	11	8							7						1		3	0.2727	0.875
		OMY057	Joseph Creek	10	10							10								0	0.0000	1.000
		OMY058	Little Minam River	5	5															0	0.0000	0.000
		OMY059	Menatchee Creek	7	5							5								2	0.2857	1.000
		OMY060	Upper Grand Ronde River	16	14							13							1	2	0.1250	0.929
		OMY061	Wallowa River	12	8							7	1							4	0.3333	0.875
		OMY062	Wenaha River	19	14							13						1		5	0.2632	0.929
		OMY063	Big Sheep Creek	18	16							16								2	0.1111	1.000
		OMY065	Lightning Creek	4	4							3							1	0	0.0000	0.750
		OMY066	Upper Imnaha River	5	3							2		1						2	0.4000	0.667
		OMY067	Big Bear Creek	25	21							20			1					4	0.1600	0.952
		OMY068	East Fork Potlatch River	16	12							11			1					4	0.2500	0.917
		OMY069	Lapwai Creek	16	9							9								7	0.4375	1.000
		OMY072	West Fork Potlatch River	8	7							7								1	0.1250	1.000
		OMY073	Little Salmon River	15	7							6							1	8	0.5333	0.857
		OMY074	Slate Creek	8	6							6								2	0.2500	1.000
			Reporting Group total	**350**	**271**	**0**	**0**	**0**	**0**	**0**	**0**	**250**	**3**	**1**	**5**	**1**	**0**	**2**	**4**	**79**	**0.2257**	**0.923**
Inland	08_YAKIMA	OMY075	Naches River - Rattlesnake Creek	4	2							1	1							2	0.5000	0.500
		OMY076	Naches River - Nile Creek	6	4								4							2	0.3333	1.000
		OMY077	Naches River - Pileup Creek	3	3							1	2							0	0.0000	0.667
		OMY078	Naches River - Quartz Creek	3	3							1	2							0	0.0000	0.667
		OMY079	North Fork Little Naches River	2	1								1							1	0.5000	1.000
		OMY080	Satus Creek	5	2								2							3	0.6000	1.000
		OMY081	Toppenish Creek	3	3								3							0	0.0000	1.000
		OMY081.2	Ahtanum Creek	4	2					1			1							2	0.5000	0.500
		OMY081.3	Yakima River - Big Creek	4	4								4							0	0.0000	1.000
		OMY081.4	Cowiche River - Crow Creek	4	4								4							0	0.0000	1.000
		OMY081.5	Teanaway River	6	4							2	2							2	0.3333	0.500
		OMY081.6	Little Rattlesnake Creek	4	4							2	2							0	0.0000	0.500

			Reporting Group total	**48**	**36**	**0**	**0**	**0**	**0**	**1**	**0**	**7**	**28**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**0.2500**	**0.778**
Inland	09_UPPCOL	OMY082	Chiwaukum Creek	5	1									1						4	0.8000	1.000
		OMY083	Upper Chiwaukum Creek	3	2							1		1						1	0.3333	0.500
		OMY084	Nason River	2	1							1								1	0.5000	0.000
		OMY085	Entiat River	19	13							10		2		1				6	0.3158	0.154
		OMY086	Methow River	9	3							1		1		1				6	0.6667	0.333
		OMY088	Omak River	9	2									2						7	0.7778	1.000
			Reporting Group total	**47**	**22**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**7**	**0**	**2**	**0**	**0**	**0**	**25**	**0.5319**	**0.318**
Inland	10_SFCLWR	OMY070	Lolo Creek	9	6										5	1				3	0.3333	0.833
		OMY090	Clear Creek	5	3										3					2	0.4000	1.000
		OMY091	Crooked River	14	14							1			11	2				0	0.0000	0.786
		OMY092	Newsome Creek	10	9										9					1	0.1000	1.000
		OMY093	Tenmile Creek	5	3										2	1				2	0.4000	0.667
			Reporting Group total	**43**	**35**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**30**	**4**	**0**	**0**	**0**	**8**	**0.1860**	**0.857**
Inland	11_UPCLWR	OMY094	Bear Creek	7	7											7				0	0.0000	1.000
		OMY095	Lower Selway River	21	20											20				1	0.0476	1.000
		OMY096	Middle Lochsa River	15	14							1				13				1	0.0667	0.929
		OMY097	Middle Lochsa River	14	14							1				13				0	0.0000	0.929
		OMY098	Upper Lochsa River	13	13										1	12				0	0.0000	0.923
		OMY099	Upper Lochsa River	25	24											24				1	0.0400	1.000
			Reporting Group total	**95**	**92**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**1**	**89**	**0**	**0**	**0**	**3**	**0.0316**	**0.967**
Inland	12_SFSALM	OMY100	East Fork South Fork Salmon River	14	10												10			4	0.2857	1.000
		OMY101	Secesh River	21	18												18			3	0.1429	1.000
		OMY102	South Fork Salmon River	5	5												5			0	0.0000	1.000
			Reporting Group total	**40**	**33**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**33**	**0**	**0**	**7**	**0.1750**	**1.000**
Inland	13_MFSALM	OMY103	Bear Valley Creek	17	17							1						16		0	0.0000	0.941
		OMY104	Big Creek	22	21							1						20		1	0.0455	0.952
		OMY105	Camas Creek	10	8												1	7		2	0.2000	0.875
		OMY106	Chamberlain Creek	19	18							1						17		1	0.0526	0.944
		OMY107	Loon Creek	13	13							1						12		0	0.0000	0.923
		OMY108	Marsh Creek	20	13													13		7	0.3500	1.000
		OMY109	Middle Fork Salmon River	23	21							2						19		2	0.0870	0.905
			Reporting Group total	**124**	**111**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**1**	**104**	**0**	**13**	**0.1048**	**0.937**
Inland	14_UPSALM	OMY110	Herd Creek	9	7							1							6	2	0.2222	0.857
		OMY111	Lemhi River	9	4							1							3	5	0.5556	0.750
		OMY112	Morgan Creek	6	5							1							4	1	0.1667	0.800
		OMY113	North Fork Salmon River	10	4							2							2	6	0.6000	0.500
		OMY114	Pahsimeroi River	10	6							1							5	4	0.4000	0.833
		OMY115	Sawtooth Hatchery	32	14							3							11	18	0.5625	0.786
			Reporting Group total	**76**	**40**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**31**	**36**	**0.4737**	**0.775**

Results

Parentage based tagging assignments of Chinook salmon in harvest mixtures

Of the 4,354 harvest Chinook analyzed, PBT identified 1,169 hatchery-origin individuals that could be confidently assigned back to 44 hatchery broodstock sources (i.e., 30 Snake River hatcheries and 14 Columbia River hatcheries) spawned in 2010-2012 (Table 13). The majority of PBT assigned individuals (83.32%) were from the 2011 brood year (i.e., 4-years-old), with a smaller fraction represented by 3-year-old (brood year 2012; 13.86%) and 5-year-old (brood year 2010; 2.82%) fish. Of the 4-year-old fish, the majority (29.77%) were assigned to the Rapid River Fish Hatchery, followed by the McCall Fish hatchery (17.04%) and Dworshak National Fish Hatchery (16.53%) – all from the Snake River drainage. Relatively few 4-year-old fish were assigned to hatcheries in the Columbia River (2.57%; Klickitat Fish Hatchery).

Wind River spring-run Chinook salmon sport harvest

Both PBT and GSI assignments were used to analyze the Wind River spring-run Chinook salmon mark-selective sport fishery. We estimated stock composition to investigate how expansion of the Chinook salmon sport fishing "bubble" boundary around the mouth of the Wind River may be affecting proportions of non-local (i.e., Carson Hatchery) Chinook salmon that are harvested by using the context of stock proportions in other spring management period fisheries and at Bonneville Dam. None of the samples from the Wind River sport mark-selective fishery assigned via PBT. Although we expect that Chinook salmon harvested in this fishery will assign to the Carson hatchery, our PBT baseline for Carson hatchery broodstocks only extends back to 2012 (Table 3) and we were only likely to be able to assign jacks from the 2015 fishery; the 2016 fishery is likely the first year in which PBT assignments will be detected.

We also applied GSI to examine the stock composition of the Wind River fishery. We found that the 10_UCOLSP reporting group (includes upper Columbia River hatcheries as well as Carson Hatchery, Walla Walla Hatchery, and Umatilla Hatcheries) represented the greatest proportion of harvest (94.8%) followed to a much lesser extent by the 12_HELLSC (5.17%) and 07_DESCP (1.72%) reporting groups (Figure 8). Assignment of the 12_HELLSC genetic stock can be the result of misassignment that occurs between the two genetically similar stocks: Carson_H/10_UCOLSP vs. 12_HELLSC. This misassignment is known to occur based on comparisons of GSI results and coded wire tags. For example, in 2014 there were 20 coded wire tags that were analyzed with PBT and GSI in the Wind River harvest. Of these 20 coded wire tag origins, 18 of them were concordant with GSI/PBT assignments (90% concordance rate). The two misassignments occurred between 10_UCOLSP vs. 12_HELLSC stocks. Similarly, in 2013, there were 12 coded wire tags that were analyzed with GSI and represented in the Wind River harvest. Of these 12 coded wire tag origins, nine of them were concordant with the GSI assignment estimates (75% concordance rate). The three misassignments occurred between those same two stocks.

905 Table 13: Summary information on the number and origin of PBT assigned Chinook salmon in 2015 fishery mixtures. HOR= hatchery origin (i.e., adipose-clipped), NOR=natural origin (adipose non-clipped). HOR and NOR categories could be separated for various fisheries during spring, summer
906 and fall harvests.

					Spring harvest						Summer harvest						Fall harvest						Total								
		Hatchery	Hatchery	PBT tag	Commercial	Sport	Test		Tribal Ceremonial		Commercial		Sport		Tribal		Commercial		Sport		Tribal		Commercial		Sport		Test		Tribal		Grand
Hatchery	Stock	location	broodstock	rate	HOR	HOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	HOR	NOR	total
Carson National Fish Hatchery	Mixed origins (Snake and Mid-/Upper Columbia Rivers)	Columbia River	OtsCAR12	0.9000	5	7									1								5	0	7	0	0	0	1	0	13
Cleawater Fish Hatchery	South Fork Clearwater River	Snake River	OtsCLWH11S	0.9615	16	33	5								3								16	0	33	0	5	0	3	0	57
			OtsCLWH12S	0.9202	1	2																	1	0	2	0	0	0	0	0	3
Lookingglass Fish Hatchery	Catherine Creek	Snake River	OtsCTHW11S	0.9216	3				1														3	0	0	0	0	0	1	0	4
Dworshak National Fish Hatchery	Clearwater River	Snake River	OtsDWOR10S	0.9848	5	2			1														5	0	2	0	0	0	1	0	8
			OtsDWOR11S	0.9899	28	76	15		11	1	2		4		22	2							30	0	80	0	15	0	33	3	161
			OtsDWOR12S	0.9926	12	12									3								12	0	12	0	0	0	3	0	27
Lookingglass Fish Hatchery	Grande Ronde River	Snake River	OtsGRUW11S	0.9953	1	1	1									1							1	0	1	0	1	0	0	1	4
Lookingglass Fish Hatchery	Imnaha River	Snake River	OtsIMNW11S	0.9443	27	20		1			3		7		14								30	0	27	0	0	1	14	0	72
McCall Fish hatchery	Johnson Creek	Snake River	OtsJHNW11S	0.9747											1	6							0	0	0	0	0	0	1	6	7
Klickitat Fish Hatchery	Klickitat River	Columbia River	OtsKH10S	0.9746	1																		1	0	0	0	0	0	0	0	1
			OtsKH11S	0.8072	11	6	2								6								11	0	6	0	2	0	6	0	25
			OtsKH12S	0.8300	1																		1	0	0	0	0	0	0	0	1
Lookingglass Fish Hatchery	Lookingglass Creek	Snake River	OtsLOOK11S	0.9747	5	10			1														5	0	10	0	0	0	1	0	16
			OtsLOOK12S	0.9835	4		1						2		3								4	0	2	0	1	0	3	0	10
Lookingglass Fish Hatchery	Lostine River	Snake River	OtsLSTW10S	0.9434	1																		1	0	0	0	0	0	0	0	1
			OtsLSTW11S	0.9153	10																		10	0	0	0	0	0	0	0	10
Lyons Ferry Fish Hatchery	Mixed origins (Snake and Upper Columbia Rivers)	Snake River	OtsLYON11S	0.8988													2		1		2	4	2	0	1	0	0	0	2	4	9
			OtsLYON12S	0.9523				1															0	0	0	0	0	1	0	0	1
			OtsLYON12S_1	0.9860														1			1	1	0	1	0	0	0	0	1	1	3
McCall Fish hatchery	South Fork Salmon River	Snake River	OtsMCCA10S	0.9958	3	2										1							3	0	2	0	0	0	0	1	6
			OtsMCCA11S	0.9904	46	42	2	1			9	1	16		31	18							55	1	58	0	2	1	31	18	166
			OtsMCCA12S	0.9710		2					1		3	1	1	1							1	0	5	1	0	0	1	1	9
Methow Fish Hatchery	Upper Methow and Twisp Rivers	Columbia River	OtsMETH12	0.9300	1										1								1	0	0	0	0	0	1	0	2
Perce Tribal Hatchery	Mixed origins (Snake and Clearwater River)	Snake River	OtsNPFH12S_1	0.9937														1		1		3	0	1	0	1	0	0	0	3	5
Pahsimeroi Hatchery	Salmon River	Snake River	OtsPAHH11S	0.9662	18	16					1		6		7	1							19	0	22	0	0	0	7	1	49
Parkdale Fish Facility	Hood River	Columbia River	OtsPFF12	0.8900	3	1							2										3	0	3	0	0	0	0	0	6
Clearwater Fish Hatchery-Powell Facility	Lochsa River	Snake River	OtsPOWP11S	0.9869	15	30	7	1	4	3					2								15	0	30	0	7	1	6	3	62
			OtsPOWP12S	0.9866	7	1									3								7	0	1	0	0	0	3	0	11
Priest Rapids Hatchery	Mixed origins (Mid-/Upper Columbia Rivers)	Columbia River	OtsPRH12	0.6264													3	2	6	1	3	4	3	2	6	1	0	0	3	4	19
Yakama Nation Prosser Hatchery	Mixed origins (Little White Salmon and Yakima Rivers)	Columbia River	OtsPRO12	0.8950																		1	0	0	0	0	0	0	0	1	1
Rapid River Fish Hatchery	Rapid River	Snake River	OtsRAPH10S	0.9898	1	9	1		1														1	0	9	0	1	0	1	0	12
			OtsRAPH11S	0.9835	51	170	34	1	20						14								51	0	170	0	34	1	34	0	290
			OtsRAPH12S	0.9536	8	6																	8	0	6	0	0	0	0	0	14
Round Butte Fish Hatchery	Deschutes River	Columbia River	OtsRB12	0.9200								1	1										0	1	1	0	0	0	0	0	2
Sawtooth Fish Hatchery	Salmon River	Snake River	OtsSAWT10S	0.9944	2	3																	2	0	3	0	0	0	0	0	5
			OtsSAWT11S	0.9697	12	10									16	4							12	0	10	0	0	0	16	4	42
			OtsSAWT12S	0.9369	2	3					2		3		5	1							4	0	6	0	0	0	5	1	16
Tucannon Fish Hatchery	Tucannon River	Snake River	OtsTUCW12S	0.9523												1							0	0	0	0	0	0	0	1	1
Umatilla Hatchery - Three Mile Dam	Umatilla River	Columbia River	OtsUMA12_sp	0.9200	3	1	1									1							3	0	1	0	1	0	0	1	6
	Little White Salmon River	Columbia River	OtsUMA12_sufa	0.9900													2		2				2	0	2	0	0	0	0	0	4
Wells Fish Hatchery	Upper Columbia River	Columbia River	OtsWELLS12	0.9947									1		1								0	0	1	0	0	0	1	0	2
Warm Spring National Fish Hatchery	Deschutes River	Columbia River	OtsWSNFH12	0.6000	3																		3	0	0	0	0	0	0	0	3
Yakima River Fish Hatchery	?	Columbia River	OtsYR12	0.9937	1	2																	1	0	2	0	0	0	0	0	3

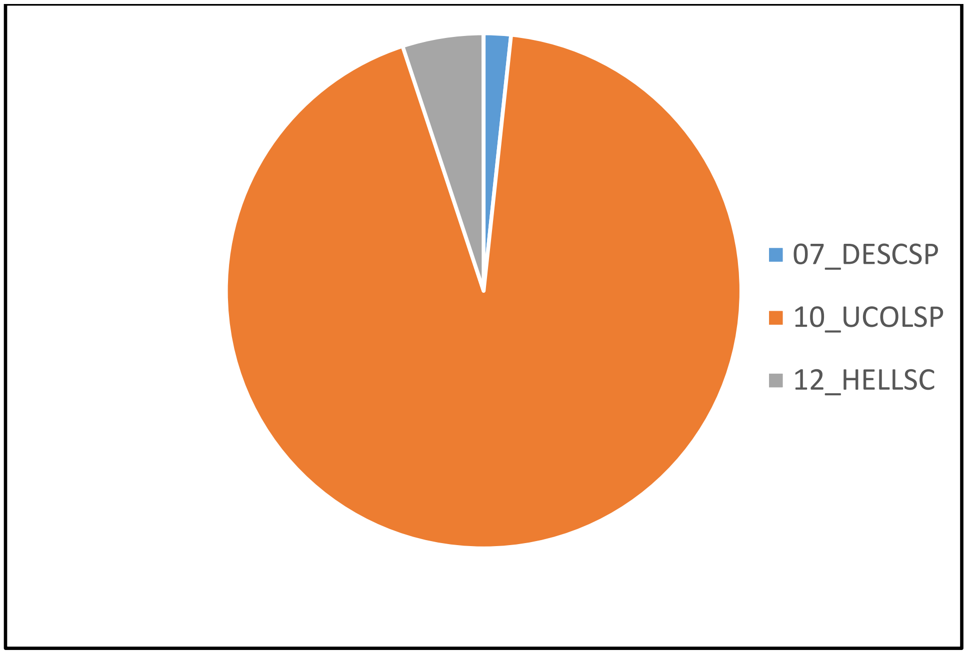


Figure 8: Genetic stock composition of the Wind River sport mark-selective harvest in 2015.

This analysis cannot currently quantify Carson Hatchery fish that are harvested in the bubble fishery, nor can it determine how many wild fish from other areas are handled in the fishery. Further, we cannot conclude whether changing the bubble fishery boundary has resulted in any change in impacts compared to previous years with the smaller bubble, because we did not sample in years prior to the bubble expansion.

Comparison of stock composition among spring management period Chinook salmon fisheries

Analysis of adipose-clipped Chinook salmon from multiple fishery mixtures in the spring management period (April to June 15th) identified relatively larger proportions of individuals that assigned via PBT to Snake River hatcheries. With the exception of the Wind River sport mark-selective harvest, Chinook salmon from Snake River hatcheries comprised the largest component of each harvest, and accounted for 37-43% of fish harvested in Region B, and 39-64% of fish harvested in Region A from commercial, sport, and Test fisheries (Figure 9). Chinook salmon from Snake River hatcheries also comprised the largest fraction of fish taken in Tribal ceremonial (55%) and Tribal harvest (62%) fisheries in Zone 6 (Figure 9). These proportions are broadly consistent with the large proportion of hatchery origin Chinook salmon passing Bonneville Dam in 2015 that assigned via PBT to Snake River hatcheries (58%). As noted in the previous section, and unlike the other fisheries assessed, the Wind River sport fishery was not composed primarily of fish from Snake River hatcheries, but rather of fish that assigned via GSI to Columbia River reporting groups (Figure 9); primarily 10_UCOLSP (Figure 8). PBT assignments to Columbia River hatcheries ranged from 0-5.5% and reflect the incomplete nature of our PBT baseline for Columbia River hatchery broodstocks (i.e., for several Columbia River hatcheries we are currently only able to assign jacks and 4-year-old fish). However, our PBT

baselines for Columbia River broodstocks are expanding, and we will be able to assign hatchery origin fish to additional Columbia River hatcheries in the near future.

Chinook from the interior ocean type lineage (OT) comprised a small fraction (0-8.5%) of Chinook salmon taken in spring harvests, and approximated that encountered passing Bonneville Dam (3.5%) during the spring management period (Figure 9). With the exception of the Wind River sport mark-selective harvest, Chinook from the interior stream type lineage (ST) comprised 19-45% of harvest samples, and approximated that observed at Bonneville Dam during the spring management period. We observed a steady decrease in the proportion of harvests comprised of the lower Columbia (LC) Chinook lineage up to Bonneville Dam (Figure 9). The proportion of LC lineage Chinook in Region B fisheries was 28-34%. This decreased to 2-21% for Region A fisheries, and to 0.1% at Bonneville Dam. No LC lineage Chinook were taken in Zone 6 Tribal fisheries above Bonneville Dam during the spring management period (Figure 9).

Analysis of adipose-intact fisheries revealed that PBT assignments to Snake River hatcheries represented 22-34% of the fish harvested (Figure 10); a lower fraction than that observed for comparable adipose-clipped fisheries (as expected) (Figure 9), but consistent with assignments for adipose intact fish passing Bonneville Dam (20%). The LC lineage was only encountered in the test fishery during the spring management period, and the OT lineage was only observed at Bonneville Dam and in the Zone 6 tribal harvest. The ST lineage comprised the greatest proportion of adipose-intact fisheries (45-75%), and approximated that encountered at Bonneville Dam during the spring management period (Figure 10).

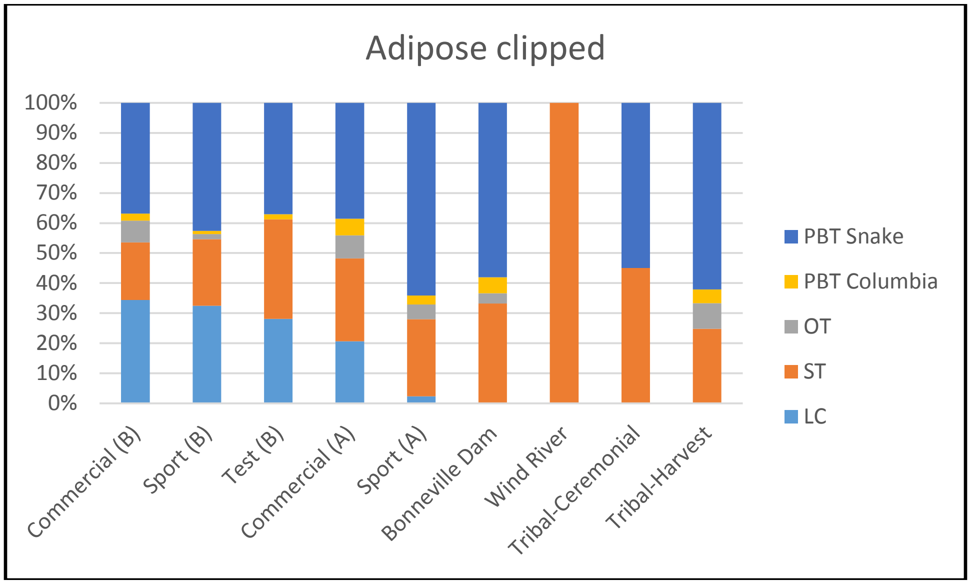


Figure 9**:** Stock composition of spring management period adipose-clipped Chinook salmon harvest mixtures. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

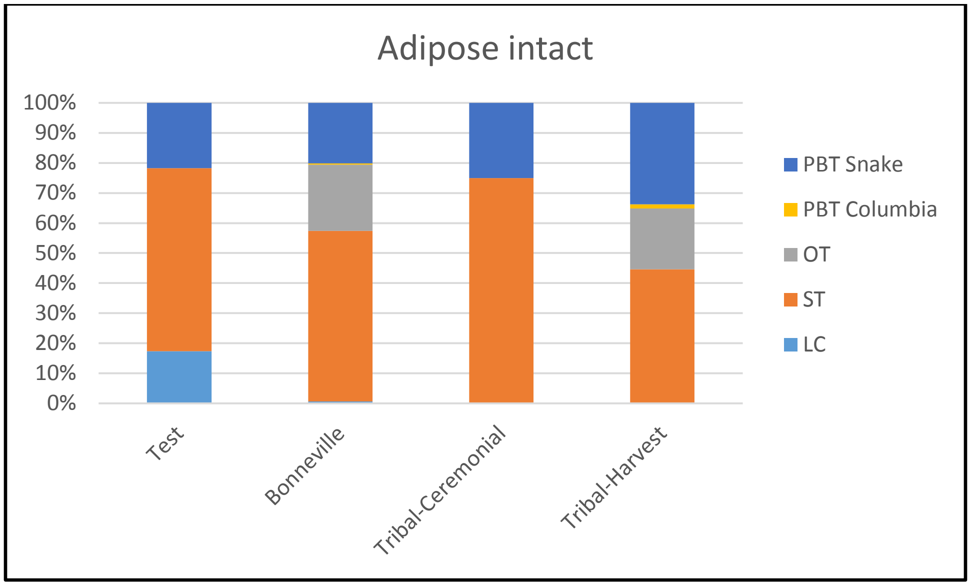


Figure 10: Stock composition of spring management period adipose-intact Chinook salmon harvest mixtures. ‘PBT Snake’ and ‘PBT Columbia’ include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

Comparison of percent stock composition of upriver spring Chinook salmon stocks (ST) among summer-management period Chinook salmon fisheries

Analysis of Chinook salmon fisheries in the summer management period (June 16 – August 1) addressed the following objectives: 1) estimate stock composition for the mark selective sport fishery and commercial fishery below Bonneville Dam, 2) compare stock composition of adipose-clipped versus adipose-intact fish from the commercial harvest below Bonneville Dam, 3) characterize temporal changes in stock composition across the season. While jack Chinook salmon are not harvested at high rates in fisheries and there are no specific harvest limits for them, jack Chinook salmon if sampled could have been incorporated into this analysis.

We observed similar stock compositions for adipose-clipped Chinook salmon taken in Lower Columbia River sport and commercial fisheries from Region B (Figure 11). However, Chinook stocks from the OT lineage comprised a greater proportion of the sport harvest (45%) than the commercial fishery (30%); a similar pattern was observed for Region A (46% vs. 33%, respectively). Additionally, while Chinook salmon stocks from the LC lineage comprised a slighter greater proportion of the commercial harvest (49%) in Region B than the sport fishery (38%), this difference was greater in region A (48% vs. 9%, respectively). A similar proportion of adipose-clipped fish from Region B sport (12%) and commercial (16%) fisheries assigned to Snake River hatcheries (Figure 11). However, for Region A, a greater proportion of adipose-

clipped fish from the sport harvest assigned to Snake River hatcheries (41%) compared to the commercial harvest (17%). We observed no appreciable differences between these fisheries (either within or between regions) in the relative proportion of fish assigned to Columbia River hatcheries or stocks from the ST lineage (Figure 11).

We observed notable differences in the stock composition of adipose-clipped and adipose-intact Chinook salmon taken in the commercial fishery below Bonneville Dam. These differences were consistent across regions (Figure 11). As expected, a greater proportion of adipose-clipped fish harvested in the commercial fishery assigned to Snake River hatcheries (16-17%) than adipose-intact fish (0-2%). Similarly, a greater proportion of the adipose-clipped harvest from the commercial fishery assigned to stocks from the LC lineage (48-49%) than adipose-intact fish (23-28%). However, we also observed that a smaller proportion of the adipose-clipped commercial harvest was comprised of the OT lineage (30-33%) than the adipose-intact harvest (68-74%) (Figure 11). We observed no appreciable differences between the adipose-clipped and adipose-intact commercial harvest (either within or between regions) in the relative proportion of fish assigned to Columbia River hatcheries or stocks from the ST lineage (Figure 11).

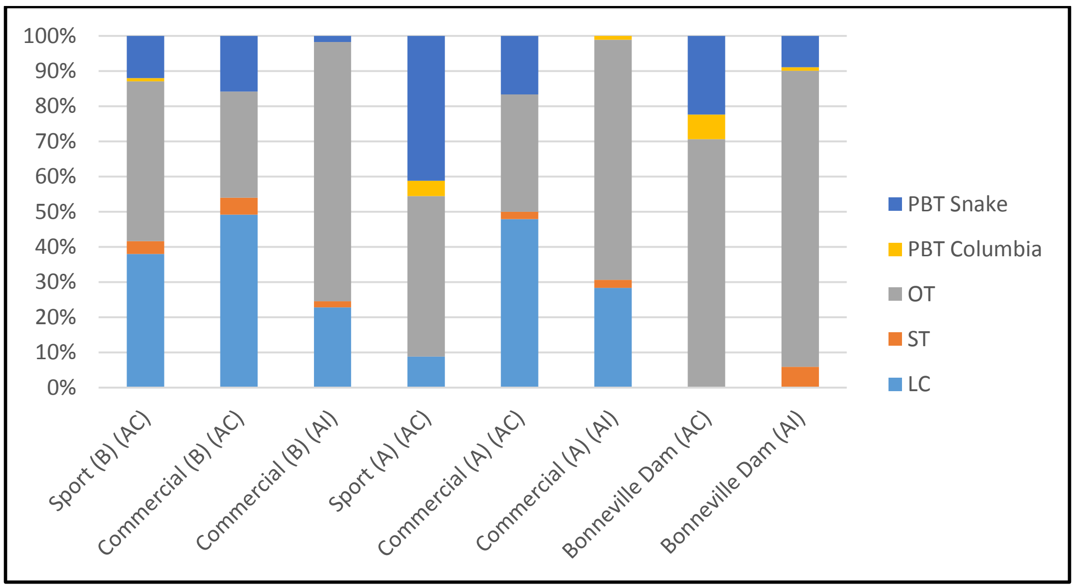
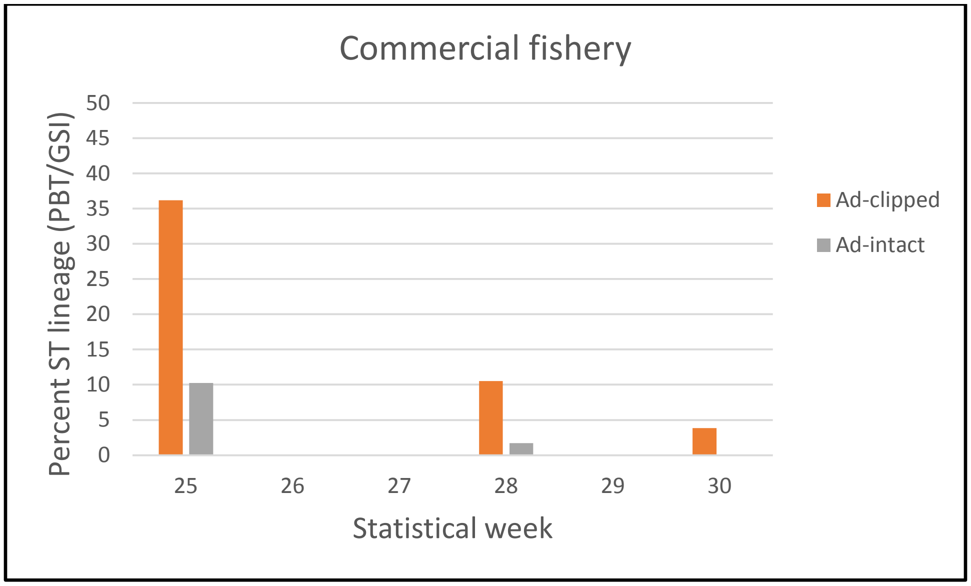


Figure 11: Stock composition of summer management period Chinook salmon fisheries and Bonneville Dam. 'AC' is adipose-clipped; 'AI' is adipose intact. 'PBT Snake' and 'PBT Columbia' include assignments to all Snake River and Columbia River hatcheries that are in our PBT baseline. Interior ocean type (OT), interior stream type (ST), and lower Columbia lineage include GSI assignments to reporting groups within our GSI baseline.

We compared changes in the % stock composition of the stream-type lineage of adipose-clipped vs. adipose-intact Chinook salmon over the course of the summer management period in the lower Columbia River commercial fishery relative to that passing Bonneville Dam. We detected declines in the proportion of ST lineage Chinook salmon, for both adipose-clipped and adipose-intact fish, in the commercial harvest and at Bonneville Dam over the summer management period (Figure 12). However, there was a modest increase in the proportion of the ST lineage for adipose-clipped fish at Bonneville Dam in statistical week 26. Meaningful comparisons are made challenging by the absence of data continuity over the time series owing to fisheries closures and cessation of sampling at the Bonneville AFF in response to elevated water temperatures.

Stock composition of the fall-run mark-selective Chinook salmon sport fishery

We estimated stock composition of the mark selective sport fishery in the lower Columbia River in 2015. Major reporting groups in order of decreasing proportion were: 18_UCOLSF (73%), 03_WCASFA (12%), 19_SRFALL (9%), 05_SPCRTU (6%) (Figure 13). These results are broadly consistent with the 2014 fall sport harvest.



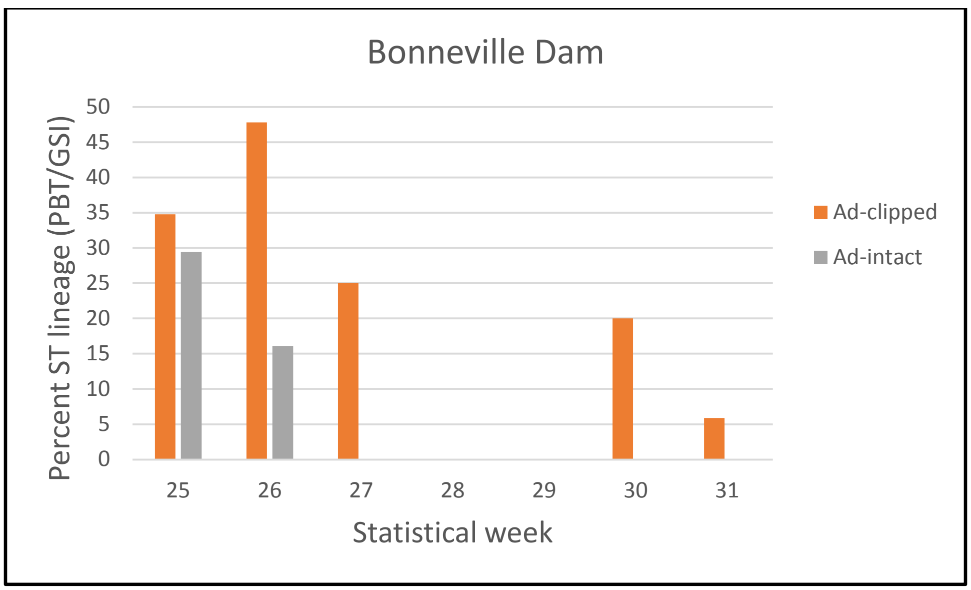


Figure 12: Temporal patterns of the percent of Chinook salmon ST lineage in adipose-clipped and adipose intact mixture samples from the lower Columbia River commercial fishery (top panel) and Bonneville Dam (bottom panel) during the summer management period (June 16-August 31).

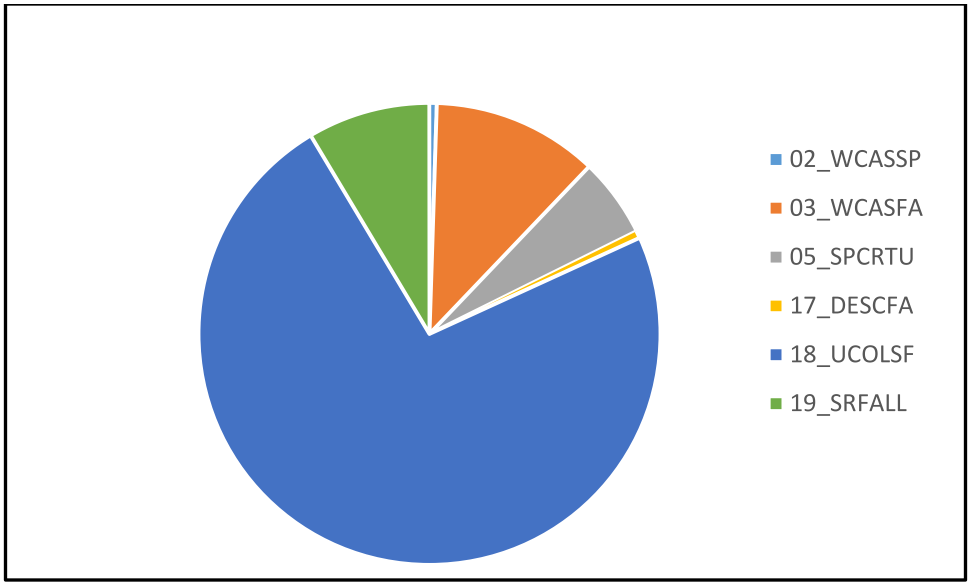


Figure 13: Genetic stock composition of the lower Columbia River fall-run mark-selective Chinook salmon sport harvest in 2015. Note: the assignment of West Cascade spring stock (02_WCASSP) may owe to misassignment between the spring and fall runs from that region, strays from the net pen rearing at Youngs Bay, or other unknown reasons.

Comparison of stock composition among sockeye salmon fisheries

Sockeye salmon were sampled from the lower Columbia River below Bonneville Dam in the lower river sport and commercial fishery and Zone 6 tribal fishery, and were assigned to three Columbia River genetic stocks (Table 14). The Zone 6 sport fishery was not sampled. Low sample numbers of Snake River sockeye make it difficult to estimate narrow confidence intervals for estimates of this stock (Table 15).

The timing of the sockeye salmon fisheries may influence the harvested proportion of each stock, and is consistent with run-timing distributions observed in our previous reports, particularly that the Wenatchee stock has relatively early run-timing compared to other stocks. The Snake River stock was only represented by 38 fish in the Zone 6 fishery samples (Table 14) making run-timing estimates imprecise for this stock. Of the 38 Snake River sockeye salmon identified with GSI, the largest number (20) were sampled in week 28 and they were estimated to be in highest relative stock proportion (4.7%) in that same week (Figure 14). Week 26 had the highest proportions of Wenatchee stock (Figure 14). Notable differences in stock representation between Bonneville Dam and the Zone 6 tribal harvest were observed for the Wenatchee stock (44 vs. 35%) and for the Okanogan stock (55 vs. 63%) in the harvest vs. Bonneville Dam mixture samples, respectively (Table 15).

Table 14: Summary of sample sizes and stock assignments for sockeye salmon fisheries by weekly strata.

		Statistical week								
Fishery	**Stock**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
Commercial	Okanogan	2	14	27	0	17	8	3	0	0
	Snake	0	1	1	0	1	0	0	0	0
	Wenatchee	1	7	13	0	8	4	1	0	0
Sport	Okanogan	1	24	9	13	8	0	1	0	0
	Snake	0	0	0	0	0	0	0	0	0
	Wenatchee	1	21	8	12	7	0	0	0	0
Zone 6	Okanogan	0	162	88	188	243	135	56	2	0
	Snake	0	0	1	4	20	9	4	0	0
	Wenatchee	0	91	105	156	166	73	32	1	0

Table 15: Comparison of stock-specific abundance and percent composition among sockeye salmon fisheries. The mean stock abundance estimate is provided for each fishery harvest and includes 95% confidence intervals.

	Mean (95% C.I.)			Stock proportion		
Mixture source	**Wenatchee**	**Okanagan**	**Snake**	**Wenatchee**	**Okanagan**	**Snake**
Commercial	139 (99 – 181)	293 (251 – 333)	13 (0 – 29)	31.27%	65.90%	2.83%
Sport	478 (380 – 574)	544 (448 – 643)	10 (0 – 29)	46.30%	52.75%	0.95%
Zone 6	13,142 (12,336 – 14,026)	16,407 (15,525 – 17,191)	546 (368 – 739)	43.67%	54.52%	1.81%
Total Harvest	13,759 (13,025 – 14,480)	17,244 (16,522 – 17,959)	569 (351 – 824)	40.51%	56.72%	2.76%
Bonneville Dam	178,325 (159,747 – 198,421)	323,797 (302,554 – 342,981)	7919 (2,949 – 13,906)	34.96%	63.48%	1.55%

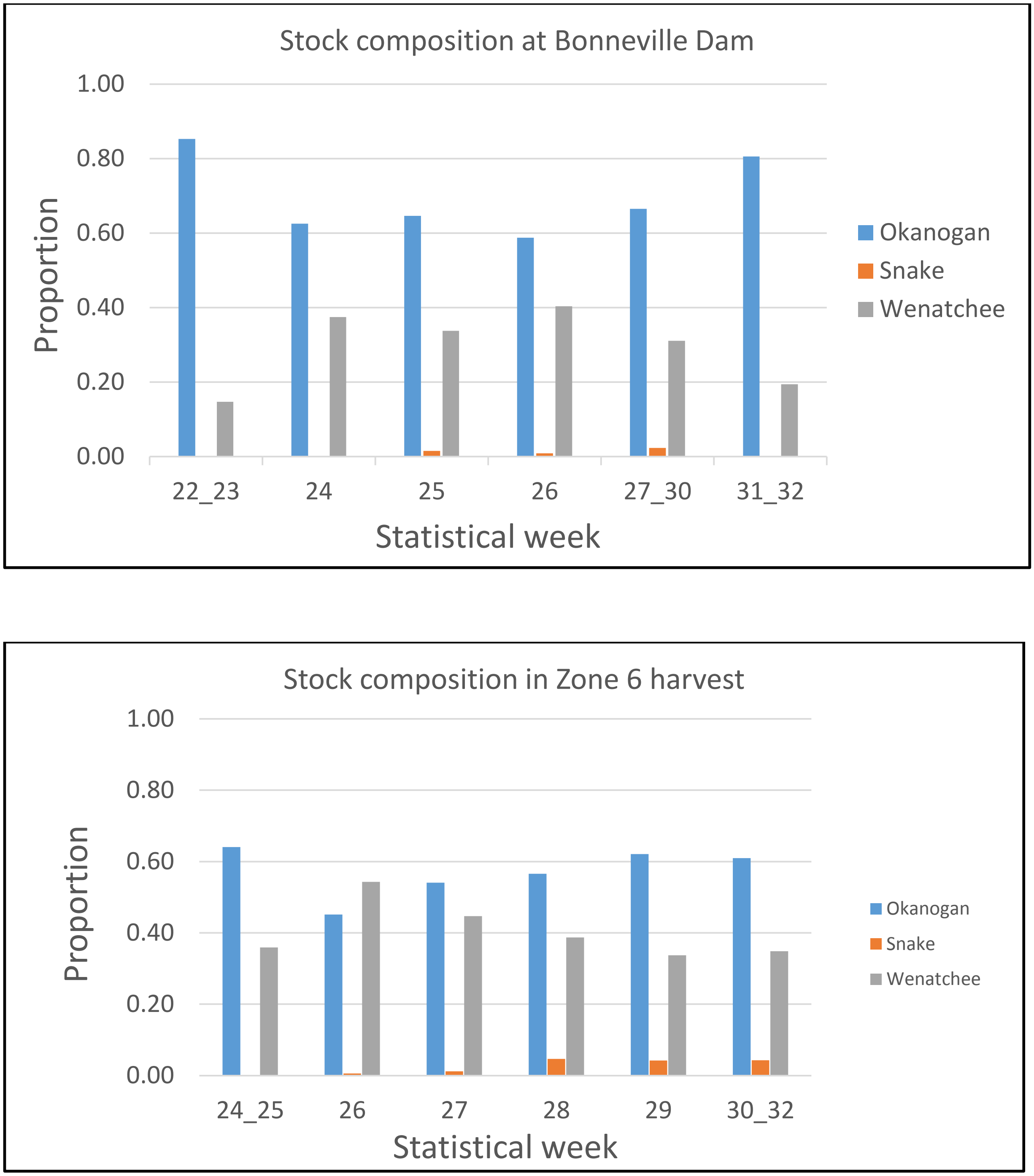


Figure 14: Stock composition of sockeye salmon at Bonneville Dam (top panel) and in the Zone 6 tribal harvest (bottom panel) across weekly strata.

Discussion

Management implications

This study utilized both genetic stock identification (GSI) and parentage based tagging (PBT) in combination to estimate stock composition of mainstem Columbia River Chinook salmon and sockeye fisheries. This is the third year in which we were able to assign all three major age classes of spring Chinook from Snake River hatcheries and the first year in which we could assign 3-year old fish to Columbia River hatcheries as a consequence of our expanded PBT baseline. Ongoing expansion of the PBT baseline will allow us the ability to assign Snake River hatchery jacks of the fall-run Chinook salmon as well as all other hatchery jacks originating above Bonneville Dam (migration year 2016), and so future years of analyses will include more emphasis on fall-run harvest. Expansion of the PBT baseline to include not only hatcheries of Chinook salmon and steelhead above Bonneville Dam, but also hatcheries throughout the range of these species could eventually lead to replacing the coded wire tag program for monitoring of in-river harvest stock composition of these species if increases in funding were available and fishery managers thought it were needed. This report includes the third genetic analysis on sockeye salmon harvest. Our results demonstrated differences in stock composition of the sockeye salmon harvest as compared to the total run estimated at Bonneville Dam, but there are questions about the validity of the estimates especially at Bonneville given the potential for sampling error around rare stocks like Snake R. sockeye salmon. We will continue to perform GSI on sockeye salmon harvest in the future to gain further insight into these patterns.

One higher level management question was possible to address in this section:

1) **Harvest RM&E: F&W Program Management Question: What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?**

The in-river estimates of stock composition, stock-specific abundance, escapement, catch, and age distribution were addressed for part of the treaty mainstem spring-management period fisheries Chinook salmon harvests above Bonneville Dam along with fisheries below Bonneville Dam, the non-treaty summer-management fisheries below Bonneville Dam which are a portion of the total non-treaty summer fisheries, and the mainstem treaty sockeye salmon harvests above and non-treaty harvest below Bonneville Dam. For the spring management period of Chinook salmon, we continue to observe a spatial pattern for the stock composition of lower Columbia River stocks which appear more abundant downstream from the Willamette River mouth as compared to upstream of this point which is consistent with a long history of CWT data. We observed differences in the composition of hatchery stocks represented in spring vs. summer management period harvest of Chinook salmon, and run-timing plays an important role in this difference (i.e., late-running stocks appear more abundant among the upriver spring-type lineage that are caught in the summer management period). This pattern is consistent when compared to known origin PIT tagged adult and jack fish tagged as juveniles. Known origin adult age upriver spring and Snake River spring Chinook salmon are almost all past Bonneville by June 15 in most years. However, specific conclusions relative to the harvest impacts on spring run Chinook salmon cannot be made from this genetic analysis as jacks are included in the current study.

This study also addressed some issues that relate to mark-selective fisheries. For example, we examined stock composition of the Wind River mark-selective spring-run Chinook salmon sport fishery and provided context of stock composition observed among other fisheries during this management period. The number of PBT-assigned Snake R. fish that were harvested in the Wind River sport fishery was less than all other fishery samples we analyzed, including the adipose-intact samples from Bonneville Dam and tribal harvests during the same set of weeks. For the 2016 analyses, our Columbia River PBT baseline should be sufficient to assign 4 year old fish from the Wind River sport fishery to the Carson hatchery. It was not possible to conclude whether or not the Wind River sport fishery harvest composition has significantly changed through time given this boundary change.

The sockeye salmon tribal fishery is managed in a way that attempts to harvest as many harvestable sockeye salmon as possible under the allowed harvest rate schedule in the U.S. v. Oregon Management Agreement. This 2015 year of analysis of the sockeye salmon harvest corroborates our 2014 and 2013 harvest analyses, which suggested there may be some over representation of the Wenatchee sockeye stocks in the Zone 6 harvest as compared to the stock proportions that are present at Bonneville Dam. The results for Snake River sockeye salmon are dependent upon representative sampling at Bonneville Dam, but low sample rate and the rarity of this stock led to uncertainty and high variation around estimates of Snake River sockeye salmon from Bonneville Dam. Sampling protocols at Bonneville Dam may have higher representation of young fish as compared to harvest mixtures. Timing of the fishery may also influence the proportion of each stock, and is consistent with run-timing distributions we observed in previous reports; the Wenatchee stock has relatively early run-timing but the timing of the Snake River stock is uncertain due to inconsistent results between PIT-tag and GSI methods. Future analysis will be needed to examine these patterns for consistency and delve into explanations.

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

Introduction

The Columbia River Basin supports ESA listed natural origin 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 and PBT) analyses of Chinook and sockeye salmon fisheries harvests in Section 3, certain stocks seem to have strong spatial and temporal associations. However, because the type of fishing gear, harvest regulations, and the locations targeted varies considerably among fisheries, samples from a representative mixture of all hatchery- and natural-origin stocks at a fixed location is expected to more accurately estimate abundance and characterize run-timing distributions of stocks. One potentially ideal fixed location for such sampling is Bonneville Dam, but trapping limitations at this location continue to pose a major challenge for sampling. In addition to information on abundance and run-timing, biological data including fork length and age can be examined with estimated stock of origin 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 (i.e., A- and B-run). These life-history categories have been observed to be differentially characterized by run-timing at Bonneville Dam (e.g., B-run typically arrives after Aug. 25th), fork length (e.g., by definition, B-run fish are greater than 78 cm), and ocean age (e.g., most B-run fish tend to spend 2 or more years in saltwater) and all of these types of data have been collected for steelhead in this study.

Project objectives and higher level harvest management questions

Here we analyze fish across the entire run of steelhead, 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 greater than 180 loci per species. GSI of sockeye salmon requires fewer markers than for other species in this study, and a set of 90 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, combination of PIT-tag data, etc.), the general approach to estimating abundance and characterizing run-timing distributions was applied consistently across species. For all three species, we have demonstrated that these genetic baselines are generally accurate for assigning unknown origin fish, but the genetic similarity of some stocks requires large reporting groups comprised of broad geographic areas (i.e., mid-Columbia R. and lower Snake R. for spring Chinook salmon). Since Bonneville Dam is the most downstream dam on the

Columbia River, the fishery mixtures obtained here represent the majority of upriver/interior Columbia River Basin stocks. This ongoing study offers a rare opportunity to monitor populations of multiple species of salmonids from a broad geographic range over several years. This long-term study will allow us to characterize trends in run timing and abundance of steelhead, Chinook and sockeye salmon and provide this data to fisheries managers. However, the genetic stock units ('reporting groups') are not the same units that groups of fish are currently managed for due to levels of genetic differentiation that can be detected among baseline stocks (see results under Objective 2 for details). Thus, fisheries managers continue to explore how to best incorporate genetic monitoring results with more traditional monitoring/tagging programs.

Harvest RM&E: F&W Program Management Questions:

- **i) What are the status and trend of adult productivity of fish populations?**
- **ii) What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?**

Analysis of the 2011 dataset by Hess et al. (2012) was the first year we were able to apply 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 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 (Steele et al. 2013). The ability of PBT to identify a fish's source hatchery has been shown to be equally accurate as traditional tags such as CWTs (Steele et al. 2013), and PBT provides assignments to specific hatcheries rather than larger reporting groups used in GSI methods (see Figure 1). However, these tools can provide the greatest benefit when applied in combination, as GSI has the ability to provide information on natural origin fish throughout the Columbia River basin, while PBT is most effective for hatchery origin fish. The current PBT baseline was recently expanded beyond Snake River hatcheries to include others above Bonneville Dam. However, this effort is ongoing, and while hatcheries continue to be added to our PBT baseline annually, GSI remains a necessary tool for both hatchery and natural origin fish that originate from outside the Snake River basin. This report is the third year in which all major age classes of steelhead (i.e. 1-, 2-, and 3- ocean ages) and Chinook salmon (3-, 4-, and 5-year olds) can be assigned using the PBT baseline of Snake River hatcheries, and the first year in which these can be assigned to some Columbia River hatcheries. This study integrates PBT and GSI results to provide the greatest amount of stock information ever available for hatchery and natural origin steelhead and Chinook salmon passing Bonneville Dam.

Time line for completion of objectives

Objectives will be ongoing and GSI results updated each year for harvest analyses of salmon and steelhead throughout the accords-funding. As new genetic techniques are developed they will be applied to this project and results will be compared between years to determine the extent of improvements.

Methods

Sample Collection

Tissue samples were obtained from adult steelhead (n=898), Chinook (n = 3,268) and sockeye salmon (n=812) adults in 2015 during migration runs at Bonneville Dam. This sampling effort is covered under the 2008-2017 *US vs.* Oregon harvest biological opinion for sampling at Bonneville Dam.

Sampling for Chinook salmon at Bonneville Dam began during statistical week 16 (April 12, 2014). Sampling occurred at the Adult Fish Facility (AFF) located on the northern end of Bonneville Dam. Fish were sampled 4–5 d 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 h 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. Just 2.5% of the total Spring management period adult Chinook salmon count had passed Bonneville by the sampling start date (April 12). While samples were taken from the majority of the total spring Chinook salmon run, some early timed stocks may be slightly under-estimated in the results. Restrictions imposed by USACE and NMFS on sampling at the Bonneville AFF result in sample rates for Chinook, sockeye, and steelhead that are often low. The average sample rate (weeks 16–25) for the adult and jack spring Chinook run in 2015 was 0.68%. The average sample rate for adult and jack summer Chinook was 0.18%, but sampling was limited to statistical weeks 25–27 and 30-31 (Table 1).

Based on numbers of fish collected, samples were pooled into weekly strata for Chinook (Table 1), monthly strata for steelhead (Table 2), or a combination thereof for sockeye salmon (Table 3) spanning the majority of the run-year from April to October. We followed a similar protocol as the Monitoring Methods Protocol "Snake River steelhead and Chinook salmon stock composition estimates (2010-026-00) v1.0".

Molecular markers

Genetic markers are provided in Hess et al. 2013 for steelhead. The GT-seq panel of 298 Chinook salmon are provided in Section 1.

Statistical analyses

Snake River Chinook salmon and steelhead were analyzed for Parentage assignments using SNPPIT software v1.0 (ID: 1341) (Published). The program ONCOR was used to estimate the most likely population-of-origin for the sockeye salmon samples. Individuals were assigned using a 'best estimate' approach Assigning individual samples using Individual Assignment (IA) genetic methods v1.0 (ID: 1334) (Published). We used GSIsim for Mixture modeling to estimate stock proportions v1.0 (ID: 1333) (Published) to estimate stock composition of Bonneville Dam mixture strata for Chinook salmon and steelhead. Additional detail regarding the specific application to Bonneville Dam are published in Hess et al. 2013.

Table 1: Sample numbers by weekly strata for Chinook salmon that were DNA sampled or tallied for abundance at Bonneville Dam in 2015.

		Statistical week	Fish count	Sample (N)					
				Clipped (HOR)		Non-clipped (NOR)		Sample	
				GSI	PBT	GSI	PBT	Total	Rate
Management period	Spring	16	22800	21	16	11	3	51	0.22%
		17	52509	65	65	20	7	157	0.30%
		18	68011	86	108	63	11	268	0.39%
		19	23358	69	94	40	13	216	0.92%
		20	18671	52	79	52	9	192	1.03%
		21	17270	55	102	84	18	259	1.50%
		22	20717	28	44	56	13	141	0.68%
		23	19915	30	64	67	15	176	0.88%
		24	26178	21	28	84	5	138	0.53%
		25sp	7036	8	3	10	0	21	0.30%
	Summer	25su	18336	17	8	33	5	63	0.34%
		26	32041	15	11	29	4	59	0.18%
		27	25893	9	3	4	0	16	0.06%
		28	15370	0	0	0	0	0	0
		29	18750	0	0	0	0	0	0
		30	9204	8	2	9	0	19	0.21%
		31	11042	20	1	26	1	48	0.43%
	Fall	32	7205	7	0	19	0	26	0.36%
		33	11228	0	0	0	0	0	0.00%
		34	23267	10	2	32	0	44	0.19%
		35	67883	23	2	68	1	94	0.14%
		36	162934	47	0	115	3	165	0.10%
		37	266367	60	1	113	3	177	0.07%
		38	189650	60	4	144	2	210	0.11%
		39	147672	41	3	148	2	194	0.13%
		40	71451	50	2	137	3	192	0.27%
		41	44034	43	0	151	3	197	0.45%
		42	21422	17	1	125	2	145	0.68%
		Total	1420214	862	643	1640	123	3268	0.23%

Note: Statistical week 16 is 4/12/15–4/18/15 and 42 is 10/11/15–10/17/15. 'Fish count' is based on tallies of Chinook salmon adults and jacks provided by the Fish Passage Center (http://www.fpc.org) observed by the Corps of Engineers at their fish counting window. 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 (Spring management period), June 16th to July 31st (Summer management period), and August 1st to December 31 (Fall management period) which are used to categorize spring-, summer-, and fall-run Chinook salmon. The number of sampled fish that were assigned via PBT or GSI are shown.

Table 2: Sample numbers by monthly strata for steelhead that were DNA sampled or tallied for abundance at Bonneville Dam in 2015.

Management Period	4-wk Strata	Bonneville Fish Window Count			Sample (N)				Sample	
					Clipped		Non-clipped			
		Total	Clipped	Non-clipped	GSI	PBT	GSI	PBT	Total	rate
Skamania Summer	15_18	860	401	459	6	3	6	0	15	1.7%
	19_22	928	657	271	18	4	5	0	27	2.9%
	23_26	4689	2689	2000	9	3	10	0	22	0.5%
Summer A/B Index	27_30	32350	13159	19191	2	7	16	0	25	0.1%
	31_34	124493	78990	45503	26	128	157	6	317	0.3%
	35_38	69474	50861	18613	27	125	55	21	228	0.3%
	39_42	26035	18676	7359	10	182	41	31	264	1.0%
	Total	258829	165433	93396	98	452	290	58	898	0.35%

Note: Statistical week 15 is 4/5/15–4/11/15 and 42 is 10/11/15–10/17/15. 'Fish count' is based on tallies of adipose-clipped and non-clipped adult steelhead provided by the Fish Passage Center (http://www.fpc.org) observed by the Corps of Engineers at their fish counting window. 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-June 30th and July 1st-October 31st which are used to categorize Skamania and summer steelhead, respectively. The sample numbers were split into two categories according to whether samples had been taken from fish that were adipose clipped or non-clipped, and then further split according to the number of samples that were either assigned via PBT or GSI.

Table 3**:** Sample numbers for genetic stock assignments of sockeye salmon that passed Bonneville Dam in 2015.

Statistical week grouping	Fish Count	Sample (N)				Sample rate (%)
		OKA	RED	WEN	Total	
22_23	2808	23	0	4	27	0.96%
24	21918	69	0	42	111	0.51%
25	84383	167	4	87	258	0.31%
26	181971	134	2	92	228	0.13%
27_30	215119	111	4	52	167	0.08%
31_32	3842	17	0	4	21	0.55%
Total	510041	521	10	281	812	0.16%

Note: Statistical week 22 is 5/24/15–5/30/15 and 32 is 8/2/15–8/8/15. 'Fish count' is based on tallies of sockeye salmon adults provided by the Fish Passage Center (http://www.fpc.org) observed by the Corps of Engineers at their fish counting window. GSI stocks are Okanagan (OKA), Snake River (RED), and Wenatchee (WEN). The number of samples for a given statistical week or grouping was used to calculate sample rate. Very few sockeye salmon were sampled from the Snake River stock (n=10), which greatly limited inferences regarding run-timing and abundance of this stocks.

Results

Estimated relative abundance of Chinook salmon stocks in 2015

There were 13 major (i.e., abundance >1000 fish) hatchery origin Chinook salmon stocks represented in the total estimate relative abundance (N=557,403) of hatchery Chinook salmon passing Bonneville Dam in 2015 (Table 4; Figure 1). These stocks in order of decreasing magnitude were 18_UCOLSF (194,513), 12_HELLSC (104,367), 05_SPCRTU (81,009), 19_SRFALL (63,265), 10_UCOLSP (43,307), 13_SFSALM (19,941), 07_DESCP (17,678), 16_UPSALM (13,450), 06_KLICKR (6,131), 09_YAKIMA (4,696), 17_DESCFA (3,811), 15_MFSALM (1,316), and 11_TUCANO (1,264) (Table 4; Figure 1). These estimates include relative abundance for PBT-assigned fish (adipose clipped and non-clipped) and adipose clipped fish that were assigned via GSI. PBT assignments improved our ability to accurately identify hatchery origin fish and estimate total stock abundance (Table 4). Further, using PBT assignments we can now provide relative abundance (Table 5; Figure 2) and run-timing estimates for particular hatchery broodstocks (Table 5).

Table 4: Stock-specific relative abundance and run-timing distribution of hatchery origin (adipose clipped and non-clipped) Chinook salmon passing Bonneville Dam in 2015.

	Estimated abundance					Run-timing distribution						
			Management Period			Ordinal day						
			Spring	Summer	Fall		1st	3rd	5th	95th	Median	Interquartile
Reporting Group	Mean	95% CI	Jan. 1-Jun. 15	Jun. 16- Aug. 1	Aug. 1-Dec. 1	Median	quartile	quartile	percentile	percentile	date	range (days)
01_YOUNGS	719	49 – 2728	0	0	719	239	237	240	235	240	08/27/15	3
02_WCASSP	229	10 – 1984	0	229	0	209	207	210	206	212	07/28/15	3
03_WCASFA	877	279 – 4961	0	0	877	258	256	259	255	261	09/15/15	3
04_WILLAM	103	82 – 2475	103	0	0	125	123	127	122	128	05/05/15	4
05_SPCRTU	81009	65741 – 95023	0	0	81009	254	250	261	242	272	09/11/15	11
06_KLICKR	6131*	3389 – 8122	5330	801	0	134	117	150	106	168	05/14/15	33
07_DESCSP	17678*	13756 – 21857	15507	2171	0	119	110	142	105	199	04/29/15	32
08_JOHNDR	728	35 – 3040	728	0	0	110	109	112	108	152	04/20/15	3
09_YAKIMA	4696*	3395 – 7149	4696	0	0	136	118	146	106	156	05/16/15	28
10_UCOLSP	43307*	40508 – 50153	43307	0	0	117	110	122	106	143	04/27/15	12
11_TUCANO	1264*	676 – 2934	919	345	0	150	144	156	130	169	05/30/15	12
12_HELLSC	104367*	98846 – 111701	97050	7317	0	118	111	129	106	173	04/28/15	18
13_SFSALM	19941*	15909 – 24150	14845	5096	0	155	146	166	128	180	06/04/15	20
14_CHMBLN	0	0 – 1078	0	0	0	-	-	-	-	-	-	-
15_MFSALM	1316	317 – 4270	1316	0	0	108	106	114	102	138	04/18/15	8
16_UPSALM	13450*	10440 – 18218	8717	4733	0	153	134	171	114	177	06/02/15	37
17_DESCFA	3811	6 – 4167	0	0	3811	253	247	266	242	273	09/10/15	19
18_UCOLSF	194513*	173387 – 216689	6186	32049	156279	249	238	259	169	276	09/06/15	21
19_SRFALL	63265*	41875 – 78639	366	2287	60612	252	246	256	229	269	09/09/15	10
Total	557403*		199070	55027	303306							

*Combined with PBT estimated abundance

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. The run-timing distributions are characterized by ordinal days for the median date, inter-quartile range (days), and 5th and 95th percentile. The distributions were based on the weekly estimated reporting group proportions that were applied to the daily tallies of Chinook salmon 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.

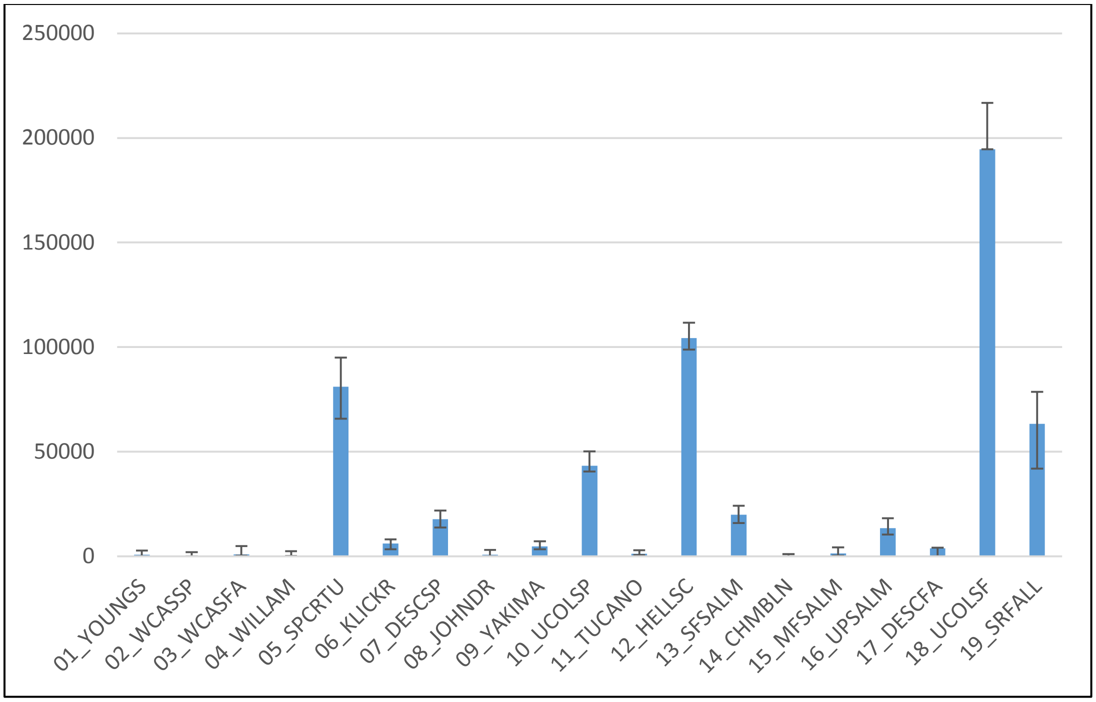


Figure 1: Relative abundance (± 95% CI) of hatchery origin Chinook (adipose clipped and non-clipped) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2015.

We detected PBT assignments for 7% (123/1763) of adipose non-clipped (i.e., presumed wild-origin) Chinook salmon sampled at Bonneville Dam in 2015. There were 14 major (i.e., abundance >1000 fish) Chinook salmon stocks represented in the total estimate relative abundance (N=818,032) of natural origin (i.e., adipose non-clipped fish that did not assign via PBT) Chinook salmon passing Bonneville Dam in 2015 (Table 6; Figure 3). These non-clipped stocks in order of decreasing magnitude were 18_UCOLSF (612,750), 19_SRFALL (114,281), 12_HELLSC (16,992), 05_SPCRTU (15,614), 10_UCOLSP (14,086), 17_DESCFA (8,776), 09_YAKIMA (7,157), 16_UPSALM (6,097), 08_JOHNDR (5,278), 13_SFSALM (5,026), 15_MFSALM (4,941), 03_WCASFA (2,110), 07_DESCP (1,907), and 14_CHMBLN (1,736). 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). Due to restrictions at Bonneville Dam, we were not able to sample Chinook during statistical weeks 28-29 (summer management period), and statistical week 33 (fall management period). Because we could not include that data in our estimates of relative abundance, the estimates that we have provided should be considered sub-total abundances for the summer and fall management periods.

Table 5: Hatchery broodstock-specific relative abundance and run-timing distributions of adipose clipped and non-clipped PBT-assigned Chinook salmon passing Bonneville Dam in 2015. Key to broodstock collection is presented in Section 3 (Table 1).

			Estimated abundance					Run-timing distribution						
					Management Period			Ordinal day						
					Spring	Summer	Fall		1st	3rd	5th	95th	Median	Interquartile
Reporting Group	Broodstock collection	Tagging rate (%)	Mean	95% CI	Jan. 1-Jun. 15	Jun. 16- Aug. 1	Aug. 1-Dec. 1	Median	quartile	quartile	percentile	percentile	date	range (days)
06_KLICKR	OtsKH10S	97.46	267	0 – 786	519	0	0	118	117	119	115	120	04/28/15	2
	OtsKH11S	80.72	4009	1713 – 5537	350	0	0	138	114	152	105	169	05/18/15	38
07_DESCSP	OtsPFF12	89.00	145	0 – 371	6836	0	0	139	137	140	136	142	05/19/15	3
	OtsRB12	92.00	2016	503 – 3631	209	0	0	169	155	175	143	210	06/18/15	20
	OtsWSNFH12	60.00	1841	587 – 3482	539	0	0	140	122	200	116	205	05/20/15	78
09_YAKIMA	OtsYR12	99.00	887	229 – 1355	99	0	0	149	139	156	131	162	05/29/15	17
10_UCOLSP	OtsCAR12	90.00	519	0 – 802	24260	0	0	129	125	144	122	149	05/09/15	19
	OtsMETH12	93.00	599	330 – 1551	2404	0	0	138	126	143	123	148	05/18/15	17
	OtsUMA12_sp	92.00	520	163 – 984	2193	0	0	133	130	136	129	141	05/13/15	6
11_TUCANO	OtsLYON12S	95.65	820	0 – 1314	2075	3103	0	148	144	167	130	170	05/28/15	23
	OtsTUCW11S	98.75	212	155 – 1365	341	0	0	150	132	153	129	156	05/30/15	21
	OtsTUCW12S	95.23	233	0 – 594	124	0	0	153	151	155	150	156	06/02/15	4
12_HELLSC	OtsCLWH10S	99.48	350	0 – 1341	267	0	0	110	109	112	108	114	04/20/15	3
	OtsCLWH11S	95.61	6836	4069 – 9333	3208	801	0	115	109	121	105	135	04/25/15	12
	OtsCLWH12S	95.93	209	0 – 782	2485	0	0	131	130	133	129	135	05/11/15	3
	OtsCTHW11S	91.67	539	71 – 1253	888	2170	0	120	117	136	116	141	04/30/15	19
	OtsDWOR10S	98.48	99	0 – 297	72	0	0	131	130	133	129	135	05/11/15	3
	OtsDWOR11S	98.69	24260	19404 – 27610	2887	951	0	116	110	120	107	138	04/26/15	10
	OtsDWOR12S	99.26	2404	1848 – 3991	0	263	5000	133	125	140	118	147	05/13/15	15
	OtsGRUW11S	98.90	2193	1159 – 4031	474	345	0	118	109	126	104	150	04/28/15	17
	OtsIMNW11S	94.42	5178	2085 – 8128	0	0	2669	172	155	179	144	183	06/21/15	24
	OtsLOOK11S	97.44	2485	1401 – 4857	64	0	0	112	108	117	104	130	04/22/15	9
	OtsLOOK12S	100.00	3058	526 – 6082	11314	2288	0	178	165	181	133	184	06/27/15	16
	OtsLSTW10S	94.34	72	0 – 587	2203	2809	0	139	137	140	136	142	05/19/15	3
	OtsLSTW11S	91.53	3839	1579 – 4860	599	0	0	154	144	163	127	175	06/03/15	19
	OtsNPFH11S	98.88	720	0 – 1574	720	0	0	119	117	139	115	148	04/29/15	22
	OtsNPFH12S	95.85	103	0 – 299	0	0	1770	131	130	133	129	135	05/11/15	3
	OtsPOWP11S	99.18	10342	7553 – 13395	103	0	0	117	109	122	105	134	04/27/15	13
	OtsPOWP12S	98.00	430	99 – 795	0	0	6208	133	130	137	129	141	05/13/15	7
	OtsRAPH11S	98.35	29903	24929 – 33773	2113	548	0	117	110	119	106	136	04/27/15	9
	OtsRAPH12S	95.36	1075	642 – 2181	145	0	0	138	130	144	123	149	05/18/15	14
13_SFSALM	OtsJHNW11S	93.90	341	154 – 1287	10342	0	0	151	127	154	123	156	05/31/15	27
	OtsJHNW12S	90.25	124	0 – 364	430	0	0	153	151	155	150	156	06/02/15	4
	OtsMCCA10S	99.37	64	0 – 6558	0	0	14698	139	137	140	136	142	05/19/15	3
	OtsMCCA11S	99.32	13602	8880 – 13954	29903	0	0	153	145	161	129	174	06/02/15	16
	OtsMCCA12S	100.00	5012	3752 – 10217	1075	0	0	172	158	179	145	183	06/21/15	21
16_UPSALM	OtsPAHH11S	97.21	2661	1437 – 4213	503	1513	0	156	138	165	129	176	06/05/15	27
	OtsSAWT11S	96.59	5201	3232 – 1365	3422	1779	0	138	125	169	112	176	05/18/15	44
	OtsSAWT12S	100.00	4501	2379 – 984	2096	2405	0	166	149	174	137	203	06/15/15	25
18_UCOLSF	OtsPRH12	62.64	14698	9343 – 19645	212	0	0	261	255	268	244	275	09/18/15	13
	OtsWELLS12	99.47	262	0 – 343	233	0	0	149	146	152	143	156	05/29/15	6
19_SRFALL	OtsLYON11S	89.88	5263	3251 – 9470	520	0	0	257	232	264	227	268	09/14/15	32
	OtsLYON12S_1	98.60	2669	306 – 6558	262	0	0	250	245	253	242	278	09/07/15	8
	OtsNPFH11S_1	89.88	1770	0 – 4450	1183	657	0	255	239	258	236	261	09/12/15	19
	OtsNPFH12S_1	99.37	6208	2317 – 10545	887	0	0	251	246	254	238	278	09/08/15	8
	Total		168539		118559	19633	30344							

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.

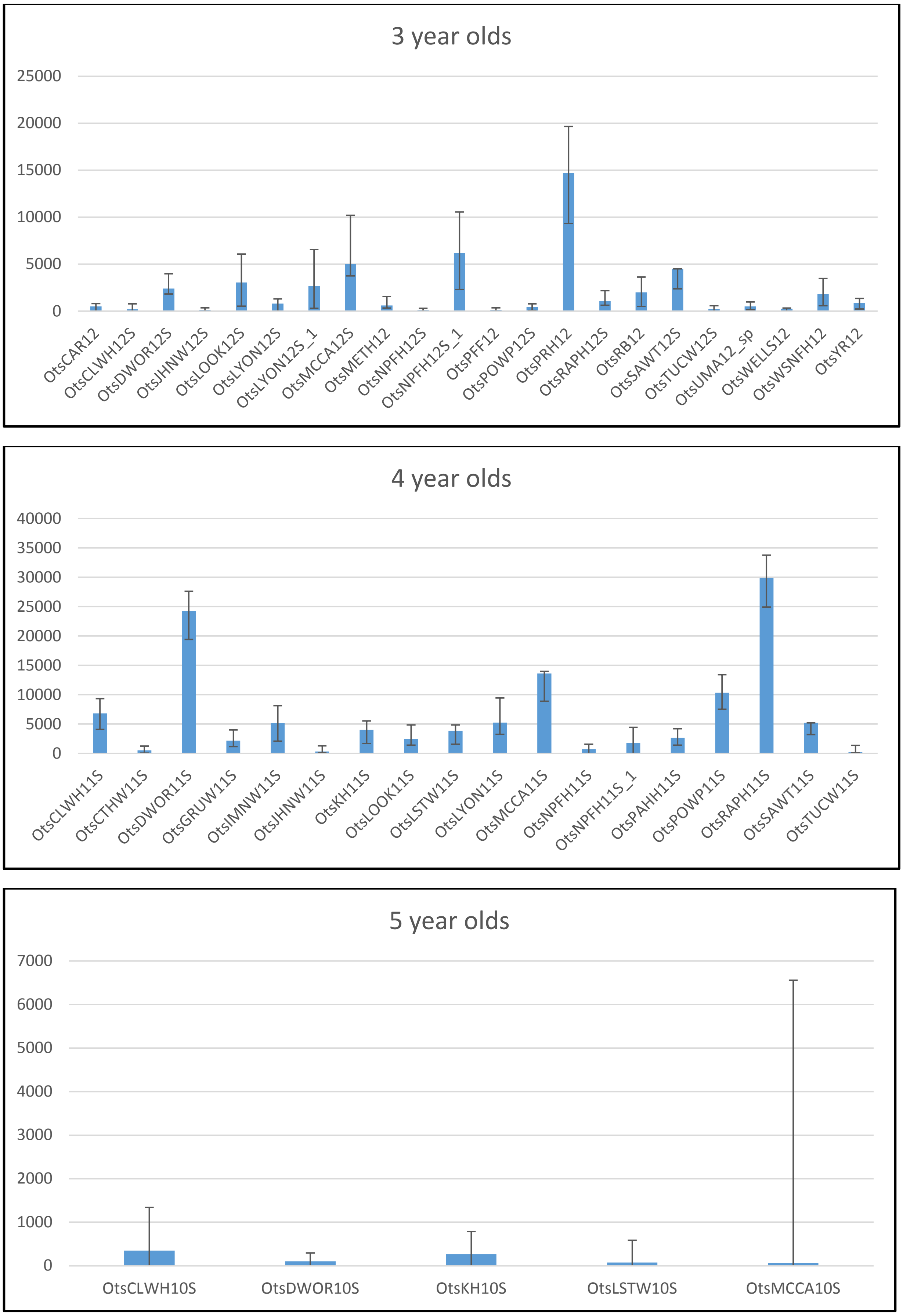
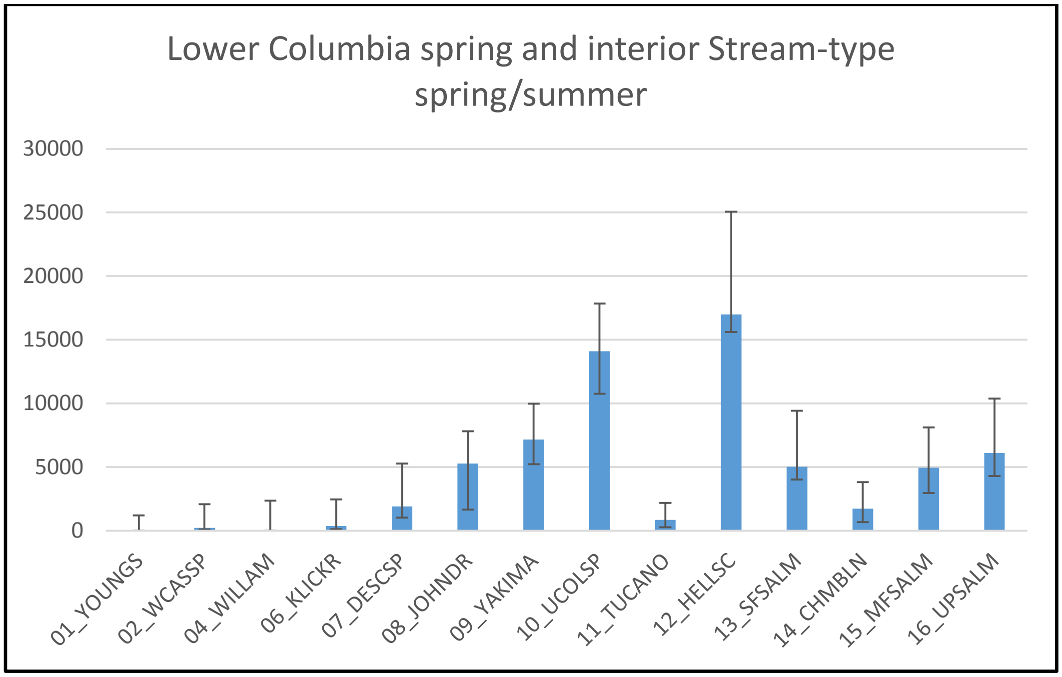


Figure 2: Relative abundance (± 95% CI) of hatchery origin Chinook (adipose clipped and non-clipped) sampled at Bonneville Dam in 2015 that assigned via PBT to 45 hatchery broodstocks of origin by age class. The 2012 age-class (3-year old fish; top panel), 2011 age class (4-year old fish), and 2010 age class (3-year old fish) are shown. Key to broodstock collection is presented in Section 3 (Table 1)



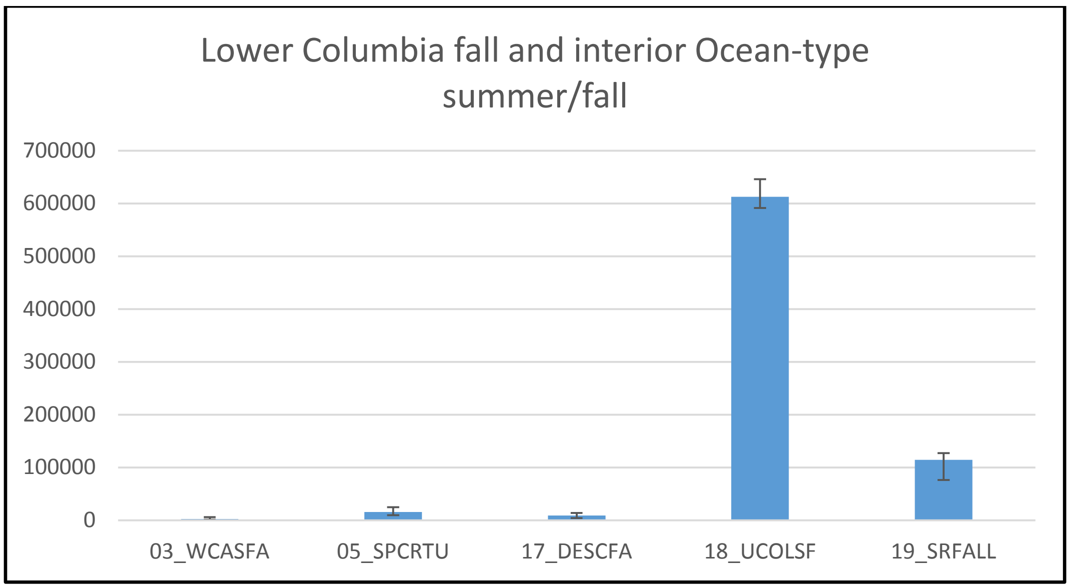


Figure 3: Relative abundance (± 95% CI) of natural origin (adipose non-clipped) Chinook sampled at Bonneville Dam in 2015 assigned to genetic stock of origin. Lower Columbia spring and interior stream-type spring/summer Chinook reporting groups (top panel), and lower Columbia fall and interior Ocean-type Chinook reporting groups (bottom panel) are shown.

Table 6: Relative abundance and run-timing distributions of natural origin (adipose non-clipped) Chinook salmon stocks passing Bonneville Dam in 2015.

	Estimated abundance					Run-timing distribution						
			Management Period			Ordinal day						
			Spring	Summer	Fall		1st	3rd	5th	95th	Median	Interquartile
Reporting Group	Mean	95% CI	Jan. 1-Jun. 15	Jun. 16- Aug. 1	Aug. 1-Dec. 1	Median	quartile	quartile	percentile	percentile	date	range (days)
01_YOUNGS	0	0 – 1204	0	0	0	-	-	-	-	-	-	-
02_WCASSP	230	98 – 2083	230	0	0	136	132	139	129	142	05/16/15	7
03_WCASFA	2110	1049 – 6074	0	0	2110	240	238	277	235	281	08/28/15	39
04_WILLAM	0	15 – 2355	0	0	0	-	-	-	-	-	-	-
05_SPCRTU	15614	9379 – 24579	0	0	15614	249	244	254	239	268	09/06/15	10
06_KLICKR	379	118 – 2459	379	0	0	157	150	160	137	163	06/06/15	10
07_DESCSP	1907	1023 – 5281	1907	0	0	117	109	128	106	146	04/27/15	19
08_JOHNDR	5278	1651 – 7822	3659	1618	0	126	117	178	109	183	05/06/15	61
09_YAKIMA	7157	5215 – 9981	7157	0	0	119	117	135	109	159	04/29/15	18
10_UCOLSP	14086	10760 – 17846	14086	0	0	118	111	129	104	152	04/28/15	18
11_TUCANO	853	262 – 2183	853	0	0	119	117	127	116	138	04/29/15	10
12_HELLSC	16992	15604 – 25070	15862	1131	0	128	112	150	105	166	05/08/15	38
13_SFSALM	5026	4019 – 9411	3986	1040	0	159	151	163	140	175	06/08/15	12
14_CHMBLN	1736	661 – 3805	1101	635	0	155	119	172	116	176	06/04/15	53
15_MFSALM	4941	2965 – 8119	4097	844	0	137	122	149	116	173	05/17/15	27
16_UPSALM	6097	4298 – 10383	6097	0	0	151	135	158	122	162	05/31/15	23
17_DESCFA	8776	3940 – 13716	0	162	8613	258	255	263	230	285	09/15/15	8
18_UCOLSF	612570	591152 – 645567	17921	35779	558869	254	245	263	173	279	09/11/15	18
19_SRFALL	114281	76055 – 127156	60	848	113373	249	242	257	231	272	09/06/15	15
Total	818032											

Run-timing of Chinook salmon stocks in 2015

We obtained sufficient sample sizes to characterize the run-timing distributions of 18 hatchery origin (adipose clipped and non-clipped) Chinook salmon stocks (Table 4; Figure 4). The run timing for five hatchery origin spring Chinook stocks (i.e., 04_WILLAM, 08_JOHNDR, 09_YAKIMA, 10_UCOLSP, and 15_MFSALM) were found to terminate within the spring management period (i.e., the 95th percentile of their run distribution occurred on or before June 15th; ordinal day 166). The run timing for six hatchery origin spring Chinook stocks (i.e., 02_WCASSP, 06_KLICKR, 07_DESCP, 11_TUCANO, 12_HELLSC, 13_SFSALM), and one hatchery spring/summer Chinook stock (i.e., 16_UPSALM) were found to terminate within the summer management period (i.e., the 95th percentile of their run distribution occurred on or before July 31st; ordinal day 212). The run-timing for the remaining hatchery summer/fall Chinook (i.e., 18_UCOLSF) and hatchery fall Chinook stocks (i.e., 01_YOUNGS, 03_WCASFA, 05_SPCRTU, 17_DESCFA, and 19_SRFALL) all had median dates on or after 8/27/15 (Table 4; Figure 4).

We obtained sufficient sample sizes to characterize the run-timing distributions of 17 natural origin (adipose non-clipped) Chinook salmon stocks (Table 6; Figure 5). The run timing for seven natural origin spring Chinook stocks (i.e., 02_WCASSP, 06_KLICKR, 07_DESCP, 09_YAKIMA, 10_UCOLSP, 11_TUCANO, 12_HELLSC) and one spring/summer Chinook stock (i.e., 16_UPSALM) were found to terminate within the spring management period (i.e., the 95th percentile of their run distribution occurred on or before June 15th; ordinal day 166). The run timing for four natural origin spring Chinook stocks (i.e., 08_JOHNDR, 13_SFSALM, 14_CHMBLN, and 15_MFSALM) were found to terminate within the summer management period (i.e., the 95th percentile of their run distribution occurred on or before July 31st; ordinal day 212). The run-timing for the remaining natural origin summer/fall Chinook (i.e., 18_UCOLSF) and natural origin fall Chinook salmon stocks (i.e., 03_WCASFA, 05_SPCRTU, 17_DESCFA, and 19_SRFALL) all had median dates on or after 8/28/15 (Table 6; Figure 5).

Using the PBT-assigned Chinook salmon, we also characterized the run timing distributions for hatchery broodstocks in our PBT baseline (Table 5; Figure 6). Among relatively abundant broodstocks (≥1000 fish), the run-timing of eight broodstock sources (i.e., OtsLOOK11S, OtsPOWP11S, OtsCLWH11S, OtsRAPH11S, OtsRAPH12S, OtsDWOR11S, OtsDWOR12S, OtsGRUW11S) were found to terminate within the spring management (i.e., the 95th percentile of their run distribution occurred on or before June 15th; ordinal day 166). The run-timing for 11 broodstock sources (i.e., OtsKH11S, OtsMCCA11S, OtsMCCA12S, OtsLSTW11S, OtsPAHH11S, OtsSAWT11S, OtsSAWT12S, OtsIMNW11S, OtsLOOK12S, OtsWSNFH12s, and OtsRB12) were found to terminate within the summer management period (i.e., the 95th percentile of their run distribution occurred on or before July 31st; ordinal day 212). The run-timing for the five remaining broodstock sources (i.e., OtsPRH12, OtsLYONS11S, OtsLYON12S_1, OtsNPFH11S_1, and OtsNPFH12S_1) all had median dates on or after 9/7/15 (Table 5; Figure 6).

Parentage based tagging analyses of Chinook salmon in 2015

We were able to assign 771 adult and jack Chinook salmon sampled at Bonneville Dam in 2015 to 45 different hatchery broodstock sources from 2010-2012. The majority (i.e., 691; 89%) assigned to 34 Snake River hatchery broodstock sources, while the remaining 80 fish assigned to 11 Columbia River hatchery broodstock sources (Table 7). The Snake River and Columbia River hatchery broodstock sources were aggregated into appropriate GSI reporting groups in order to integrate the relative abundance estimates from this analysis with relative abundance from GSI analyses. Tagging rates varied across hatchery brood stock sources from 60% to 100%, with six hatchery broodstock sources having tagging rates <90% (i.e., OtsWSNFH12, OtsPRH12, OtsKH11, OtsPFF12, OtsLYON11S, OtsNPFH11S_1) (Table 7).

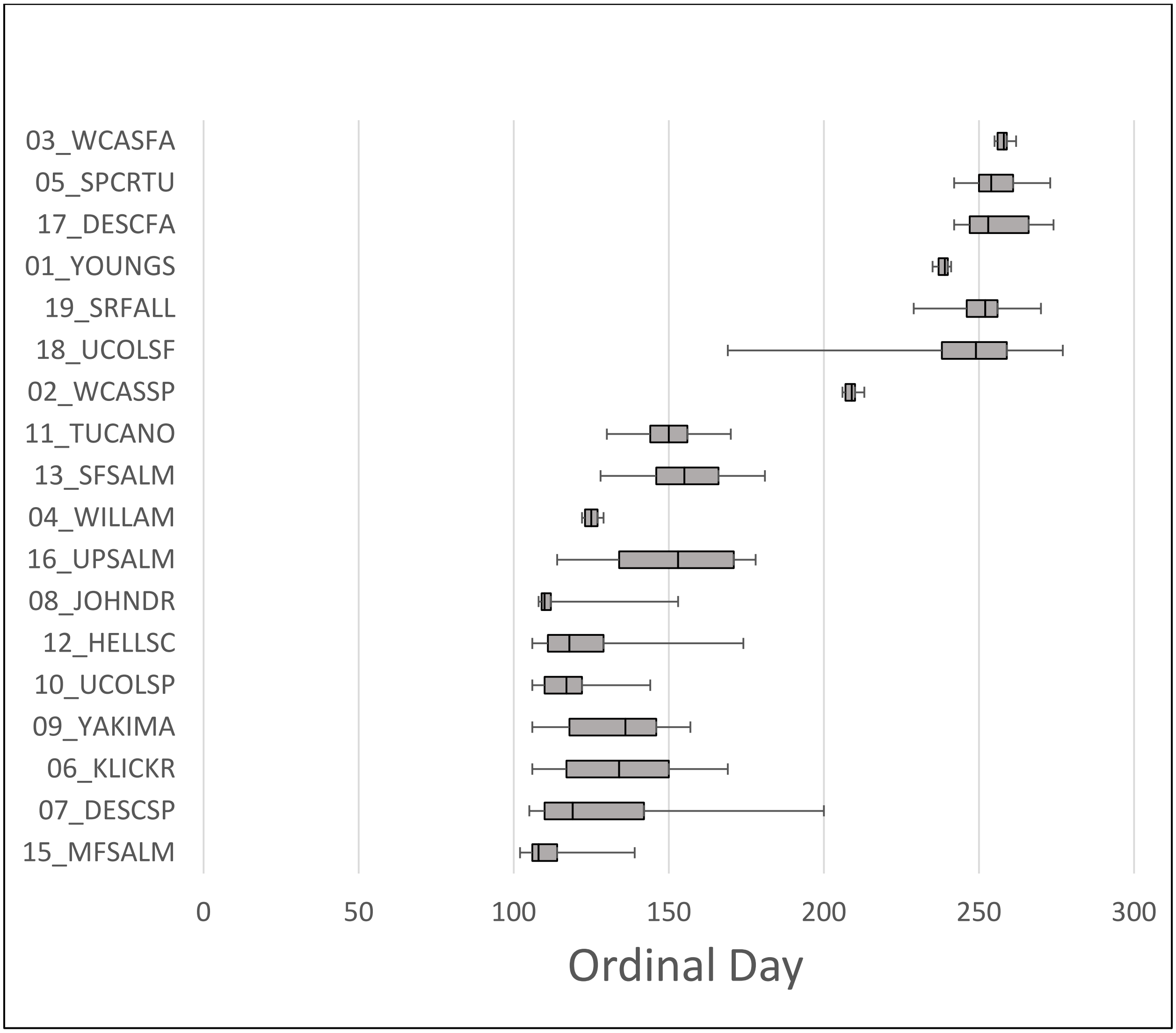


Figure 4: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for hatchery origin Chinook (adipose clipped and non-clipped) that were sampled at Bonneville Dam in 2015 and assigned to stock of origin.

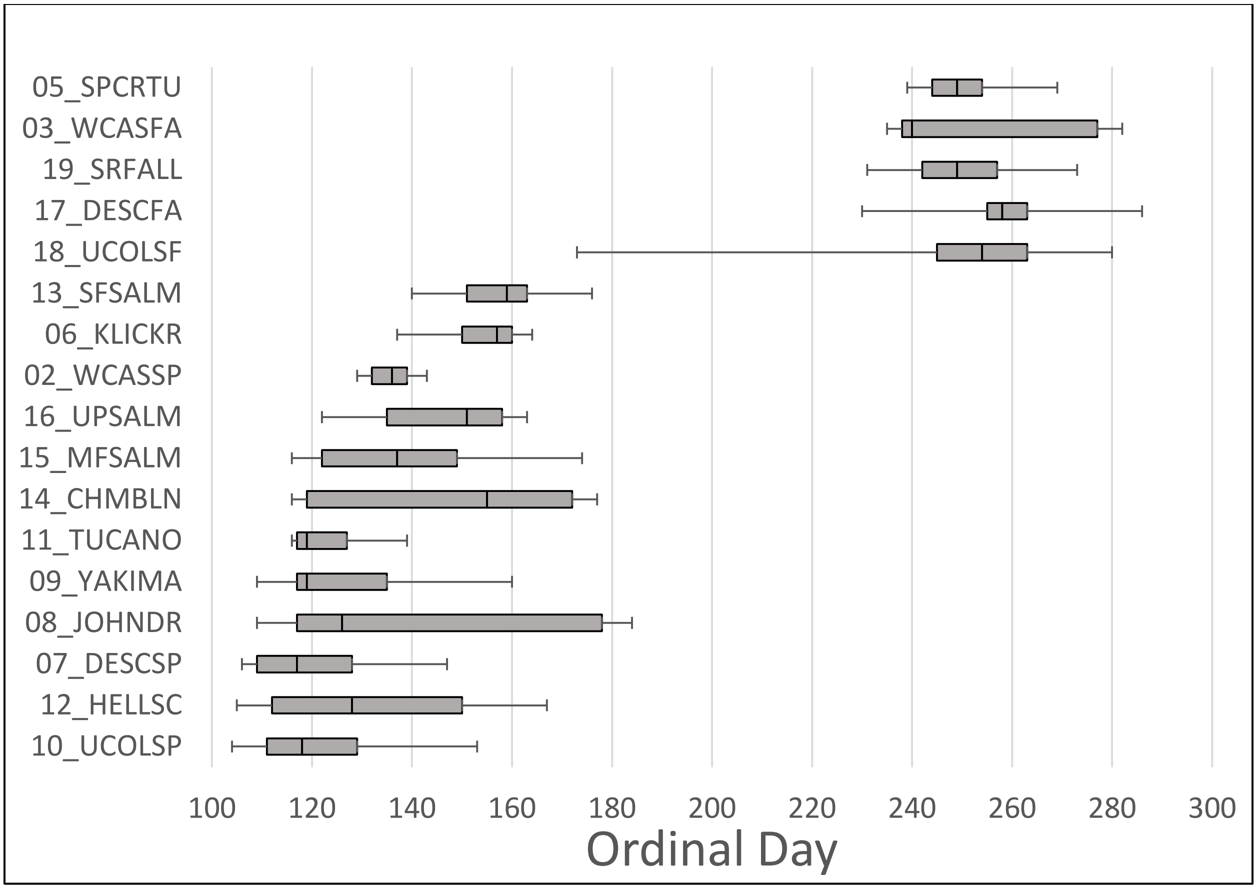


Figure 5: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for natural origin Chinook (adipose non-clipped) that were sampled at Bonneville Dam in 2015 and assigned to stock of origin.

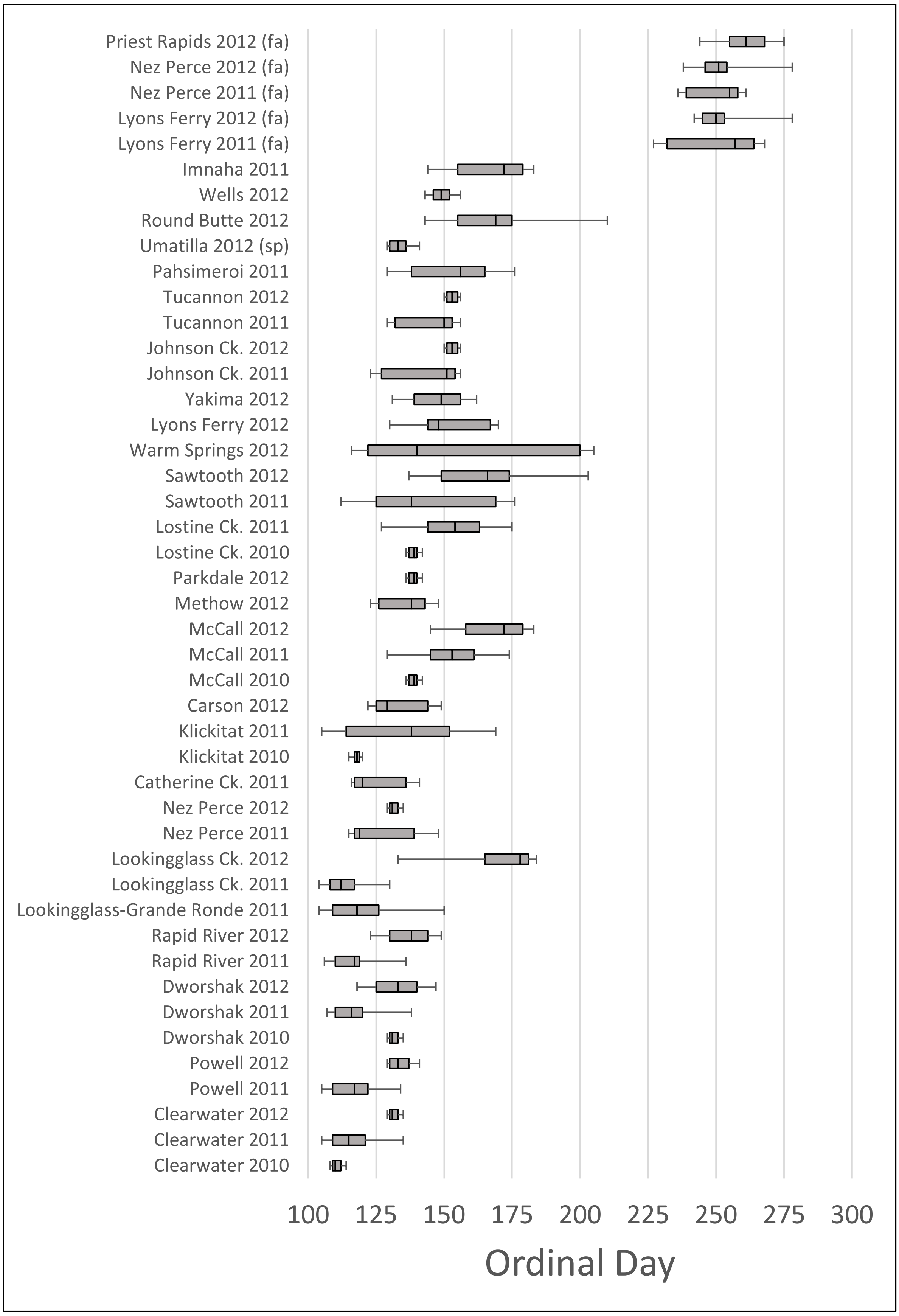


Figure 6: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for hatchery origin Chinook (adipose clipped and non-clipped) assigned to PBT broodstock of origin that were sampled at Bonneville Dam in 2015. Hatcheries and their broodyears are ordered from earliest to latest run-timing, with fall Chinook hatchery stocks at the top.

Table 7: Summary information about the PBT Chinook salmon hatchery broodstock sources and number of assignments. Key to broodstock collection is presented in Section 3 (Table 1).

Broodstock collection	Basin	Reporting Group	Tagging rate (%)	Age	Adipose fin		Total
					Clipped	Non-clipped	
OtsKH10S	Columbia	06_KLICKR	97.46	5	1		1
OtsKH11S	Columbia	06_KLICKR	80.72	4	20	1	21
OtsPFF12	Columbia	07_DESCSP	89.00	3	2		2
OtsRB12	Columbia	07_DESCSP	92.00	3	8		8
OtsWSNFH12	Columbia	07_DESCSP	60.00	3	7		7
OtsYR12	Columbia	09_YAKIMA	99.00	3	7	1	8
OtsCAR12	Columbia	10_UCOLSP	90.00	3	4		4
OtsMETH12	Columbia	10_UCOLSP	93.00	3	1	5	6
OtsUMA12_sp	Columbia	10_UCOLSP	92.00	3	6		6
OtsLYON12S	Snake	11_TUCANO	95.65	3		5	5
OtsTUCW11S	Snake	11_TUCANO	98.75	4		2	2
OtsTUCW12S	Snake	11_TUCANO	95.23	3		2	2
OtsCLWH10S	Snake	12_HELLSC	99.48	5	1		1
OtsCLWH11S	Snake	12_HELLSC	95.61	4	31		31
OtsCLWH12S	Snake	12_HELLSC	95.93	3	2		2
OtsCTHW11S	Snake	12_HELLSC	91.67	4	4		4
OtsDWOR10S	Snake	12_HELLSC	98.48	5	1		1
OtsDWOR11S	Snake	12_HELLSC	98.69	4	108	8	116
OtsDWOR12S	Snake	12_HELLSC	99.26	3	24	1	25
OtsGRUW11S	Snake	12_HELLSC	98.90	4	5	8	13
OtsIMNW11S	Snake	12_HELLSC	94.42	4	20		20
OtsLOOK11S	Snake	12_HELLSC	97.44	4	9		9
OtsLOOK12S	Snake	12_HELLSC	100.00	3	9		9
OtsLSTW10S	Snake	12_HELLSC	94.34	5	1		1
OtsLSTW11S	Snake	12_HELLSC	91.53	4	24		24
OtsNPFH11S	Snake	12_HELLSC	98.88	4		4	4
OtsNPFH12S	Snake	12_HELLSC	95.85	3		1	1
OtsPOWP11S	Snake	12_HELLSC	99.18	4	36	14	50
OtsPOWP12S	Snake	12_HELLSC	98.00	3	3	2	5
OtsRAPH11S	Snake	12_HELLSC	98.35	4	132	6	138
OtsRAPH12S	Snake	12_HELLSC	95.36	3	11		11
OtsJHNW11S	Snake	13_SFSALM	93.90	4		4	4
OtsJHNW12S	Snake	13_SFSALM	90.25	3		1	1
OtsMCCA10S	Snake	13_SFSALM	99.37	5	1		1
OtsMCCA11S	Snake	13_SFSALM	99.32	4	72	28	100
OtsMCCA12S	Snake	13_SFSALM	100.00	3	11	7	18
OtsPAHH11S	Snake	16_UPSALM	97.21	4	19		19
OtsSAWT11S	Snake	16_UPSALM	96.59	4	25	6	31
OtsSAWT12S	Snake	16_UPSALM	100.00	3	22	1	23
OtsPRH12	Columbia	18_UCOLSF	62.64	3	9	6	15
OtsWELLS12	Columbia	18_UCOLSF	99.47	3	1	1	2
OtsLYON11S	Snake	19_SRFALL	89.88	4	3	5	8
OtsLYON12S_1	Snake	19_SRFALL	98.60	3	1	2	3
OtsNPFH11S_1	Snake	19_SRFALL	89.88	4	1	1	2
OtsNPFH12S_1	Snake	19_SRFALL	99.37	3	1	6	7

Estimated relative abundance of steelhead stocks in 2015

There were five major stocks (abundance >1000) represented in the total estimated relative abundance (N=166,201) of hatchery origin steelhead passing Bonneville Dam in 2015 (Table 8). These stocks in order of decreasing magnitude were 07_MGILCS (63,072), 14_UPSALM (54,210), 10_SFCLWR (28,871), 09_UPPCOL (12,746), and 03_SKAMAN (7,010) (Table 8; Figure 7). These estimates include relative abundance estimated from PBT-assigned fish that were mostly adipose clipped; however, a portion of the PBT-assigned fish were found to be non-clipped. Therefore, PBT assignments improved our ability to accurately identify hatchery-origin steelhead and estimate total stock relative abundance. Further, using PBT assignments we can now provide relative abundance (Table 9; Figure 8) and run-timing estimates for particular hatchery broodstocks (Table 9). There were 15 major hatchery broodstock sources (abundance >1000) represented in the total estimated relative abundance of hatchery origin steelhead passing Bonneville Dam in 2015 (Table 9). These stocks in order of decreasing magnitude were OmyDWOR12S (21,400), OmyPAHH13S (19,310), OmySAWT13S (16,612), OmyWALL13S (14,944), OmyWEL13 (10,701), OmyLYON13S (10,516), OmyWALL12S (9,665), OmyLYON12S (9,451), OmyRB13 (6,603), OmyDWOR13S (6,573), OmyPAHH12S (6,437), OmyLSCR13S (6,227), OmyOXBO13S (5,878), OmyOXBO12S (2,877), and OmySAWT12S (2,107).

We detected PBT assignments for 16.7% (58/348) of adipose non-clipped (i.e., presumed wild-origin) steelhead sampled at Bonneville Dam in 2015. There were 11 major stocks (abundance >1000) represented in the total estimated relative abundance (N=139,120) of natural origin (i.e., adipose non-clipped fish that did not assign via PBT) steelhead passing Bonneville Dam in 2015 (Table 10; Figure 9). These stocks in order of decreasing magnitude were 07_MGILCS (89,315), 09_UPPCOL (12,837), 13_MFSALM (6,732), 14_UPSALM (6,582), 10_SFCLWR (5,609), 11_UPCLWR (5,583), 08_YAKIMA (5,349), 06_KLICKR (2,480), 12_SFSALM (1,559), 02_LOWCOL (1,324), and 03_SKAMAN (1,087).

Table 8: Stock-specific relative abundance and run-timing distribution of hatchery origin (adipose clipped and non-clipped) steelhead passing Bonneville Dam in 2015.

	Estimated abundance				Run-timing distribution						
			Management Period		Ordinal day						
			Skamania	Summer		1st	3rd	5th	95th	Median	Interquartile
Reporting Group	Mean	95% CI	Apr. 1-Jun. 30	July 1- Oct. 31	Median	quartile	quartile	percentile	percentile	date	range (days)
01_WCOAST	0	0 – 96	0	0	-	-	-	-	-	-	-
02_LOWCOL	291	18 – 1405	93	199	242	117	251	100	259	08/30/15	134
03_SKAMAN	7010	3843 – 7323	2022	4988	196	176	205	132	224	07/15/15	29
04_WILLAM	0	0 – 779	0	0	-	-	-	-	-	-	-
05_BWSALM	0	0 – 152	0	0	-	-	-	-	-	-	-
06_KLICKR	0	0 – 1111	0	0	-	-	-	-	-	-	-
07_MGILCS	63072*	54490 – 74322	792	62281	222	211	233	193	257	08/10/15	22
08_YAKIMA	0	1 – 1202	0	0	-	-	-	-	-	-	-
09_UPPCOL	12746*	7011 – 19277	0	12746	223	212	239	196	259	08/11/15	27
10_SFCLWR	28871*	24370 – 33922	0	28871	260	245	270	231	284	09/17/15	25
11_UPCLWR	0	0 – 595	0	0	-	-	-	-	-	-	-
12_SFSALM	0	0 – 380	0	0	-	-	-	-	-	-	-
13_MFSALM	0	0 – 817	0	0	-	-	-	-	-	-	-
14_UPSALM	54210*	46010 – 62289	46	54164	232	219	250	207	272	08/20/15	31
Total	166201										

*Combined with PBT estimated abundance

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 steelhead that were observed passing Bonneville Dam at the fish counting window. The run-timing distributions are characterized by ordinal days for the median date, inter-quartile range (days), and 5th and 95th percentile. The distributions were based on the weekly estimated reporting group proportions that were applied to the daily tallies of steelhead 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.

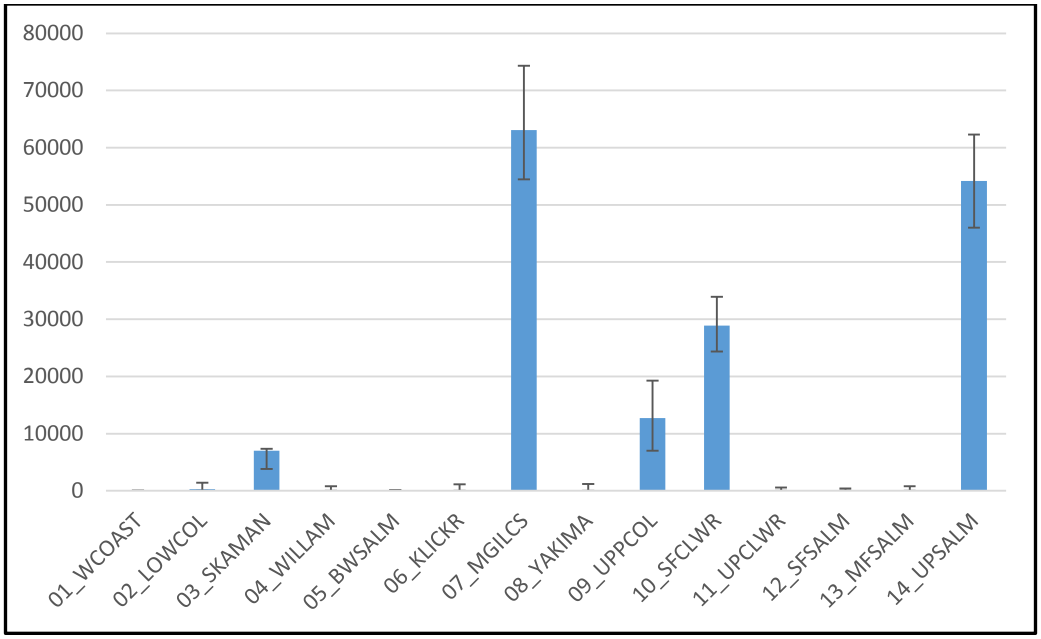


Figure 7: Relative abundance (± 95% CI) of hatchery origin steelhead (adipose clipped and non-clipped) assigned to genetic stock of origin that were sampled at Bonneville Dam in 2015.

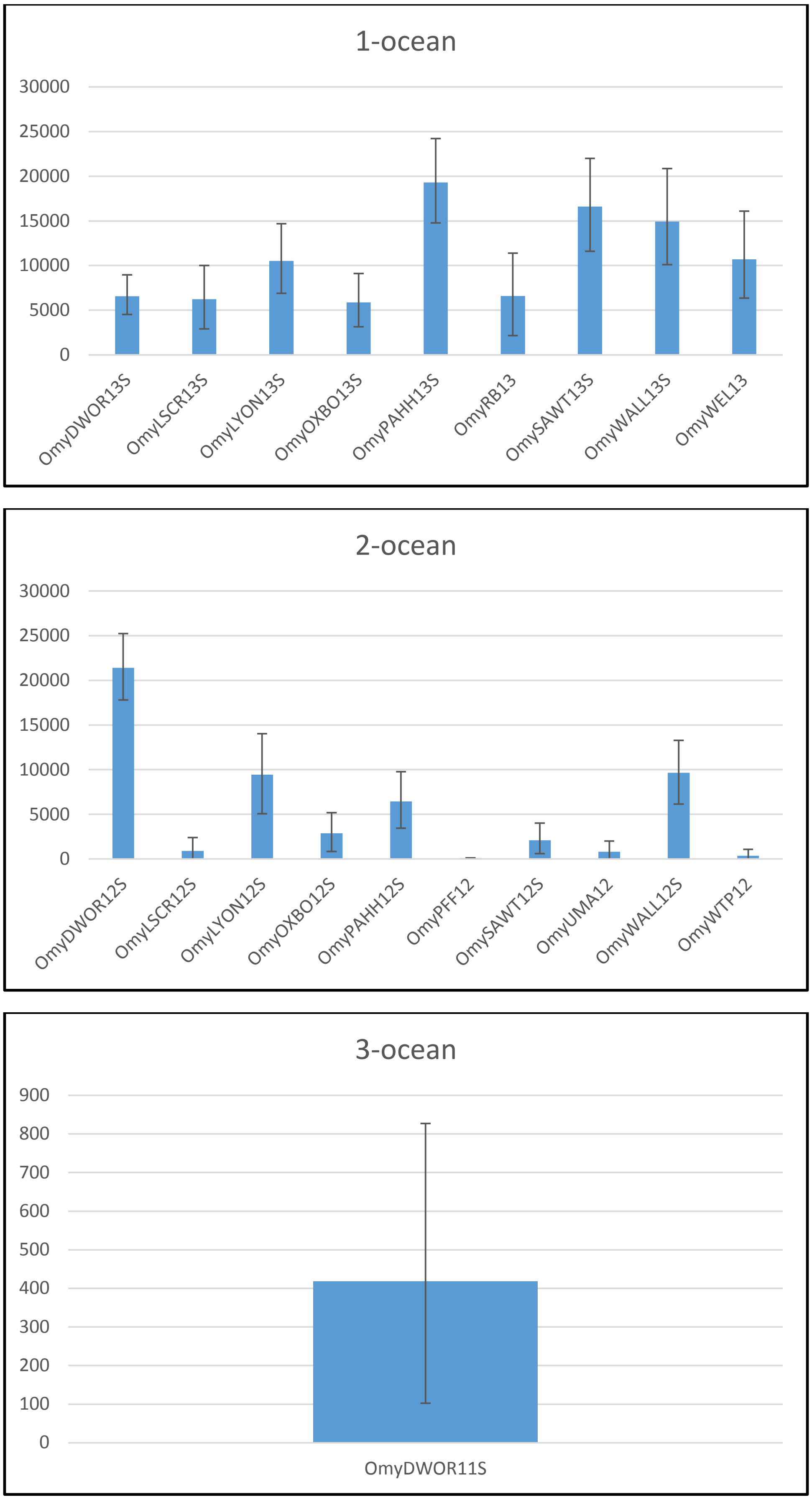


Figure 8: Relative abundance (± 95% CI) of hatchery origin steelhead (adipose clipped and non-clipped) sampled at Bonneville Dam in 2015 that assigned via PBT to 20 hatchery broodstocks of origin. The 2013 age-class (1-ocean fish; top panel), 2012 age class (2-ocean fish), and 2010 age class (3-ocean fish) are shown. Key to broodstock collection is presented in Section 3 (Table 1).

Table 9: Hatchery broodstock-specific relative abundance and run-timing distributions of adipose clipped and non-clipped PBT-assigned steelhead passing Bonneville Dam in 2015. Key to broodstock collection is presented in Section 3 (Table 3).

			Estimated abundance				Run-timing distribution						
					Management Period		Ordinal day						
					Skamania	Summer		1st	3rd	5th	95th	Median	Interquartile
Reporting Group	Broodstock collection	Tagging rate	Mean	95% CI	Apr. 1-Jun. 30	July 1- Oct. 31	Median	quartile	quartile	percentile	percentile	date	range (days)
02_LOWCOL	OmyPFF12	95.5%	93	0 – 139	93	0	110	104	117	96	121	20-Apr	13
07_MGILCS	OmyLSCR12S	95.6%	902	0 – 21403	0	902	227	218	244	209	257	15-Aug	26
	OmyLSCR13S	72.8%	6227	2901 – 10010	0	6227	223	214	232	208	256	11-Aug	18
	OmyLYON12S	100.0%	9451	5072 – 14016	935	8517	213	198	233	169	255	1-Aug	35
	OmyLYON13S	100.0%	10516	6896 – 14701	0	10516	225	216	243	208	260	13-Aug	27
	OmyRB13	84.2%	6603	2169 – 11389	167	6436	205	197	221	184	239	24-Jul	24
	OmyUMA12	100.0%	805	0 – 2019	0	805	227	218	244	209	257	15-Aug	26
	OmyWALL12S	99.2%	9665	6150 – 13285	0	9665	228	218	246	209	262	16-Aug	28
	OmyWALL13S	91.0%	14944	10113 – 20853	83	14861	221	211	228	197	257	9-Aug	17
09_UPPCOL	OmyWEL13	98.7%	10701	6367 – 16092	74	10627	221	211	231	195	259	9-Aug	20
	OmyWTP12	88.1%	354	0 – 1097	0	354	247	242	253	236	259	4-Sep	11
10_SFCLWR	OmyDWOR11S	95.3%	419	103 – 828	0	419	271	265	278	262	286	28-Sep	13
	OmyDWOR12S	96.3%	21400	17798 – 25225	0	21400	258	244	269	224	283	15-Sep	25
	OmyDWOR13S	96.5%	6573	4519 – 8959	0	6573	262	248	272	238	284	19-Sep	24
14_UPSALM	OmyOXBO12S	98.4%	2877	835 – 5176	0	2877	231	219	246	209	257	19-Aug	27
	OmyOXBO13S	97.1%	5878	3142 – 9115	0	5878	226	217	246	209	271	14-Aug	29
	OmyPAHH12S	98.1%	6437	3440 – 9775	46	6391	224	215	239	208	259	12-Aug	24
	OmyPAHH13S	99.5%	19310	14780 – 24212	0	19310	241	222	254	210	277	29-Aug	32
	OmySAWT12S	100.0%	2107	596 – 4032	0	2107	238	221	251	210	270	26-Aug	30
	OmySAWT13S	99.7%	16612	11612 – 22002	73	16539	232	216	249	199	270	20-Aug	33
Total			151875		1471	150404							

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. 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.

Table 10: Relative abundance and run-timing distributions of natural origin (adipose non-clipped) steelhead stocks passing Bonneville Dam in 2015.

	Estimated abundance				Run-timing distribution						
			Management Period		Ordinal day						
Reporting Group	Mean	95% CI	Skamania Apr. 1-Jun. 30	Summer July 1- Oct. 31	Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Interquartile range (days)
01_WCOAST	0	0 – 242	0	0	-	-	-	-	-	-	-
02_LOWCOL	1324	210 – 2668	395	930	213	168	223	109	231	1-Aug	55
03_SKAMAN	1087	447 – 3169	843	244	170	160	177	137	279	19-Jun	17
04_WILLAM	66	0 – 749	66	0	110	104	117	96	121	20-Apr	13
05_BWSALM	606	4 – 2515	0	606	222	213	228	208	275	10-Aug	15
06_KLICKR	2480	979 – 4812	970	1510	221	169	253	138	278	9-Aug	84
07_MGILCS	89315	81666 – 102444	1271	88044	222	211	233	192	261	10-Aug	22
08_YAKIMA	5349	1228 – 7600	0	5349	220	211	228	195	251	8-Aug	17
09_UPPCOL	12837	7184 – 23410	201	12636	227	218	244	208	257	15-Aug	26
10_SFCLWR	5609	3023 – 9172	0	5609	250	243	258	236	277	7-Sep	15
11_UPCLWR	5583	3328 – 8996	0	5583	260	244	270	222	284	17-Sep	26
12_SFSALM	1559	388 – 2886	0	1559	245	224	263	211	281	2-Sep	39
13_MFSALM	6723	3295 – 10554	0	6723	225	216	244	209	264	13-Aug	28
14_UPSALM	6582	5 – 8836	0	6582	231	214	250	196	272	19-Aug	36
Total	139120		3747	135373							

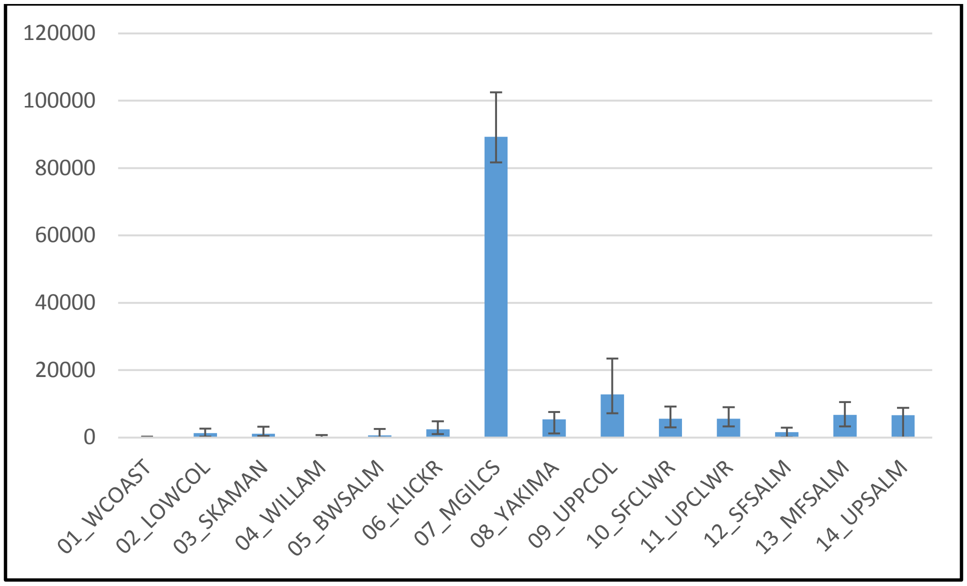


Figure 9: Relative abundance (± 95% CI) of natural origin (adipose non-clipped) steelhead sampled at Bonneville Dam in 2015 assigned to genetic stock of origin.

Run-timing of steelhead stocks in 2015

We characterized the run-timing distributions of the six major hatchery steelhead stocks (Table 8; Figure 10) and 13 major natural origin steelhead stocks in 2015 (Table 10; Figure 11). Very few stocks of winter-run steelhead exist above Bonneville Dam and our sampling program at Bonneville AFF does not trap or collect fish between December and March when winter-run steelhead would be most likely to occur. Thus, winter-run stocks are expected to be rare in our samples from Bonneville but could occur from hatchery-origin fish from PFF in the Hood River (winter-run broodstock) or natural origin fish from sub-basins nearest upstream such as Klickitat, Hood, and Fifteenmile rivers. For hatchery origin steelhead, the 02_LOWCOL stock was characterized by an early run timing, but prolonged run duration (interquartile range dates: 4/27/15-9/8/15; ordinal days: 117-251). The 03_SKAMAN hatchery stock has previously been characterized as part of an early run-timing category, and in 2015 had a median run-timing date of 7/15/2015 (ordinal day 196). An intermediate run-timing category has also been described, and includes the following three major steelhead stocks (ordered by median date): 07_MGILCS (8/10/15; ordinal day 222), 09_UPPCOL (8/11/15; ordinal day 223), and 14_UPSALM (8/20/15; ordinal day 232). Finally, a late run-timing category consists of the 10_SFCLWR stock (9/17/15; ordinal day 260), and is typically thought to be characteristic of B-run steelhead that return after August 25th at Bonneville Dam (Table 8; Figure 10).

The natural origin steelhead stocks (Table 10; Figure 11) generally fit the same run-timing categories as characterized for hatchery origin steelhead. The 13 major natural origin stocks ordered by median run-timing date were: 04_WILLAM (4/20/15; ordinal day 110), 03_SKAMAN (6/19/15; ordinal day 170), 02_LOWCOL (8/1/15; ordinal day 213), 08_YAKIMA (8/8/15; ordinal day 220), 06_KLICKR (8/9/15; ordinal day 221), 05_BWSALM (8/10/15; ordinal day 222), 07_MGILCS (8/10/15; ordinal day 222), 13_MFSALM (8/13/15; ordinal day 225), 09_UPPCOL (8/15/15; ordinal day 227) , 14_UPSALM (8/19/15; ordinal day

231), 12_SFSALM (9/2/15; ordinal day 245), 10_SFCLWR (9/7/15; ordinal day 250), and 11_UPCLWR (9/17/15; ordinal day 260) (Table 10; Figure 11).

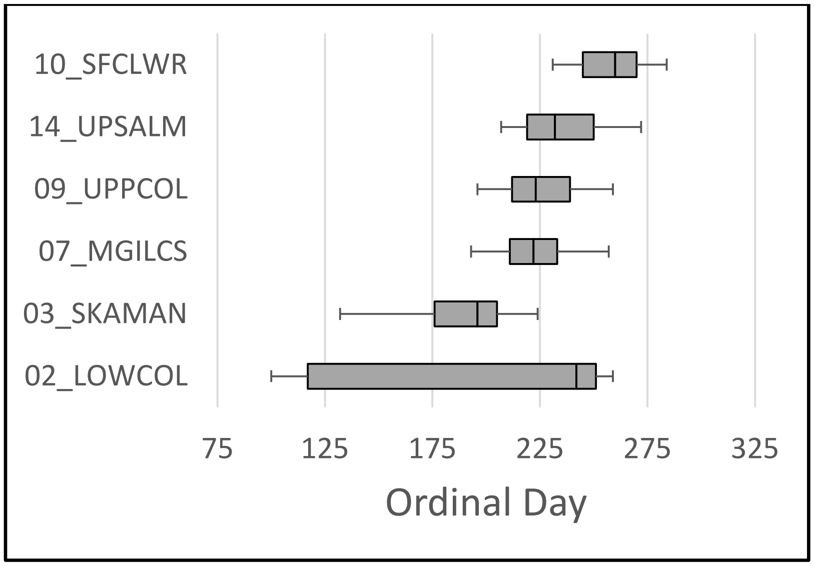


Figure 10: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for hatchery origin steelhead (adipose clipped and non-clipped) that were sampled at Bonneville Dam in 2015 and assigned to stock of origin.

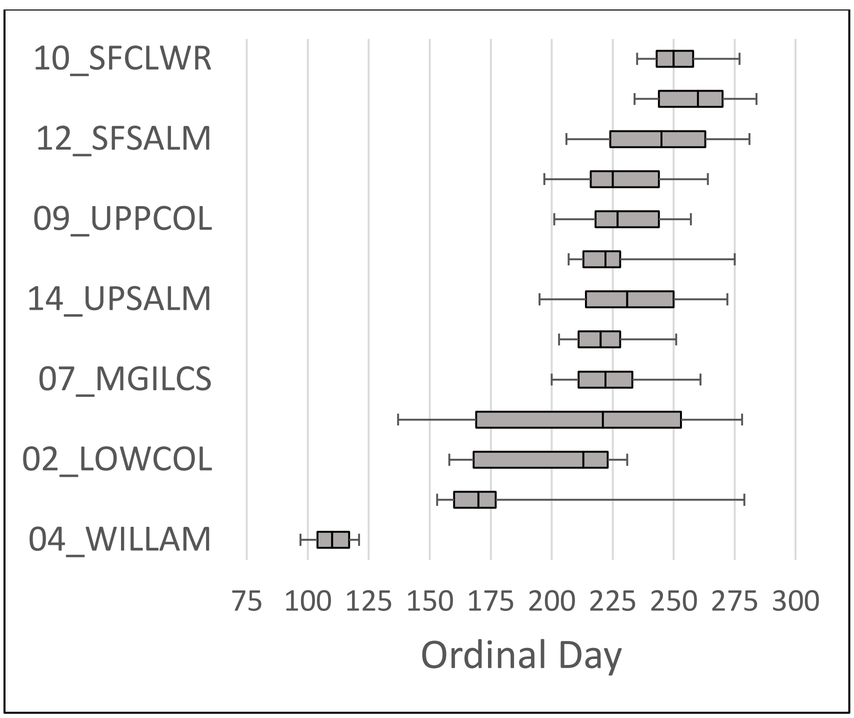


Figure 11: Run-timing distributions (median ordinal day, interquartile range, and 5th and 95th percentile) for natural origin steelhead (adipose non-clipped) that were sampled at Bonneville Dam in 2015 and assigned to stock of origin.

Using the PBT-assigned steelhead, we also characterized the run-timing distributions for hatchery broodstock sources in our PBT baseline (Table 9; Figure 12). For this analysis we grouped the 15 major hatchery broodstock sources (abundance >1000) into categories according to their median run-timing date. Only the OmyRB13 broodstock source had a median run-timing date before August 1^{st}. Most broodstock sources had median run-timing dates from August 1 – August 29 (i.e., OmyLYON12S, OmyLYON13S, OmyWALL12S , OmyWALL13S, OmyWEL13, OmyLSCR13S, OmyPAHH12S, OmyPAHH13S, OmyOXBO12S, OmyOXBO13S, OmySAWT12S, OmySAWT13S). Only OmyDWOR12S and OmyDWOR13S had median run-timing distributions after September 1^{st}.

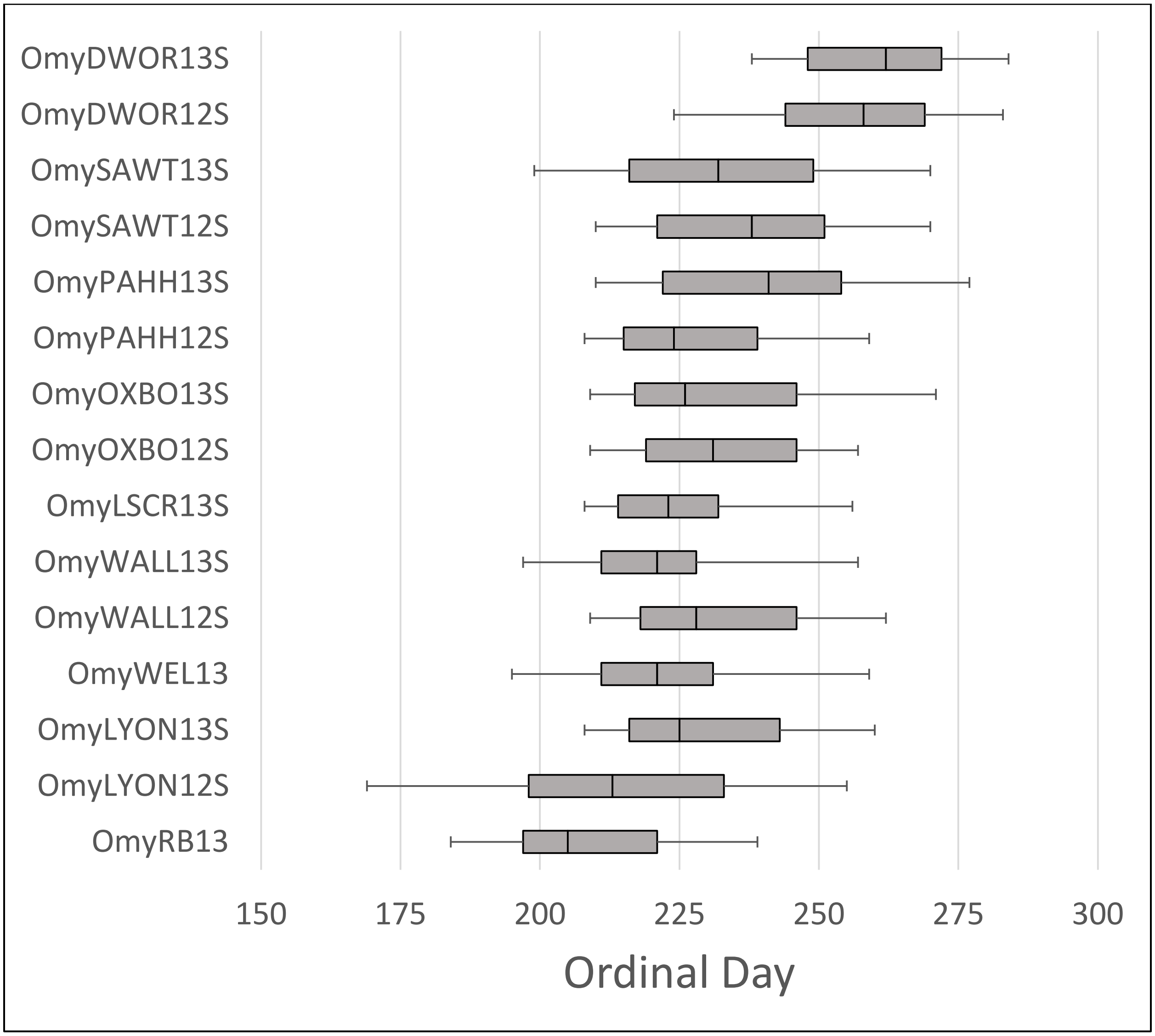


Figure 12: Run-timing distributions (median ordinal day, interquartile range, and 5^{th} and 95^{th} percentile) for hatchery origin steelhead (adipose clipped and non-clipped) assigned to PBT broodstock of origin that were sampled at Bonneville Dam in 2015. Hatcheries and their broodyears are ordered from earliest to latest run-timing. Key to broodstock collection is presented in Section 3 (Table 3).

Parentage based tagging analyses of steelhead in 2015

We were able to assign 510 steelhead sampled at Bonneville Dam in 2015 to 20 hatchery broodstock sources (2011-2013) throughout the Columbia River basin (Table 9). The largest portion of the PBT-assigned fish originated from the Dworshak Hatchery (n=97, 19%), followed by Pahsimeroi (n=87; 17%), Wallowa (n=82; 16%), Lyons Ferry (n=66; 13%), and Sawtooth (n=61; 12%) hatcheries. Using these known hatchery-of-origin steelhead, we compared the individual assignments from GSI analysis, and used these assignments to help classify them into reporting groups (Table 9). Those groupings were used to combine results of PBT-hatchery abundance estimates with the GSI estimated abundance of hatchery stocks (Table 8). Tagging rates varied across source hatcheries from 72.8% (OmyLSCR13S) to 100% (OmyLYON12S, OmyLYON13S, and OmySAWT12S) (Table 9; Table 11).

We examined which of the hatchery sources were contributing the size range of fish typically classified as B-run steelhead (Table 11). Fish with a fork length ≥78 cm were found to primarily originate from the Dworshak broodstock source, and most of these were from the 2012 (2-ocean age) spawn year (Table 11). The 2-ocean age is typical of B-run life history. Further, the regions of the South Fork and Upper Clearwater R. and Middle Fork and South Forth Salmon R. are generally thought to be the largest sources of B-run steelhead. Dworshak broodstock fit within the South Fork Clearwater R. genetic stock and so are expected to produce large, older steelhead.

Table 11: Summary information for Columbia River and Snake River hatchery broodstock sources for steelhead sampled at Bonneville Dam in 2015.

Reporting Group	Broodstock collection	Tagging rate	Age	Sex and size				
				Females		Males		Total
				N	≥78 cm length	N	≥78 cm length	N
02_LOWCOL	OmyPFF12	95.5%	2-ocean	1	0.0%	1	0.0%	2
07_MGILCS	OmyLSCR12S	95.6%	2-ocean	2	0.0%	0	0.0%	2
	OmyLSCR13S	72.8%	1-ocean	5	0.0%	6	0.0%	11
	OmyLYON12S	100.0%	2-ocean	16	0.0%	8	0.0%	24
	OmyLYON13S	100.0%	1-ocean	14	0.0%	14	0.0%	28
	OmyRB13	84.2%	1-ocean	2	0.0%	6	0.0%	8
	OmyUMA12	100.0%	2-ocean	2	0.0%	0	0.0%	2
	OmyWALL12S	99.2%	2-ocean	19	0.0%	8	0.0%	27
	OmyWALL13S	91.0%	1-ocean	13	0.0%	18	0.0%	31
09_UPPCOL	OmyWEL13	98.7%	1-ocean	12	0.0%	12	0.0%	24
	OmyWTP12	88.1%	2-ocean	1	0.0%	0	0.0%	1
10_SFCLWR	OmyDWOR11S	95.3%	3-ocean	2	100.0%	2	100.0%	4
	OmyDWOR12S	96.3%	2-ocean	86	43.0%	43	83.0%	129
	OmyDWOR13S	96.5%	1-ocean	4	0.0%	40	0.0%	44
14_UPSALM	OmyOXBO12S	98.4%	2-ocean	5	0.0%	2	0.0%	7
	OmyOXBO13S	97.1%	1-ocean	4	0.0%	14	0.0%	18
	OmyPAHH12S	98.1%	2-ocean	10	0.0%	7	0.0%	17
	OmyPAHH13S	99.5%	1-ocean	33	0.0%	40	0.0%	73
	OmySAWT12S	100.0%	2-ocean	3	0.0%	4	0.0%	7
	OmySAWT13S	99.7%	1-ocean	22	0.0%	29	0.0%	51

Estimated relative abundance and run-timing of sockeye salmon stocks in 2015

Relative stock abundance for sockeye salmon was estimated over a course of 10 statistical weeks (i.e. weeks 22-32) and were grouped to obtain a minimum of n=20 per group. A total of 812 sockeye salmon were sampled at Bonneville Dam in 2015 and were assigned to one of three genetic stocks (i.e., Okanogan, Wenatchee, and Snake) (Table 12). The Okanogan stock had the highest relative abundance (323,797), followed by the Wenatchee (178,325) and Snake River stock (7,919) (Figure 13).

We characterized the run-timing distributions of the three main genetic stocks of sockeye salmon (Figure 14). These stocks could be ordered by median run -timing date as follows: Wenatchee (6/24/15; ordinal day 175), Okanagan (6/25/15; ordinal day 176), and Snake River (6/28/15, ordinal day 179) (Table 12).

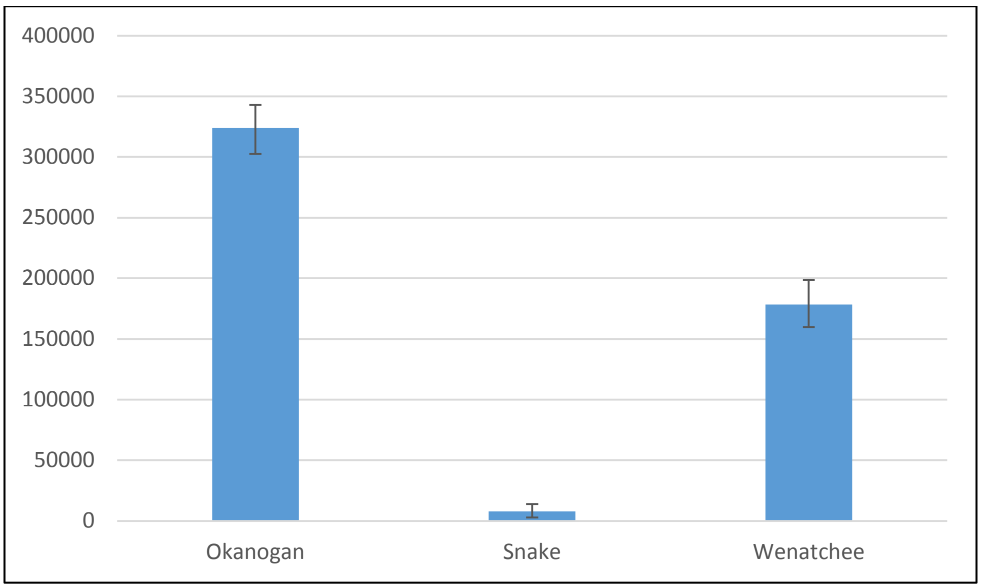


Figure 13: Relative abundance (± 95% CI) of sockeye salmon stocks sampled at Bonneville Dam in 2015.

Table 12: Relative abundance and run-timing distributions of sockeye salmon stocks passing Bonneville Dam in 2015.

Reporting Group	N	Estimated abundance		Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Interquartile range
		Mean	95% CI							
Okanogan	521	323797	302554-342981	176	171	182	164	195	06/25/15	11
Snake	10	7919	2948-13906	179	173	185	167	196	06/28/15	12
Wenatchee	281	178325	159747-198420	175	171	181	164	193	06/24/15	10
Total	812									

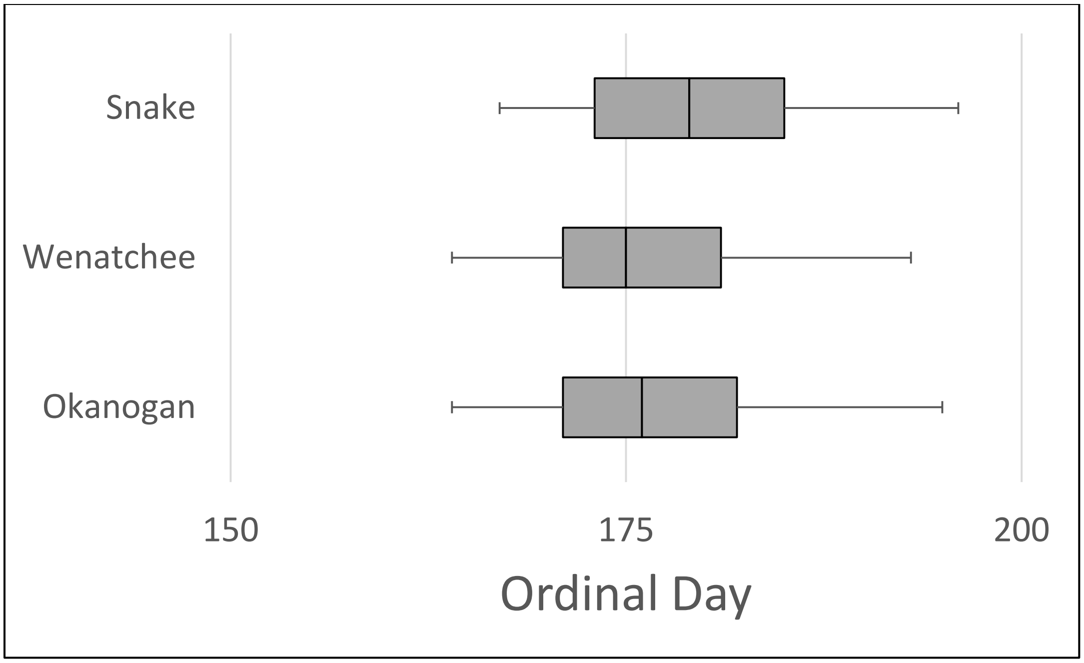


Figure 14: Run-timing distributions (median ordinal day, interquartile range, and 5^{th} and 95^{th} percentile) for sockeye salmon that were sampled at Bonneville Dam in 2015 and assigned to stock of origin.

Discussion

Parentage based tagging (PBT) and genetic stock identification (GSI) may be considered as methods that could replace the central functions of the coded wire tag program and could be a replacement for adipose fin marking to identify hatchery origin fish. However, this replacement would be contingent on continued genotyping of hatchery broodstock, fish passing Bonneville Dam, and from harvested fish. For ocean fisheries management, additional hatcheries throughout the range of Chinook salmon would have to contribute broodstock samples to this PBT baseline in order for the method to serve ocean fisheries management and the need to monitor total fishery impacts for stocks including Columbia River stocks of fall Chinook (tules and upriver brights) harvested in ocean fisheries. The genetic methods provide a substantial amount of information when they are combined and used to analyze Columbia River Chinook salmon and steelhead passing Bonneville Dam. PBT improves the accuracy for defining hatchery-origin and by subtraction, total natural-origin stocks. Genetic monitoring combining PBT and GSI is one of a number of possible tools that can be used to identify hatchery and natural fish at various resolutions. Other methods include, CWT's, PIT tags, VIE tags, and otolith marks. Adipose fin clips can be used to differentiate hatchery fish from wild fish either when fish are clipped at 100% or through expansions if stocks are not clipped at 100%. PBT can further discriminate among hatchery stocks within 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 provide information that would

not be possible with PBT, which is identification of all non-Snake River hatchery stocks as well as all natural-origin stocks.

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. We were able to address the following **F&W Program Management Questions:**

What are the status and trend of adult productivity of fish populations?

What are your in-river monitoring results and what are your estimates of stock composition and stock-specific abundance, escapement, catch, and age distribution?

Trapping at Bonneville Dam can only be done at very low rates due to restrictions placed on trap operations by USACE and NFMS. Low sample rates inhibit getting a representative sample of various stocks of fish. Higher sample rates would improve the precision of the estimates of fish at Bonneville Dam. Some fisheries were also sampled at very low rates.

We identified 13 hatchery stocks (45 hatchery broodstock sources) and 14 wild stocks of Chinook salmon estimated to have relative abundances ≥1,000 fish passing Bonneville Dam in 2015. It may interest fisheries managers to know that the run-timing of the "spring-run" stocks contributed to the total abundance of adult and jack Chinook salmon that pass through the Columbia River mainstem in two management periods (i.e., spring and summer).

We identified five hatchery stocks (20 hatchery broodstock sources) and 11 wild stocks of steelhead estimated to have relative abundances ≥1,000 fish passing Bonneville Dam in 2015. We found that genetic stocks seemed to fit well into the historical management categories, especially the hatchery stocks. Genetic stocks included an early Skamania summer-run, an intermediate run-timing category that contains most wild and hatchery steelhead stocks, and a late run-timing category with stocks that exhibit median dates after August 25th and includes South Fork Clearwater R. (Dworshak Hatchery), as well as wild stocks from upper Clearwater R, and SF Salmon 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 (≥78 cm) steelhead were found primarily to originate from Dworshak hatchery broodstock. These fish were also relatively old (2-ocean-age) and were derived from the Clearwater R., which is one of the regions expected to produce "B-run" steelhead. It is notable that the MGILCS reporting group represents some fish both within and outside the Snake River steelhead DPS, but does not represent all of the fish within the Snake River DPS.

This was the fourth year we were able to analyze sockeye salmon using GSI. We estimated relative stock composition and stock abundance for sockeye passing Bonneville Dam in 2015, and found that the Okanogan stock has the greatest relative abundance, followed by the Wentachee and Snake River stocks. We also found that the migratory run timing of these stocks overlaps broadly at Bonneville Dam.

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Overall Conclusion

This project combines four inter-related studies from the Fish & Wildlife Program Accords that address the following 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, sockeye salmon and kokanee, and coho salmon; 3) implement Genetic Stock Identification (GSI) programs for mainstem Chinook salmon, sockeye salmon, and steelhead fisheries and 4) GSI of fish passing Bonneville Dam (steelhead, sockeye, and Chinook salmon).

As described in Section 1, SNP panels continue to be expanded with GTseq that enables genotyping large sample sizes (>125,000 fish genotyped in 2016). This new genotyping protocol has greatly increased our laboratory's efficiency by allowing large numbers of fish to be genotyped with large numbers of SNP loci but at lower costs. For genetic baseline expansion (Objective 2), PBT hatcheries above Bonneville were genotyped to enable more thorough assignment of hatchery origin fish. In addition, GSI baselines are being developed to include two RAD-seq projects that have been initiated and will provide high density geographic coverage of Chinook salmon and steelhead populations in the Columbia River Basin. SNPs identified through these latter efforts will be useful in characterizing genetic diversity of hatchery and wild Chinook salmon and steelhead stocks. This study included two broad applications of stock identification; namely, stock composition of fisheries for Chinook salmon, sockeye salmon, and steelhead (Objective 3), and stock composition of Chinook salmon, 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 will be focused on identifying stocks of natural-origin fish.

Our GSI analyses of harvest included stock composition results for the spring, summer, and fall management periods of Chinook salmon fisheries in the lower Columbia River mainstem. Among selective fisheries issues, we continue to provide results to address a recent concern of fishery managers related to an expansion of the Chinook salmon sport fishing boundary around the mouth of the Wind River. Although our results could not be used to conclude that the Wind River sport fishery continues to primarily target its intended stock despite the boundary change; we did find that the composition of this harvest was quite different from other fisheries conducted during the spring management period.

Sockeye salmon and steelhead fisheries were analyzed and our stock composition results will provide additional information to managers of these fisheries. However, the sockeye salmon results indicate an increase in sample size may be warranted to make accurate estimates of rare stocks such as Snake River sockeye salmon. Although it was possible to estimate stock proportions of Snake River sockeye salmon, the low sample sizes precluded our ability to conclude whether there are significant differences in proportions of this stock among fisheries and at Bonneville Dam.

For Objective 4, we used a combination of GSI and PBT to estimate run-timing distributions and relative abundance of hatchery and wild Chinook salmon and steelhead stocks in 2015. For sockeye salmon, we used GSI to estimate relative stock abundance and run-timing distributions. The stock-specific data on abundance and run-timing of these species were used as a context for interpreting harvest stock composition.