

**2013 Annual Report**  
**GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS**

BPA Project # 2008-907-00

**Report covers work performed under BPA contract #(s) 56518**

**Report was completed under BPA contract #(s) 56518**

4/1/2014 - 3/31/2015

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This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

This report should be cited as follows: Jon E. Hess Nathan R. Campbell Andrew P. Matala Shawn R. Narum, GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS, 4/1/2014 - 3/31/2015 Annual Report, 2008-907-00

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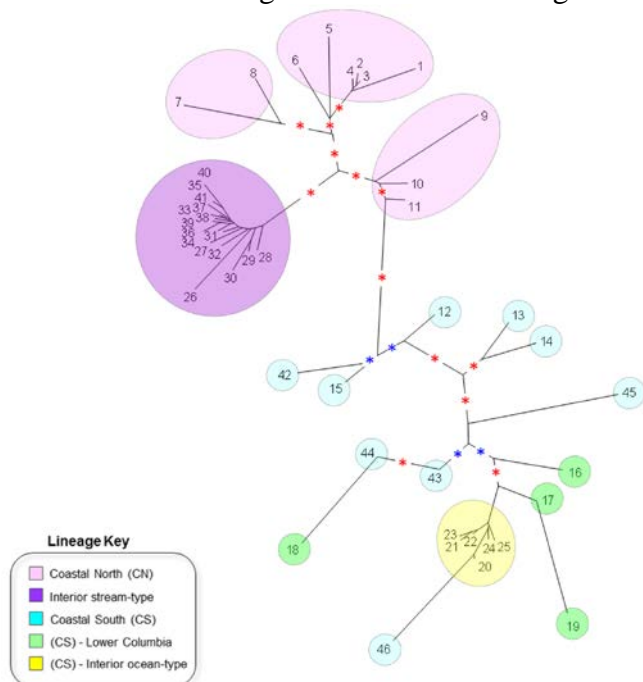
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## Executive Summary

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 fourth year of this project, SNP discovery and evaluation goals were achieved with a total of three projects that included one project each on Chinook salmon, steelhead trout, and Pacific lamprey that identified SNP markers using restriction associated DNA sequence (RAD-seq) technology.

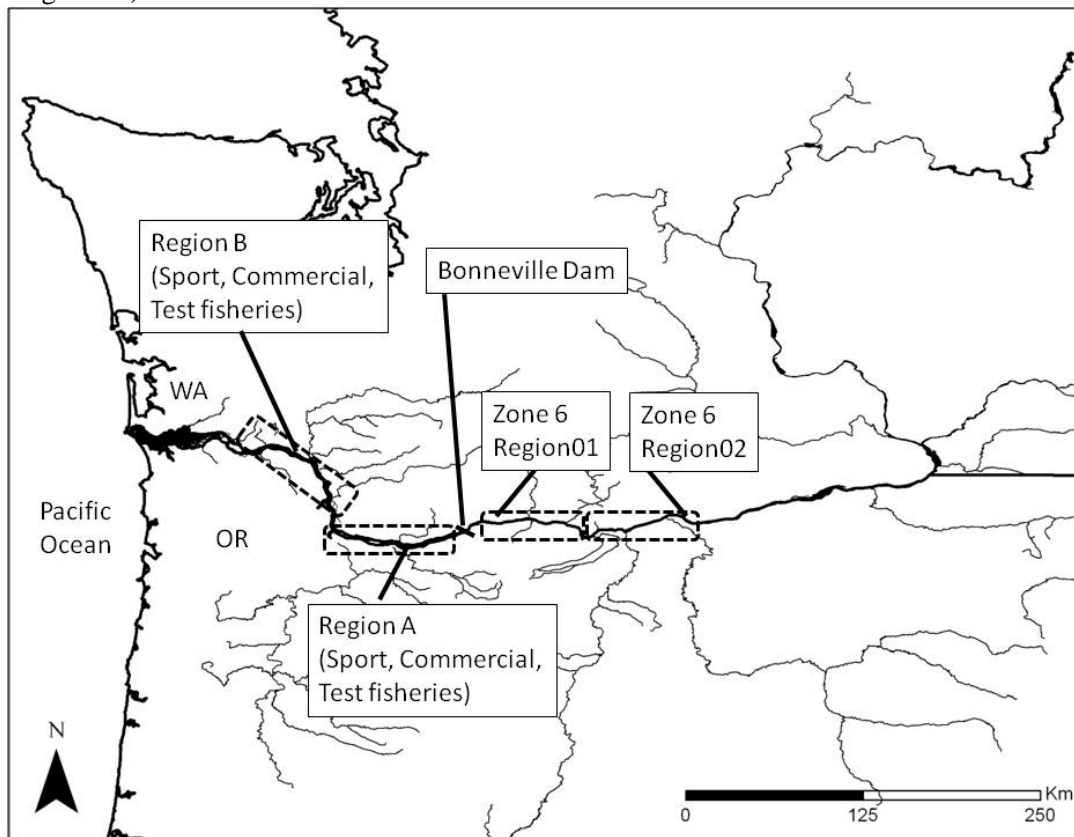
**Objective 2) expand and create genetic baselines for multiple species including Chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*O. mykiss*), sockeye salmon and kokanee (*O. nerka*), and coho salmon (*O. kisutch*).** There are three GSI baselines available that are representative of salmonid populations from the entire Columbia River Basin including Chinook salmon (N=79 collections), steelhead (N=145 collections), and *O. nerka* (N=29 collections). This report includes a description of minor changes to the Chinook salmon GSI baseline that utilizes 192 SNP markers (including a 96-SNP panel optimized for parentage based tagging (PBT) and a 96-SNP panel optimized for genetic stock identification (GSI)). We also characterize the addition of a significant number of collections to the 96-SNP baseline for *O. nerka*, which is utilized for GSI of three major Columbia River sockeye stocks. Further, a RAD-seq project on Chinook salmon identified 20,000+ quality-filtered SNPs across 50 collections distributed throughout the Northeastern Pacific range (Fig. 1). These SNPs were useful in characterizing genetic diversity of hatchery and wild Chinook salmon stocks, and may help to further characterize genetic variation among these stocks.



**Figure 1. Neighbor joining tree of Chinook salmon collections genotyped with >20,000 SNPs with RAD-seq.** Collection reference numbers correspond with Section 2: Table 1. Asterisks indicate 100% bootstrap support (red) and >95% bootstrap support (blue) based on 1000 replicate data sets.

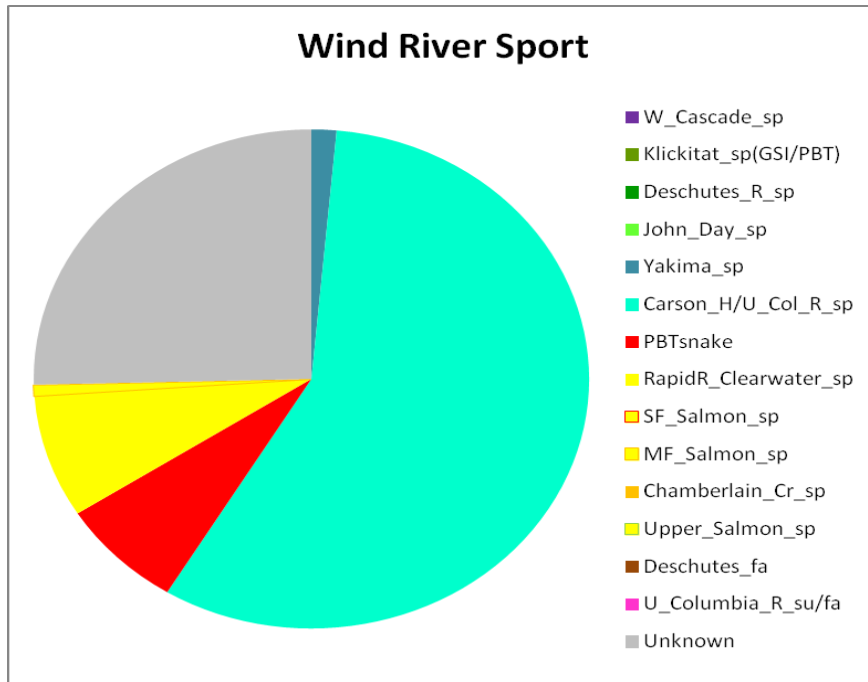


**Objective 3) implement GSI programs for mainstem Chinook salmon, sockeye salmon, and steelhead trout fisheries (Fig. 2).** Chinook salmon and steelhead trout fishery applications of GSI were integrated with the relatively new genetic technology of PBT. Long histories of exogenous stock transfers from specific hatchery programs along with closely related natural stocks often prevents effective application of GSI in assigning origins of hatchery fish. This limitation can be overcome by the use of PBT. Currently PBT allows monitoring of all Snake River hatchery Chinook salmon and steelhead trout stocks, and does not require adipose fin clipping. The role of PBT continues to expand and beginning with spawn year 2013, most Chinook salmon and steelhead trout broodstock from hatcheries above Bonneville Dam have been sampled and may be incorporated into the PBT baseline if funding is available to genotype them. GSI still will be useful for filling in information gaps that remain after PBT has been more broadly implemented (e.g. determining the stock identity of wild fish and non-tagged hatchery-origin fish).



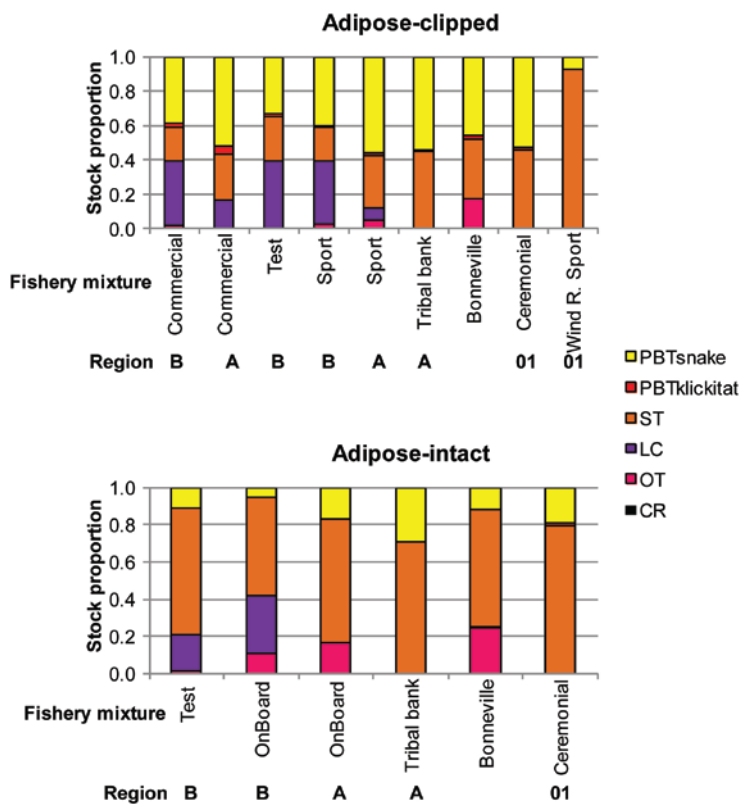
**Figure 2. Sources of fishery mixtures in the lower Columbia River mainstem.**

Using a combination of PBT and GSI approaches we estimated stock composition of several Chinook salmon fisheries and Bonneville Dam sampled during the spring management period (January 1 – June 15). This included: 1) sport, commercial, and tribal bank fisheries below Bonneville Dam, 2) non-lethal interrogation of fish at Bonneville Dam, and 3) a tribal ceremonial fishery in Zone 6 above Bonneville Dam. Further, 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. These comparisons among fisheries showed that a distinctive characteristic of the Wind River sport fishery is its large proportion of Carson Hatchery/Upper Columbia River hatchery stock (Fig. 3). This genetic stock includes Carson Hatchery fish, which is the intended target of this fishery; however, genetic similarity between Carson Hatchery and Upper Columbia



River hatcheries and others in the middle Columbia River created uncertainty regarding the specific stock composition results. An additional distinction of the Wind River harvest was its relatively low proportion of Snake River hatchery fish that were assigned using PBT (Fig. 3).

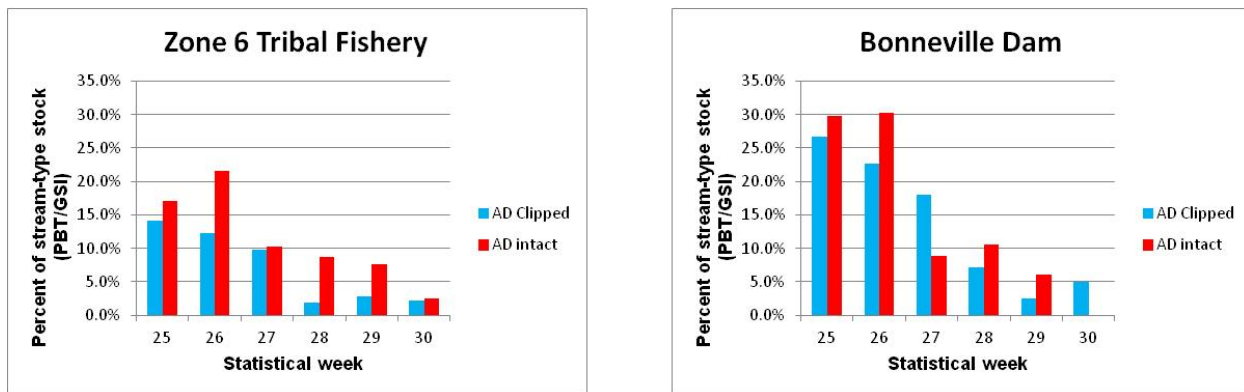
**Figure 3. Genetic stock composition of the Wind River sport harvest in 2013.**



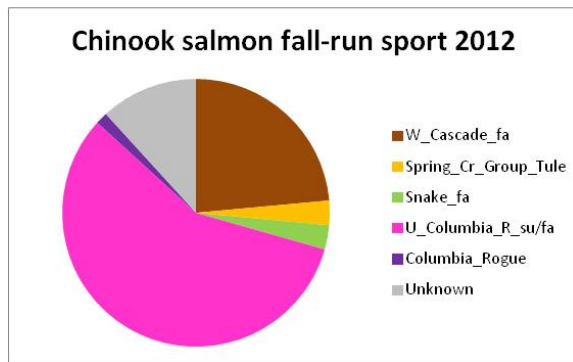
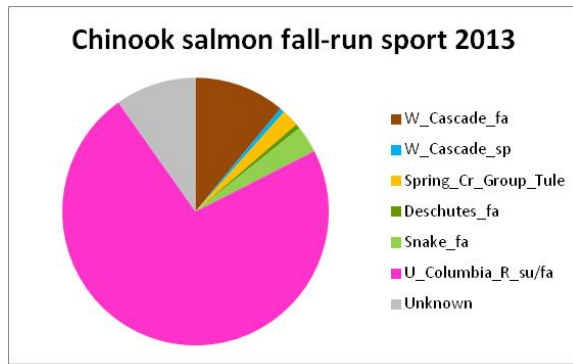
**Figure 4. Stock composition of Chinook salmon harvest mixtures and Bonneville Dam during the spring management period.** Note: “ST” includes upriver spring run and Snake River spring/summer run Chinook salmon stocks, “LC” includes all lower river stocks (e.g. Willamette, Cowlitz, Lewis, and Sandy stocks), “OT” includes upper Columbia summer and upriver fall Chinook salmon stocks, and “CR” denotes Columbia River Rogue stock reared in Youngs Bay net pens. PBT was used to assign Klickitat and Snake River hatchery spring-run Chinook salmon.

Analysis of adipose-clipped Chinook salmon from multiple fishery mixtures in the spring management period identified relatively larger proportions of lower Columbia River stocks (LC, likely dominated by the Willamette River stock) near the mouth of the Columbia River, such that region B was comprised of a range of 37– 39% and region A had a range of 8-17% of the LC stock in commercial and sport fisheries, respectively (Fig. 2, 4). PBT-assignments made it possible to further discriminate fish by their hatchery-of-origin (16 total hatcheries represented), which is a finer scale compared to the larger stock units that are resolved with GSI. Stream-type stocks (ST, i.e. upriver spring-run and Snake River spring/summer-run Chinook salmon) were dominant upstream of region B. Analysis of adipose-intact fishery mixtures showed similar patterns, however the Snake River PBT-assigned stock (hatchery-origin) was estimated in smaller proportions than in adipose-clipped samples as would be expected since PBT identifies hatchery origin fish.

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 below Bonneville Dam, 2) compare stock composition of adipose-clipped versus adipose-intact fish from the commercial harvest below Bonneville Dam, 3) characterize stock composition by pool and statistical week in Zone 6, and 4) compare temporal changes in stock composition across the return for adipose-clipped versus adipose-intact fish in Zone 6. The percentage of upriver adult and jack spring-run stocks (ST) declined in both harvest and Bonneville Dam mixtures over time, but the harvest may have generally included a lower proportion of this group compared with what actually passes the dam in any given week (Fig. 5).



**Figure 5. Temporal patterns of the percent of upriver spring-run stocks (ST) by-catch in adipose-clipped and adipose-intact mixtures of adult and jack Chinook salmon sampled in Zone 6 tribal fishery and Bonneville Dam during the summer management period in 2013. Statistical week 25 begins on June 17 and week 30 ends on July 28.**

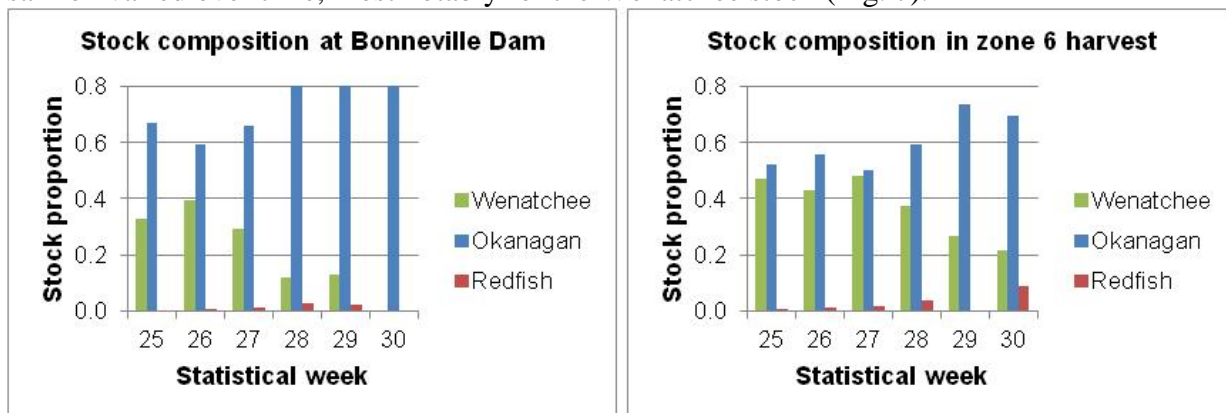


For fall-run Chinook salmon fisheries, we estimated stock composition of the mark selective sport fishery in the lower Columbia River in 2012 and 2013. No PBT assignments were observed since fall Chinook salmon were not yet included in the PBT program, but were tested to check for any spring Chinook salmon that may have been inadvertently included. Major GSI stocks from greatest to least were: U\_Columbia\_R\_su/fa (73%), W\_Cascade\_fa (11%), Snake\_fa (3%), Spring\_Cr\_Group\_Tule (2%) (Fig. 6).

**Figure 6. Genetic stock composition of the lower Columbia River fall-run Chinook salmon sport harvest in 2012 and 2013.** Note: the difference between the assignment of West Cascade spring stocks in 2013 and 2012 may owe to misassignment between the spring and fall runs from that region, strays from the net pen rearing at

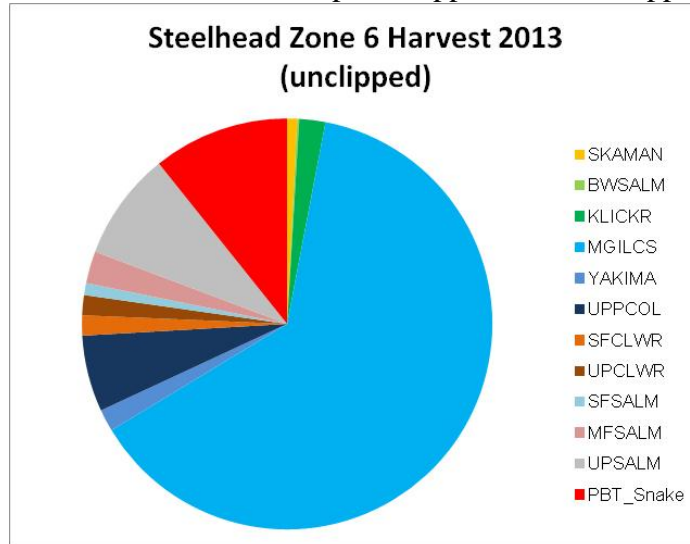
Youngs Bay, or other unknown reasons.

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 the commercial fishery were Okanogan at 64.6%, Wenatchee at 31.2%, and Snake (Redfish)= 4.2%. The overall composition of the three stocks of sockeye salmon in the Zone 6 fishery were Okanogan at 55.6%, Wenatchee at 42.8%, and Snake = 1.6%. The proportion of each stock of sockeye salmon varied over time, most notably for the Wenatchee stock (Fig. 7).



**Figure 7. Stock composition at Bonneville Dam and the Zone 6 sockeye harvest on a weekly basis.** Statistical week 25 begins on June 17 and week 30 ends on July 28.

Finally, objective 3 concluded with a stock composition analysis of unclipped steelhead harvested in the summer and fall tribal fishery in Zone 6. Despite, the absence of an adipose mark, PBT assignments were used to identify 11% of the sampled harvest as unclipped Snake River hatchery-origin (Fig. 8). These fish are likely to be primarily from supplementation releases that were not adipose-clipped and mis-clipped fish from other hatchery programs.



**Figure 8. Stock composition of adipose-intact steelhead harvested in 2013 from summer and fall fisheries in Zone 6.** Eleven Columbia River Basin stocks were represented: Skamania “SKAMAN”, Big White Salmon “BWSALM”, Klickitat “KLICKR”, MGILCS, Yakima “YAKIMA”, upper Columbia “UPPCOL”, S.F. Clearwater “SFCLWR”, upper Clearwater “UPCLWR”, S.F. Salmon “SFSALM”, M.F. Salmon “MFSALM”, and upper Salmon “UPSALM”. The MGILCS is a large group of closely related stocks that

includes the following Snake River stocks: George Cr, Asotin Cr, Alpowa Cr, and Tucannon R as well as the stocks from the Grande Ronde, Imnaha, Lower Clearwater and Lower Salmon R.; and all other middle Columbia River stocks that were not listed above.

**Objective 4) use GSI to estimate stock composition of fish passage at Bonneville Dam (steelhead trout, sockeye salmon, and Chinook salmon) and juvenile Pacific lamprey passage at Lower Granite Dam.** Fish were sampled as they migrated past Bonneville Dam. We sampled adult and jack Chinook during the spring and summer management periods and sampled steelhead in the spring, summer, and fall management periods. 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 2013. Those estimates revealed ten separate hatchery stocks and twelve distinct wild stocks of Chinook salmon that passed Bonneville Dam in numbers greater than 1,000 fish per stock. The run-timing of these stocks contributed to the total abundance of Chinook salmon that pass through the Columbia River mainstem in two management periods (spring and summer). In fact, we observed more than 5,000 natural-origin and 10,000 hatchery-origin fish assigned to spring-run Chinook salmon reporting groups (mostly Snake River origin) that return during the summer management period (June 16 – July 31) based on our estimates. Some jack aged spring and Snake River spring/summer Chinook salmon stocks are known from PIT tag data to have a very late run timing and it is likely that a significant number of these 15,000 fish were late returning jacks. Some run-timing results were consistent with those from previous analyses, e.g. Salmon River spring-run Chinook salmon have relatively late runs compared to other spring stocks.

There were five wild and five hatchery steelhead stocks with an estimated relative abundance greater than 1000 fish passing Bonneville Dam in 2013. Managers have historically grouped steelhead stocks into management periods and we observed that the run-timing of genetic stocks

appears to correspond relatively well. Historically, managers have defined an early Skamania summer-run, an intermediate run-timing category that contains most wild and hatchery steelhead stocks, and a late run-timing category that arrived after August 25<sup>th</sup>. While managers do not utilize the August 25 date break anymore, the overall trends of stocks tend to segregate into this historic pattern. The latter timed group includes South Fork Clearwater R., upper Clearwater R., and South Fork and Middle Fork Salmon R. stocks. However, this pattern was not exclusive as these three stocks also had some earlier returning fish, and late returning fish were also comprised of other stocks.

We combined PIT-tag (adults tagged at Bonneville Dam) and genetic analyses to estimate relative abundances and median run-timing dates of the following three extant anadromous sockeye salmon stocks : Okanagan (127,256; Jun. 27th), Wenatchee (53,245; Jun. 26th), and Snake River (1,677; Jul 6th). The estimated median date for Snake River sockeye calculated by this method differs from estimates based on PIT tagged juveniles which were 50% complete at Bonneville by July 1 and were 76% complete by July 6. However, the sample size for fish assigned to Snake River with GSI was very small as only 8 fish were identified from Bonneville Dam and thus limits precise estimates of run-timing. A comparison of dam counts in the Snake River compared to dam counts at Priest Rapids would suggest about 1 in 170 sockeye salmon in the basin were Snake River stock (approximately 0.6% of the total run). The low abundance of this stock makes it challenging to estimate with the methods in this study given current restrictions on numbers of fish that can be sampled at Bonneville Dam at the Adult Fish Facility (AFF) trap. Further, low sample numbers of the Snake River stock in the harvest make it difficult to estimate stock proportions with narrow confidence intervals and preclude determination of whether harvest proportions were significantly higher than the estimate for this stock at Bonneville, which was 0.9% of the sockeye run during this fishing period. Possible explanations for differences among the estimates of this stock could owe to the sampling protocols at Bonneville Dam (Fig. 7), which may have higher representation of young fish as compared to harvest mixtures.

This study also utilized a genetic baseline of adult lamprey that were translocated throughout the Snake River basin in the year 2007 and examined whether they could be assigned as parents to any Pacific lamprey juveniles collected as mortalities on the screens of turbine units at Lower Granite Dam (LGR) in 2011. A previous parentage analysis identified a cohort of translocation-offspring (broodyear 2007) as 5-year-olds that were exiting their home-tributary (Newsome Creek) in 2012. None of the 181 LGR juveniles assigned to the translocated adults. Estimation of the effective number of spawners represented by these LGR juveniles yielded 501 (95%CI 413–634). This number is extremely low given that it represents the effective spawner abundance of nearly the entire Snake River Basin.

## **Acknowledgements**

The authors of this report thank the following individuals and organizations for providing time and expertise toward this project: The Bonneville Power Administration for providing funding for this research project and to its staff for their assistance. Tissue samples were collected and provided by Bobby Begay, Steve Boe, Chris Brun, Peter Cleary, Roger Dick Jr., Roger Dick Sr., Dani Evenson, Joe Hymer, Ken Keller, Chris Kern, James Kiona, Jacinda Mainord, Alfred McConnville, Jim Nagler, Terra Schultz, David Sohapp, Jason Vogel, John Whiteaker, Marc Whitman, Bill Young, and Joe Zendt. Collection of samples from the commercial, sport, and test fishery were coordinated with Joe Hymer. We are grateful for extensive contributions in the genetics laboratory from Stephanie Harmon, Nick Hoffman, Travis Jakobson, Amanda Matala, Lori Maxwell, Megan Moore, Vanessa Morman and Jeff Stephenson. Maureen Hess contributed guidance on performing parentage based tagging analyses. Substantial data and statistical guidance was contributed by Idaho Department of Fish and Game, and the following agencies made significant biological tissue and/or DNA sample contributions: Washington Department of Fish and Game, United States Fish and Wildlife Service, Abernathy Fish Technology Center, and NOAA fisheries.

## 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 trout 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 trout (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 trout 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 trout hatchery broodstocks (Steele et al. 2011) and we will soon have a baseline that includes most Chinook salmon and steelhead trout hatcheries located above Bonneville Dam. This application has effectively tagged all Snake River hatchery Chinook salmon and steelhead trout 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 trout 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 limiting relative 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. Future applications of PBT may include other species such as Pacific lamprey, and could be 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 2013. 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 2013.

## **Section 1: SNP Discovery**

### **Introduction**

Analysis of genetic data is often used for evaluation of populations of wild and domestic organisms. The statistical power of this data for uses such as population differentiation (Genetic Stock Identification, GSI), parentage determination (Parentage Based Tagging, PBT), or physical trait mapping (Genome-Wide Association Studies, GWAS) is directly related to the quality and number of genetic markers used for genotyping. Until recently the pool of available genetic markers in salmonids was small and the selection of marker panels for genotyping often meant including markers with low statistical power. However, new sequencing technologies now provide billions of bases of DNA sequence information which can be used to identify thousands of SNPs (Single Nucleotide Polymorphisms) and provide genotypes for evaluation of their utility for genetic analysis applications. We are currently using an Illumina HiSeq instrument to generate sequencing data from RAD (Restriction site Associated DNA) libraries generated using salmonid DNA. This sequencing information can then be used to develop particular SNPs into high throughput assays should they prove to be effective for genetic analyses (population differentiation, sex determination, and trait prediction for example).

#### *Project objectives and higher level harvest management questions*

We have analyzed RAD data from a diverse set of 50 collections of Chinook salmon and are in the process of evaluating over 21,000 SNP loci in this species. Similarly, RAD libraries are being generated for both range wide evaluation of steelhead SNPs and for generation of a dense linkage map for Pacific lamprey. We plan on uploading all RAD data to a central database in order to address the following higher level question:

#### **F&W Program Management Question:**

How has your work supported exchange and dissemination of fish and wildlife data or the development of a database to manage data that may be shared regionally, relative to the RM&E data management strategies roadmap?

#### *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 included in this report. Data will be routinely uploaded to a database for sharing regionally.

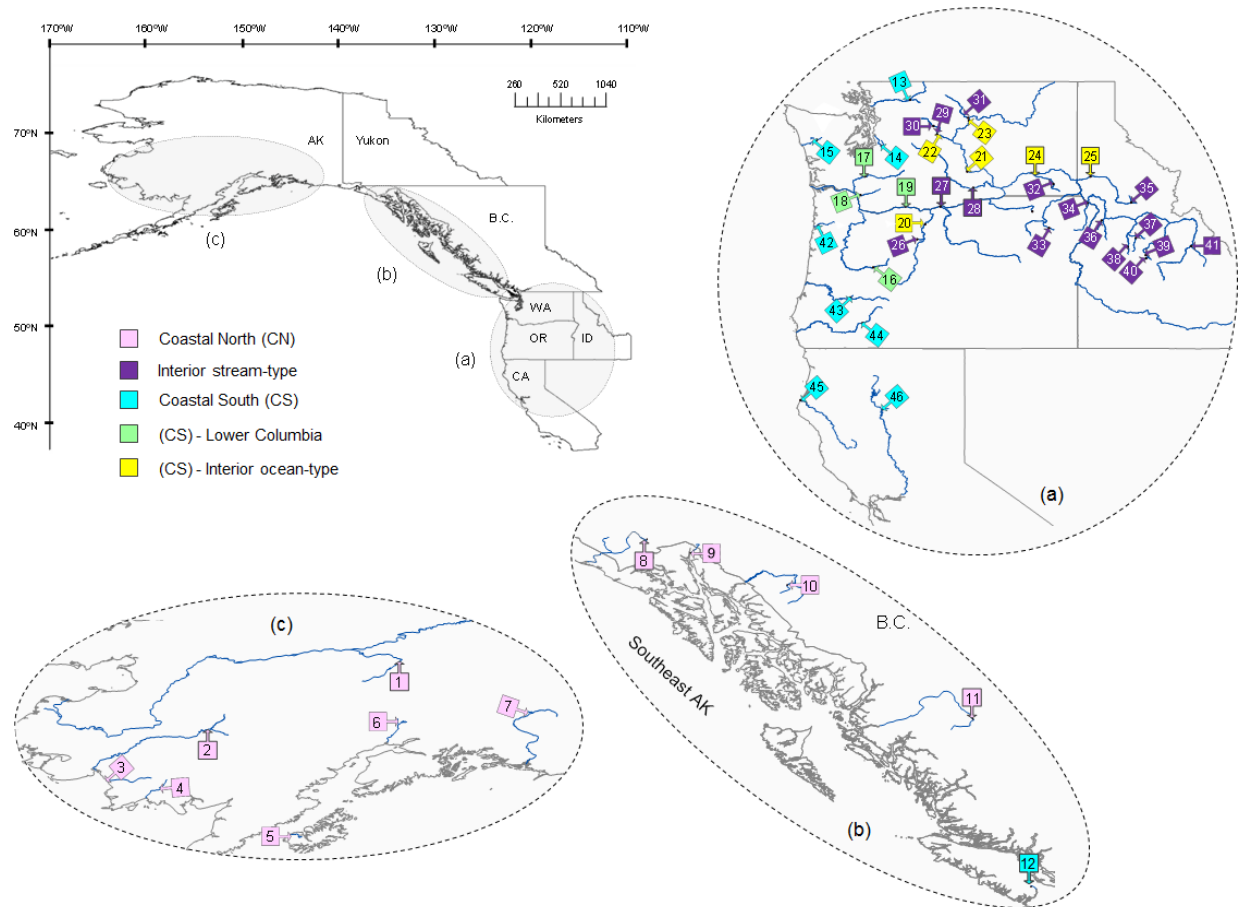


Figure 1. Chinook RAD map identifying collections by lineage. Numbered collections correspond with Table 1. Ellipses are (a) Oregon, Washington and Idaho, (b) British Columbia, and (c) Alaska. Collections are coded by lineage (see legend).

## Methods

### *Chinook RAD*

Fin clips taken from 50 collection sites within and outside the Columbia River drainage (Figure 1) were utilized for extraction of genomic DNA using Qiagen blood and tissue kits. The extracted DNA was used to create RAD libraries (*SbfI*) for sequencing using our Illumina 1500 instrument according to the methods outlined in <https://www.monitoringmethods.org/Method/Details/4144>.

The identification of SNPs within the RAD sequencing data as well as genotyping of the individual samples was carried out using the program STACKS (Catchen et al. 2013). Sequences from two individuals from each sample collection site were used for SNP identification and up to 4 SNPs were allowed per RAD tag alignment. All identified SNP sites were genotyped in each of the sequenced individuals. Individuals and SNPs that genotyped in less than 80% of the individuals sequenced were removed. In RAD tags that contained more

than 1 SNP site, only the SNP with the highest minor allele frequency was retained. Lastly, SNP loci violating Hardy-Weinberg expectations at more than 6 of the collections were discarded.

#### *Steelhead RAD*

A similar project to ‘Chinook RAD’, 50 collections of fin tissues from diverse collection sites are being targeted for generating RAD libraries in order to evaluate a large pool of SNP markers for their utility in a variety of conservation genetic analyses. We are currently in the process of DNA extraction (using Qiagen blood and tissue kits) and RAD library preparation as described in <https://www.monitoringmethods.org/Method/Details/4144> using the restriction enzyme *SbfI*.

#### *Lamprey Genetic Map*

Tissue samples of Pacific lamprey offspring (ammocoetes) along with their parents were collected at the Columbia River research laboratory (US Geological Survey). DNA was extracted from 284 tissue samples using Qiagen blood and tissue kits and 3 RAD libraries were prepared using the methods described in <https://www.monitoringmethods.org/Method/Details/4144>. Sequencing was carried out on an Illumina HiSeq 1500 instrument using single-end 100 reads. SNP identification and genotyping is now underway using the program STACKS (Catchen et al. 2013). The program OneMap (Margarido et al. 2007) will be used to generate a genetic map using the genotypes.

## **Results**

#### *Chinook RAD*

Forty-four RAD libraries were produced containing between 48 and 96 individual samples each from 50 collection sites. Libraries with 96 individuals were sequenced on 2 lanes in order to generate enough sequencing reads for genotyping. A total of 2,777 individuals were included in these libraries and sequencing generated an average of 3.1M 100 base reads per individual. Following strict filtering, a final data set containing genotypes for 21,216 SNP loci for 1,959 samples across 50 collections was produced (supplemental data).

#### *Steelhead RAD*

RAD library preparation and identification of appropriate sample collections is ongoing.

#### *Lamprey Genetic Map*

Of the 284 samples included in RAD sequencing, 279 achieved the minimum threshold of 0.8M reads required for accurate genotyping. These samples included both parents and a collection of 277 offspring.

## **Discussion**

The use of next generation sequencing technologies has revolutionized marker discovery and development. The numbers of markers identified using techniques such as RAD sequencing are orders of magnitude more than were previously possible using Sanger sequencing methods at ever decreasing costs. Moreover, RAD sequencing is also a platform for genotyping samples at several thousand SNP markers without necessarily having previously identified SNP markers. This has made it possible to conduct genetic analyses not previously possible in many non-model organisms due to that lack of available markers such as GWAS and linkage mapping. Having large pools of SNP markers identified will also allow for selection of the most informative markers for use in small panel applications such as GSI and PBT.

These efforts in generating sequencing data and the identification of SNP markers in these species will provide a resource for future genetic applications. A genetic map of the Pacific lamprey genome will allow for the identification of important genomic regions associated with various physical traits which will help inform reintroduction and hatchery production programs now underway in the Columbia River. GSI is now used to provide stock information to help manage the impact of fishing on protected stocks of Chinook salmon and steelhead. However, some stocks are too genetically similar to distinguish from one another using small SNP panels. Selection of the most informative SNPs for stock determination from a very large pool of SNPs can allow for more accurate stock composition estimates. Thus far we have reported over 21,000 SNPs from Chinook salmon and are anticipating a similar resource for steelhead. We also expect to have completed a dense genetic map for Pacific lamprey containing thousands of mapped SNP markers.

## **References**

- Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. *Molecular Ecology* 22:3124-3140.
- Margarido GRA, Souza AP, Garcia AAF (2007) OneMap: software for genetic mapping in outcrossing species. *Hereditas* 144:78-79.

## Section 2: Genetic Baseline Expansion

### Introduction

Distinct population aggregates of Chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*Oncorhynchus mykiss*), and the species *Oncorhynchus nerka* (including sockeye salmon), have evolved through the cumulative effects of selection and genetic drift (Waples 1991; Nielsen et al. 2009). Homing to spawn in natal rearing sites (philopatry) is a distinctive behavior of Pacific salmon species that has been well documented. This life history attribute can significantly restrict gene flow and thus shape productivity limitations among naturally reproducing populations (Hasler and Scholz 1983; McIssac and Quinn 1988; Quinn *et al.* 1991). Population distinctions may be more readily resolved on a large geographic scale where gene flow and reproductive restrictions are often well defined by migration distance and adjacency in stream networks, yet the distribution of suitable spawning habitat and local adaptations may produce fine scale genetic structure between stream sections or watersheds in close proximity (Beacham *et al.* 2006; Matala et al. 2012). Homing miscues (straying) are thought to be necessary to buffer loss of genetic diversity in salmon (Milner and Bailey 1989), particularly in small populations. However, the rate of straying among wild fish is generally low (Quinn 1993; Heard *et al.* 1995) and despite the resulting moderate gene flow, genetic structure between populations may persist (Neville *et al.* 2007). There is some evidence that the incidence of straying is higher among hatchery origin fish. This may be an artifact of changes in fish passage protocols, transport through the hydro system, or artificial rearing practices. An elevated rate of immigration between populations may alter genetic variation associated with local adaptation, and lead to changes in spatial and temporal variability within and/or among populations (Hess and Matala 2013).

In the Columbia River Basin, the population genetics of Chinook salmon have been studied in great detail (Narum et al. 2004; Waples et al. 2004; Beacham et al. 2006; Narum et al. 2008b; Matala et al. 2011), and steelhead to a similar degree (Winans et al. 2004; Currens et al. 2009; Blankenship et al. 2012; Narum et al. 2011). Genetic evaluations of *O. nerka* (both sockeye salmon and kokanee) have been comparatively limited in scope (Gustafson et al. 1997; Waples et al. 2011; Iwamoto et al. 2012). Conservation units such as distinct population segments (DPS) evolutionarily significant units (ESU) major population groups (MPG), and viable salmonid populations (VSP) have been established based on criteria including population ecology and viability, ancestry and descent, reproductive isolation, and inferred local adaptations (Fraser and Bernatchez 2001; Fraser et al. 2011). However, conservation is not commonly informed by direct evaluations of non-neutral variation (e.g., selection and adaptive divergence). Landscape genetics is an approach aimed at describing population differentiation relative to features in an organism's environment (Segelbacher 2010; Sepulveda-villet & Stepien 2012), including natural or human erected barriers and local climate. However, landscape genetics has been described primarily on the basis of neutral divergence (Dionne et al. 2008; Narum et al. 2008), where restricted gene flow is explained in the context of heterogeneous habitats (Latch et al. 2011). And, although local adaptation may be inferred from neutral genetic structure coincident with habitat (Olsen et al. 2011; Blankenship et al. 2012), inferences based exclusively on neutral differentiation risk incorrectly identifying the underlying processes affecting population distinctions (Funk et al. 2012; Landguth & Balkenhol 2012).

*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 single nucleotide polymorphism (SNP) genotypic data. These data serve as species-specific baselines to characterize Chinook salmon, steelhead trout, and *O. nerka* population structure throughout the Columbia River Basin. SNPs are highly prolific in the genome, with substantial coverage for linkage analyses (Moen et al. 2008). Large numbers of highly informative SNP loci can be discovered via next generation sequencing technologies such as Restriction-site Associated DNA (RAD) sequencing (Miller et al. 2007; Baird et al. 2008; Hecht et al. 2013). SNPs are amenable to superior high throughput capabilities and are relatively easily amplified and scored, 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 observed among populations may be indicative of non-neutral influences (i.e. selection and local adaptation). Techniques such as outlier detection methods, association testing, 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 (see Limborg et al 2011) between genetic variation and the physical environment. This additional information may ultimately inform conservation criteria for delineating populations across diverse landscapes. Currently our SNP baselines are being used to characterize populations in archival studies, in efforts to reintroduce fish into extirpated regions within historic ranges, hatchery-wild interactions, and using genetic stock identification (GSI) in tribal and sport harvest fisheries, and to evaluate migration timing through the hydro system.

#### *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 a database for sharing regionally.



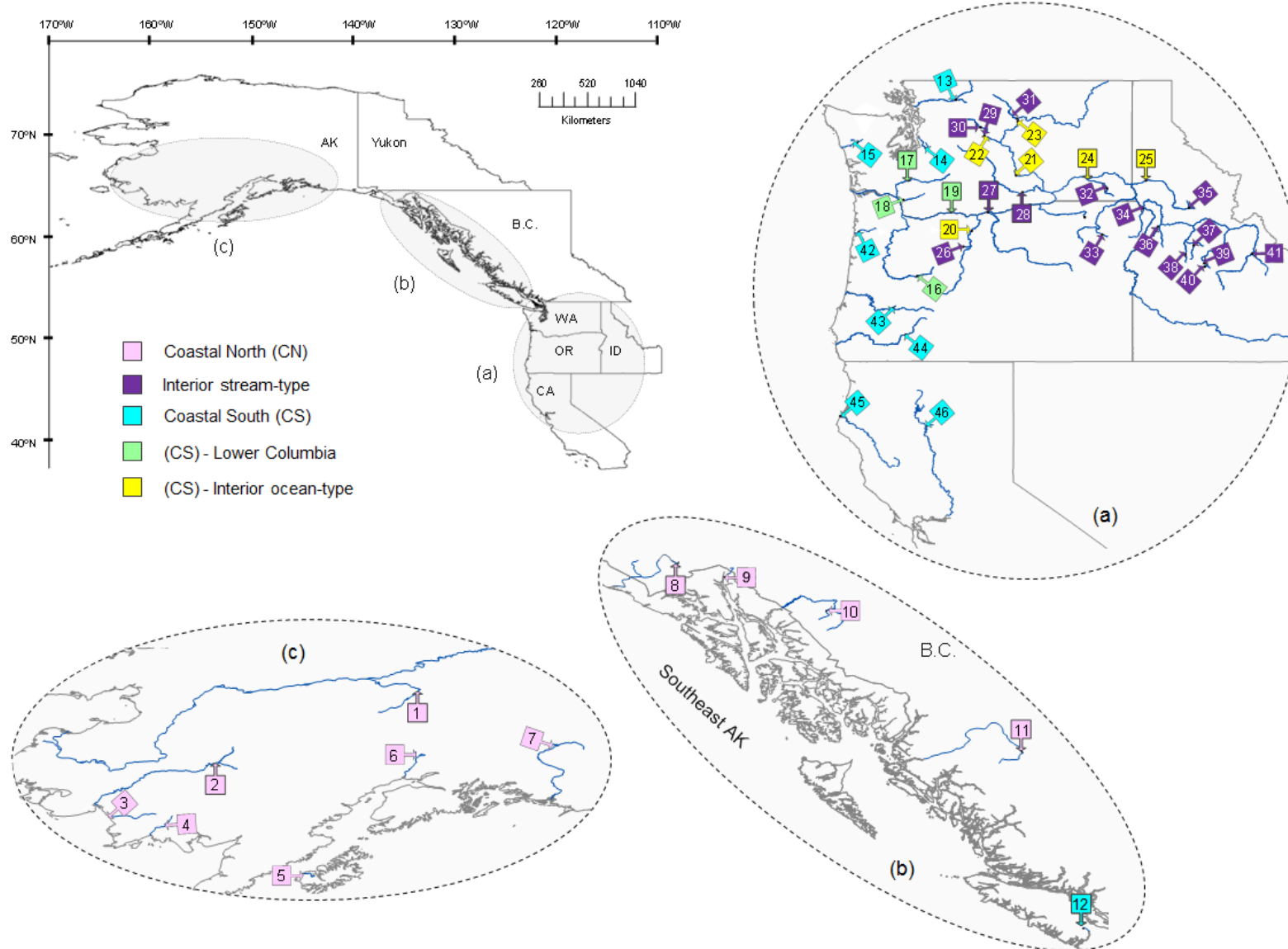


Figure 1. Chinook RAD map identifying collections by lineage. Numbered collections correspond with Table 1. Ellipses are (a) Oregon, Washington and Idaho, (b) British Columbia, and (c) Alaska. Collections are coded by lineage (see legend).

## Methods

### *Baseline sampling and expansion*

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 baselines may include life history variants such as potentially distinct populations of resident *O. mykiss* (Narum et al. 2008a; Narum et al. 2011). Existing baseline datasets, completed through 2012, are described in detail in Hess et al. (2012). Chinook salmon collections from the White River, Nason Creek, And Chiwawa River, from the Wenatchee River Subbasin were added to the 192 SNP baseline for 2013. Additional efforts were initiated in 2013 to characterization Chinook salmon throughout the Columbia River and coastwide (Figure 1), including 46 populations (see section 1 of this report), and employing a genotyped by RAD-tag sequencing approach (Table 1).

Table 1. Chinook RAD collections. Lineage is Coastal North (CN), Coastal South (CS), and Interior Stream-type (IS). Among the coastal south lineage are two Columbia River groups: lower Columbia (LC), and Interior Ocean-type (OT). Run-type is fall (F), spring (SP) and summer (SU).

Reference	POP code	POP ID	stream location	Lineage	Run-type	(N)	Lat.	Long.
1	KTS	Kantishna	Kantishnas R., AK	CN	U	42	64.7591	-149.966
2	GEO	George	George R. Weir, AK	CN	U	47	61.8975	-157.714
3	KNK	Kanektok	Kanektok R., AK	CN	U	47	59.7495	-161.927
4	TOG	Togiak	Togiak R. Weir, AK	CN	U	47	59.5323	-159.693
5	KAR	Karluk	Karluk R., AK	CN	U	41	57.5651	-154.377
6	MON	Montana	Susitna R., AK	CN	U	40	62.1818	-149.954
7	SIN	Sinona	Copper R., AK	CN	U	48	62.5723	-144.632
8	ALS	Alsek	Northern B.C. - AK border	CN	U	43	59.8667	-136.65
9	PULL	Pullen	Tahini R. stock, AK	CN	U	43	59.4516	-135.322
10	TAT	Tatsamenie	Northern B.C. - AK border	CN	U	40	58.4775	-132.385
11	MOR	Morice	Morice R., B.C.	CN	SU	48	54.4096	-126.746
12	ROB	Robertson	Robertson Cr. Hatchery, B.C.	CS	F	48	49.3167	-124.983
13	MBT	Marblemount	Marblemount Hatchery, WA	CS	SP	43	48.52	-121.43
14	SOO	Soos	Soos Cr. Hatchery, WA	CS	F	48	47.3009	-122.175
15	QUI	Quinault	Quinault Lake, WA	CS	F	48	47.4659	-123.887
16	MCK	McKenzie	McKenzie R. Hatchery, OR	LC	SP	48	44.123	-122.402
17	COW	Cowlitz	Cowlitz R. Hatchery, WA	LC	F	43	46.5101	-122.615
18	KAL	Kalama	Kalama R., WA	LC	SP	38	46.024	-122.738
19	SPR	Spring	Spring Cr. Hatchery, WA	LC	F	39	45.7277	-121.546
20	DES	Deschutes	Sherars Fs, Deschutes R., OR	OT	F	28	45.2571	-121.039
21	PRH	Priest	Priest Rapids Hatchery, WA	OT	F	46	46.64	-119.93
22	WNC	Wenatchee	Wenatchee R., Bonnaville Dam PIT-tag	OT	SU	46	47.6169	-120.723
23	WEL	Wells	Wells Hatchery, WA	OT	SU	45	47.9737	-119.888

24	LYO	LyonsFerry	Lyons Ferry Hatchery, OR	OT	F	48	45.59	-118.22
25	CLW	Clearwater	Clearwater R., Cherry Lane, ID	OT	F	33	46.5133	-116.686
42	NES	Nestucca	Nestucca R., OR	CS	F	43	45.2148	-123.844
43	ROC	Rock	Rock Cr., Umpqua R., OR	CS	SP	48	43.334	-123.003
44	COL	Cole	Cole M. Rivers Hatchery, Rogue R., OR	CS	SP	46	42.6635	-122.685
45	EEL	Eel	Eel River, OR	CS	F	34	40.6414	-124.312
46	BAT	Battle	Coleman-NFH Sacramento R., CA	CS	F	42	40.4006	-122.144
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26	WAR	WarmSPs	Warm Springs R., Deschutes R., OR	IS	SP	47	44.8624	-121.245
27	JDR	JohnDay	Bonneville Dam PIT-tag, John Day R., OR	IS	SP	12	45.7218	-120.635
28	YAK	Yakima	Prosser Dam, PIT-tag, Yakima R., WA	IS	SP	47	46.2122	-119.772
29	WEN	NasonChiwawa	Nason Cr./Chiwawa R., Wenatchee R., WA	IS	SP	33	47.6162	-120.723
30	WHI	White	White R., Wenatchee R., WA	IS	SP	18	47.8347	-120.819
31	MET	Methow	Bonneville PIT-tag, Methow R., WA	IS	SP	47	48.0489	-119.905
32	TUC	Tucannon	Tucannon R. WA.	IS	SP	46	46.3153	-117.662
33	CAT	Catherine	Catherine Cr., Grand Ronde R., OR	IS	SP	43	45.158	-117.779
34	IMN	Imnaha	Imnaha R., OR	IS	SP/SU	48	45.817	-116.765
35	NEW	Newsome	Newsome Cr., S. F. Clearwater R., ID	IS	SP	40	45.8288	-115.615
36	RAP	Rapid	Rapid River Hatchery, Salmon R., ID	IS	SP	46	45.3537	-116.395
37	JOC	Johnson	Johnson Cr., S. F. Salmon R., ID	IS	SP/SU	68	44.899	-115.492
38	MCH	McCall	McCall Fish Hatchery, S. F. Salmon R., ID	IS	SP/SU	43	44.667	-115.705
39	MSH	Marsh	Marsh Cr., M. F. Salmon R., ID	IS	SP/SU	39	44.449	-115.23
40	CAP	Capehorn	Cape Horn Cr., M. F. Salmon R., ID	IS	SP/SU	37	44.36	-115.228
41	PAH	Pahsimeroi	Pahsimeroi R., ID	IS	SP/SU	43	44.6822	-114.039

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For *O. nerka*, the baseline was expanded by eight collections (Table 2), comprised largely of transplanted kokanee stocks genotyped at 96 SNP loci described in Hess et al. (2012).

Table 2. Sockeye salmon and Kokanee collections included in the *O. nerka* SNP baseline. Life histories are either kokanee (K), or sockeye salmon (Sock). Origin is known hatchery stock (Hat), natural origin (N) or unknown (U). Collections in bold text (\*) represent additions for the 2013 report year.

ID#	Lake/River	Collection	BPA Subbasin	Year	(n)	Lat.	Long.	life history	Origin
1	Alturas Lake	Alturas Lake	Salmon	2008	59	43.909	-114.865	K	U
2	Redfish Lake	Fishhook Creek	Salmon	2007	96	44.128	-114.971	K	U
3	Pettit Lake	Pettit Lake Creek	Salmon	2003	70	43.978	-114.882	K	U
4	Redfish Lake	Captive Broodstock	Salmon	2009	86	44.131	-114.927	Sock	Hat.
5	Stanley Lake	Stanley Lake Creek	Salmon	2011	53	44.209	-115.084	K	U
6	South Fork Salmon River	Warm Lake	Salmon	2003	68	44.648	-115.671	K	N
7	Wallowa Lake	stream spawners	Grande Ronde	2011	43	45.281	-117.209	K	N
8*	<b>Wallowa Lake</b>	<b>shore spawners</b>	<b>Grande Ronde</b>	2011	23	45.281	-117.209	K	N
9	Suttle Lake	Lake Creek outlet	Deschutes	2009	99	44.427	-121.727	K	U
10	Suttle Lake	Lake Creek outlet	Deschutes	2010	92	44.427	-121.727	K	U
11	Suttle Lake	Lake Creek outlet	Deschutes	2011	100	44.427	-121.727	K	U
12*	<b>Suttle Lake</b>	<b>Link Creek</b>	<b>Deschutes</b>	2012	100	44.418	-121.756	K	U
13	Lake Billy Chinook	SWW Facility	Deschutes	2010	98	44.603	-121.280	K	U
14	Lake Billy Chinook	SWW Facility	Deschutes	2011	100	44.603	-121.280	K	U
15	Lake Billy Chinook	Metolius River Inlet	Deschutes	2009	98	44.603	-121.280	K	U
16	Lake Billy Chinook	Metolius River Inlet	Deschutes	2010	94	44.603	-121.280	K	U
17*	<b>Lake Billy Chinook</b>	<b>Metolius River Inlet</b>	<b>Deschutes</b>	2012	100	44.603	-121.280	K	U
18*	<b>Odell</b>	<b>Odell Lake</b>	<b>Deschutes</b>	2012	59	43.700	-122.034	K	Hat.
19*	<b>Paulina</b>	<b>Paulina Lake</b>	<b>Deschutes</b>	2012	99	43.713	-121.277	K	Hat.
20	Deschutes R.	Pelton Fish Trap	Deschutes	2010	23	44.603	-121.280	Sock	U
21	Deschutes R.	Pelton Fish Trap	Deschutes	2011	22	44.603	-121.280	Sock	U
22*	<b>Deschutes R.</b>	<b>Pelton Fish Trap</b>	<b>Deschutes</b>	2012	98	44.603	-121.280	Sock	U
23*	<b>Wickiup</b>	<b>Wickiup Reservoir</b>	<b>Deschutes</b>	2012	5	43.700	-121.762	K	Hat.
24*	<b>Wizard</b>	<b>Wizard Falls Hatchery</b>	<b>Deschutes</b>	2012	100	43.713	-121.277	K	Hat.
25	Meadow Creek	Meadow Creek	B.C. Okanagan	2005	46	50.217	-116.983	K	Hat.
26	Wenatchee	Tumwater Dam	Wenatchee	2004,09,11	344	47.617	-120.723	Sock	N
27	Okanagan	Wells Dam	Upper Columbia	2004,09	212	47.945	-119.866	Sock	N
28*	<b>Palmer</b>	<b>Palmer Lake</b>	<b>Upper Columbia</b>	2012	62	48.910	-119.645	K	Hat.
29	Lake Whatcom	Lake Whatcom	Puget Sound	2005	46	48.673	-122.278	K	Hat.

No expansion genotypes were produced for *O. mykiss* in 2013. However, a three year plan was laid out for RAD sequencing of Columbia River steelhead stocks (Table 3). Implementation for the first year began in January 2014, including RAD library preparation for 30 populations. Our two primary goals for these three species are: 1) annual genetic stock identification (GSI) analyses that will be used for monitoring of fishery returns through the migratory corridor, including harvest GSI in the lower Columbia River, and fish passage GSI at Bonneville and Lower Granite dams (see sections 3 & 4 of this report), 2) maintenance of a ten year SNP baseline expansion (including RAD-tag sequencing) for application in various analyses including population structure analyses, investigation of landscape genetics, and adaptive differentiation among populations.

Table 3. Steelhead RAD collections and metadata. Distinct populations segments (DPS) are: lower Columbia (LC), middle Columbia (MC), upper Columbia (UC), upper Willamette (UW) and Snake River. Origin in natural (N) or hatchery (Hat.), run is winter (WIN), summer (S) or unknown (U). Stage is adult (A), juvenile (J) or smolt (S).

pop ID	Name	tributary/ region	DPS	MPG	(n)	lat	Long	Origin	Run	Stage
<b><u>Coastal</u></b>										
POP01	Abernathy Cr.	Abernathy	LC	Cascade	164	46.226	-123.148	N	WIN	A
POP02	Kalama R.	Kalama	LC	Cascade	94	46.045	-122.804	N	S	A
POP03	Kalama R.	Kalama	LC	Cascade	94	46.045	-122.804	N	WIN	A
POP04	Skamania Stock	Clackamas	LC	Willamette - Cascade	59	45.242	-122.282	Hat.	S	A
POP05	Big White Salmon R.	Big White Salmon	MC*	Cascades Eastern Slope	78	45.799	-121.485	N	<u>U</u>	J/A
POP06	Canyon/ Luckiamute Cr.	West Side	UW	Willamette	51	44.908	-123.419	N	<u>U</u>	J
POP07	East Fork Hood R.	Hood	LC	Gorge	52	45.575	-121.627	N	WIN	S
POP08	West Fork Hood R.	Hood	LC	Gorge	35	45.605	-121.634	N	S	S
POP09	N. F. Eagle Cr.	Clackamas	LC	Willamette - Cascade	90	45.325	-122.289	N	WIN	J/S
<b><u>Inland</u></b>										
POP10	Trout Cr.	Deschutes	MC	Cascades Eastern Slope	57	44.822	-121.086	N	S	J
POP11	Fifteen Cr.	Fifteen	MC	Cascades Eastern Slope	91	45.625	-121.066	N	WIN	J
POP12	Upper M. F. J. D.	M. F. John Day	MC	John Day	107	44.620	-118.568	N	S	J
POP13	Granite Cr.	N. F. John Day	MC	John Day	74	44.838	-118.477	N	S	J
POP14	Bowman Cr.	Klickitat	MC	Cascades Eastern Slope	48	45.845	-121.042	N	S	J
POP15	Iskuulpa Cr.	Umatilla	MC	Umatilla And Walla Walla	148	45.700	-118.397	N	S	A/J
POP16	Nile/ Naches R.	Yakima	MC	Yakima	59	46.834	-120.945	N	S	J
POP17	Satus Cr.	Yakima	MC	Yakima	46	46.262	-120.112	N	S	J
POP18	Joseph/ Elk Cr.	Grande Ronde	Snake	Grande Ronde	90	46.028	-117.018	N	S	A
POP19	Wenaha R.	Grande Ronde	Snake	Grande Ronde	93	45.945	-117.451	N	S	J
POP20	Big Sheep Cr.	Imnaha	Snake	Imnaha	61	45.557	-116.835	N	S	J
POP21	Fish Cr.	M. F. Clearwater	Snake	Clearwater	99	46.334	-115.347	N	S	A
POP22	N. F. Moose	M. F. Clearwater	Snake	Clearwater	92	46.163	-114.901	N	S	J
POP23	Crooked R.	S. F. Clearwater	Snake	Clearwater	104	45.821	-115.527	N	S	A
POP24	Tucannon R.	lower Snake	Snake	Lower Snake	105	46.310	-117.657	N	S	A



POP25	Whitebird Cr.	Lower Salmon	Snake	Salmon	59	45.752	-116.320	N	S	J
POP26	Loon Cr.	M. F. Salmon	Snake	Salmon	84	44.598	-114.812	N	S	J
POP27	Secesh R./ Lick Cr.	S. F. Salmon	Snake	Salmon	84	45.027	-115.708	N	S	J
POP28	Pahsimeroi Weir	Upper Salmon	Snake	Salmon	96	44.684	-114.040	N	S	A
POP29	Methow R.	Methow	UC	Upper Columbia	90	48.476	-120.182	N	S	S
POP30	Salmon Cr.	Okanagan	UC	Upper Columbia	98	48.375	-119.591	N/Hat.	S	A
POP31	Chiwaukum R.	Wenatchee	UC	Upper Columbia	54	47.688	-120.741	N	S	J

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Note: Big White Salmon and Klickitat Rivers occur in a transition between the lower Columbia and middle Columbia DPS and could belong to either.

### *Laboratory Protocol*

Methods for DNA extraction, DNA amplification, microfluidics, and genotyping of SNP assays are available at (<https://www.monitoringmethods.org/Protocol/Details/230>). The Monitoring Methods template for section 2 of this report is ID#230, owner: Matthew Campbell. Additional species or population specific details are available in Hess et al. (2012). For methods using genotyping by sequencing, or RAD-tag sequencing see Monitoring Methods #4144, owner: Nathan Campbell. SNP discovery using the RAD-tag sequencing technique is described in section #1 of this report.

### *Statistical Analysis:*

Allele frequencies (i.e. minor allele frequency at each locus; MAF), mean pairwise  $F_{ST}$  (among-group variation), and number of samples analyzed per population per locus were calculating using the program GenAlEx version 6.2 (Peakall and Smouse 2006). The Markov Chain Monte Carlo (MCMC) approximation of Fisher's exact test implemented in GENEPOP v. 3.3 (1000 batches with 1000 iterations; Raymond and Rousset 1995) to test for deviations from Hardy-Weinberg equilibrium (HWE) expectations. Linkage disequilibrium was tested for all pairs of loci across populations using a simulated exact test in GENEPOP. Statistical significance ( $\alpha$ ) was adjusted for the number of simultaneous tests (initial  $\alpha = 0.05$ ) for both HWE and linkage tests via the B-Y FDR method (Benjamini and Yekutieli 2001) as implemented by Narum (2006) to reduce false positive tests. For RAD sequence data, allele frequencies, number of samples analyzed, Hardy-Weinberg equilibrium (HWE) tests, and observed heterozygosity were calculated at the individual and population level, at each locus using Microsoft Excel 2010. Matrices of pairwise  $F_{ST}$  (among-group variation) were calculating using GENEPOP v. 3.3. The program LOSITAN (Antao *et al.* 2008) was used to evaluate the relationship between  $F_{ST}$  and  $H_e$  (expected heterozygosity) for all loci in an island model, to identify outlier loci (candidates for selection) having excessively high or low  $F_{ST}$  compared to neutral expectations. We used data simulations based on 50,000 replicates, and a 0.995 confidence interval for all SNP loci under an infinite alleles model. Loci exceeding the 0.995 quantile were deemed outliers for putative adaptive divergence, while all loci between the 0.001 and 0.950 quantiles were deemed as putatively neutral. Pairwise matrices of Nei's genetic distances (1972) and un-rooted neighbor-joining (NJ) phylograms were generated using PHYLIP version 3.68 (Felsenstein 1992). The SEQBOOT option was implemented to generate 1000 simulated data sets, and consensus topology with bootstrap support was generated using the CONSENSE option in PHYLIP. For Chinook salmon, the NJ-trees and comparisons of pairwise  $F_{ST}$  were evaluated for all 21,605 loci and for a subset of putatively neutral loci. Bayesian cluster analyses were conducting using the analysis program STRUCTURE version 2.3.4 (Pritchard et al. 2000) to estimate the mean membership coefficients ( $Q$ ), or fractional cluster membership for *O. nerka* populations, among an inferred (tested) number of probable populations ( $k$ ) ranging from 2-12. Default settings were used for the ancestry and frequency models, with a burn-in of 50,000 and 250,000 MCMC repetitions. The most likely number of distinct populations (in the range of  $k$ ) was selected based on distinct clustering with minimal ambiguity.

## **Results**

### *Chinook salmon*

A total of 22,502 RAD-tag SNP loci were recovered. Loci were excluded if allele frequencies were fixed for a lineage or across lineages, and when greater than 30% of collections exhibited

deviation from HWE expectations, resulting in 21,619 SNPs that passed quality screening. Of these, a subset of 5,789 loci were determined to be putatively neutral, and 2,871 (13.3%) were considered as adaptation candidate loci. The mean observed heterozygosity (allelic diversity) across loci and collections ranged from 0.075 in the Spring Creek collection (#19), to 0.167 in the Tatsamenie collection (#10). Proportion of loci fixed per collection ranged from 20% (#44) to 56% (#7). The largest proportion of loci deviating from HWE expectations occurred in Clearwater R., Cherry Lane (#25; Table 4). Statistics per lineage are also provided (Table 5).

Table 4. Descriptive statistics for Chinook RAD collections.

order	POP code	%HWE (Bon)	%HWE (BY)	H <sub>O</sub>	H <sub>O</sub> /H <sub>E</sub>	#Fixed	%Fixed
1	KTS	0.4	2.7	0.1239	-0.0087	11293	50.2
2	GEO	1.3	8.0	0.1165	-0.0260	9530	42.4
3	KNK	0.4	2.8	0.1403	-0.0050	9154	40.7
4	TOG	3.0	17.3	0.0982	-0.0455	9406	41.8
5	KAR	0.5	3.1	0.1278	-0.0102	10627	47.2
6	MON	0.8	5.7	0.1205	-0.0219	10028	44.6
7	SIN	0.4	2.9	0.1152	-0.0085	12586	55.9
8	ALS	1.2	8.5	0.1032	-0.0303	11259	50.0
9	PULL	1.0	6.5	0.1213	-0.0237	10375	46.1
10	TAT	0.5	3.0	0.1665	-0.0033	6548	29.1
11	MOR	0.9	5.8	0.1504	-0.0168	6348	28.2
12	ROB	1.0	6.1	0.1506	-0.0185	6960	30.9
13	MBT	0.7	4.7	0.1502	-0.0152	7329	32.6
14	SOO	0.7	3.8	0.1610	-0.0069	6798	30.2
15	QUI	3.0	17.7	0.1267	-0.0471	6216	27.6
16	MCK	1.8	10.6	0.1210	-0.0336	8603	38.2
17	COW	2.5	14.2	0.1248	-0.0403	6298	28.0
18	KAL	1.2	5.9	0.1169	-0.0238	9870	43.9
19	SPR	5.3	26.0	0.0746	-0.0666	9788	43.5
20	DES	4.2	25.0	0.0827	-0.0789	7899	35.1
21	PRH	2.1	11.4	0.1308	-0.0310	6100	27.1
22	WNC	2.8	13.0	0.1278	-0.0340	5982	26.6
23	WEL	1.1	5.3	0.1505	-0.0115	6219	27.6
24	LYO	1.4	7.3	0.1454	-0.0195	5721	25.4
25	CLW	5.5	26.9	0.0887	-0.0771	6966	31.0
42	NES	2.7	15.5	0.1217	-0.0447	7140	31.7
43	ROC	2.1	11.7	0.1436	-0.0317	4483	19.9
44	COL	2.8	15.4	0.1215	-0.0417	6789	30.2
45	EEL	1.3	7.2	0.1117	-0.0313	9739	43.3
46	BAT	4.7	24.4	0.0779	-0.0606	10221	45.4
26	WAR	1.7	9.1	0.1197	-0.0294	8224	36.5
27	JDR	0.0	2.2	0.1301	-0.0174	9942	44.2
28	YAK	1.9	10.2	0.1286	-0.0295	6092	27.1
29	WEN	0.5	2.7	0.1550	0.0003	7809	34.7
30	WHI	0.0	1.3	0.1590	0.0071	9445	42.0
31	MET	0.0	10.2	0.1197	-0.0286	6731	29.9
32	TUC	1.1	6.5	0.1345	-0.0202	7133	31.7
33	CAT	0.7	4.3	0.1273	-0.0131	8372	37.2

34	IMN	3.6	10.8	0.1332	-0.0218	5404	24.0
35	NEW	1.6	9.3	0.1051	-0.0308	9317	41.4
36	RAP	1.3	8.2	0.1171	-0.0258	8597	38.2
37	JOC	3.9	14.5	0.1156	-0.0300	6734	29.9
38	MCH	2.5	13.3	0.1075	-0.0367	7970	35.4
39	MSH	1.5	7.1	0.1196	-0.0245	7966	35.4
40	CAP	0.5	2.8	0.1265	-0.0081	10367	46.1
41	PAH	1.2	6.8	0.1173	-0.0214	9044	40.2

Mean pairwise  $F_{ST}$  across 46 collections and 5,789 putatively neutral loci ranged from 0.128 (#22) to 0.241 (#18). Ranges in mean pairwise among-group variation varied widely, but were lowest among ocean-type and interior stream-type comparisons (Figure 2a; Table 5). Mean pairwise  $F_{ST}$  across 46 collections and 21,619 putative candidate loci for divergent selection ranged from 0.158 (#29) to 0.286 (#18).

Table 5. Descriptive statistics by lineage for Chinook RAD collections.

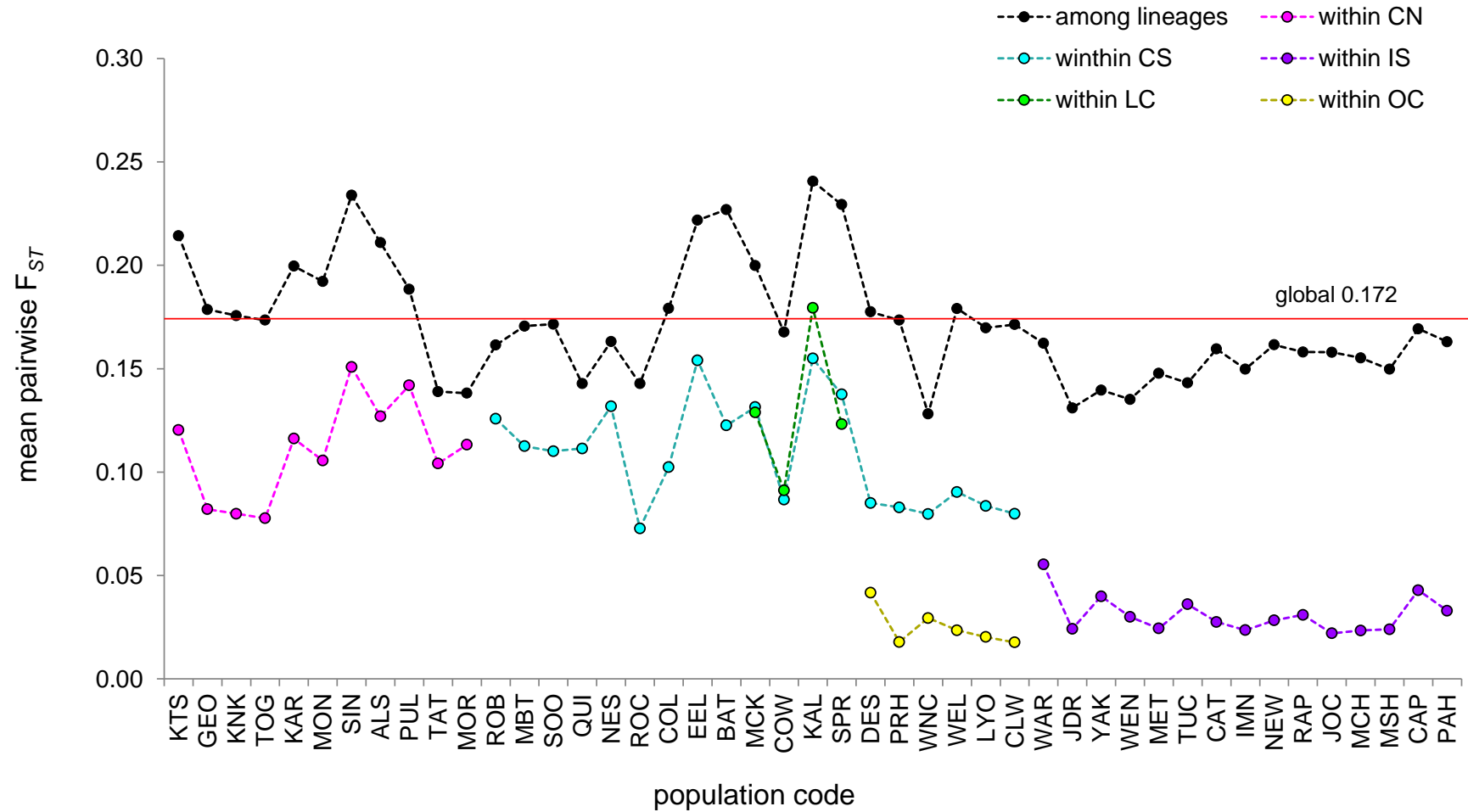
21,619 RAD-tags	CN	CS	IS	overall
# Outlier loci	406	637	430	1946
adaptive loci	---	---	---	2871
neutral loci	---	---	---	5789
Fixed Loci	3156	558	2649	6363
mean $F_{ST}$ (5,789)	0.111	0.108	0.031	0.172
mean $F_{ST}$ (21,619)	0.120	0.115	0.036	0.206
mean $H_O$	0.126	0.123	0.126	0.125
mean %HWE	6.0	13.3	7.5	9.5

Ranges in mean pairwise among-group variation by lineage varied widely, but were lowest within the ocean-type subgroup and within interior stream-type comparisons (Figure 2b; Table 5). We demonstrated genetic similarity among populations within the topology of unrooted NJ phylograms (Figure 3a & 3b). Results revealed defined clustering of collections between the three major lineages, indicating clear divergence (most with 100% bootstrap support). The coastal north group was most genetically similar to the interior stream-type. Genetic distances were generally largest between the three subgroups within the coastal south lineage. In large part, the Chinook salmon collections clustered accurately into geographic regions or major tributaries, and there were no discernible differences between topologies based on all loci or exclusively neutral loci (Figure 3). Note that 2013 expansion collections from White River, Nason Creek and Chiwawa River were genotyped for both the panel of 196 SNP loci and using RAD-tag sequencing. Results indicate no significant difference between all three Wenatchee River

populations, warranting the decision to combine all three into one analysis group for GSI analyses (see Sections 3 & 4 of this report).

Figure 2. Mean pairwise  $F_{ST}$  plots among Chinook salmon collections for (a) the set of neutral loci and (b) the set of all loci. Numbered collections are identified in Table 1. Results display among-group variation between collections across all lineages combined (black), and lineage (or subgroup) specific comparisons. The red line indicates the global  $F_{ST\ value}$  across collections and loci.

a.



b.

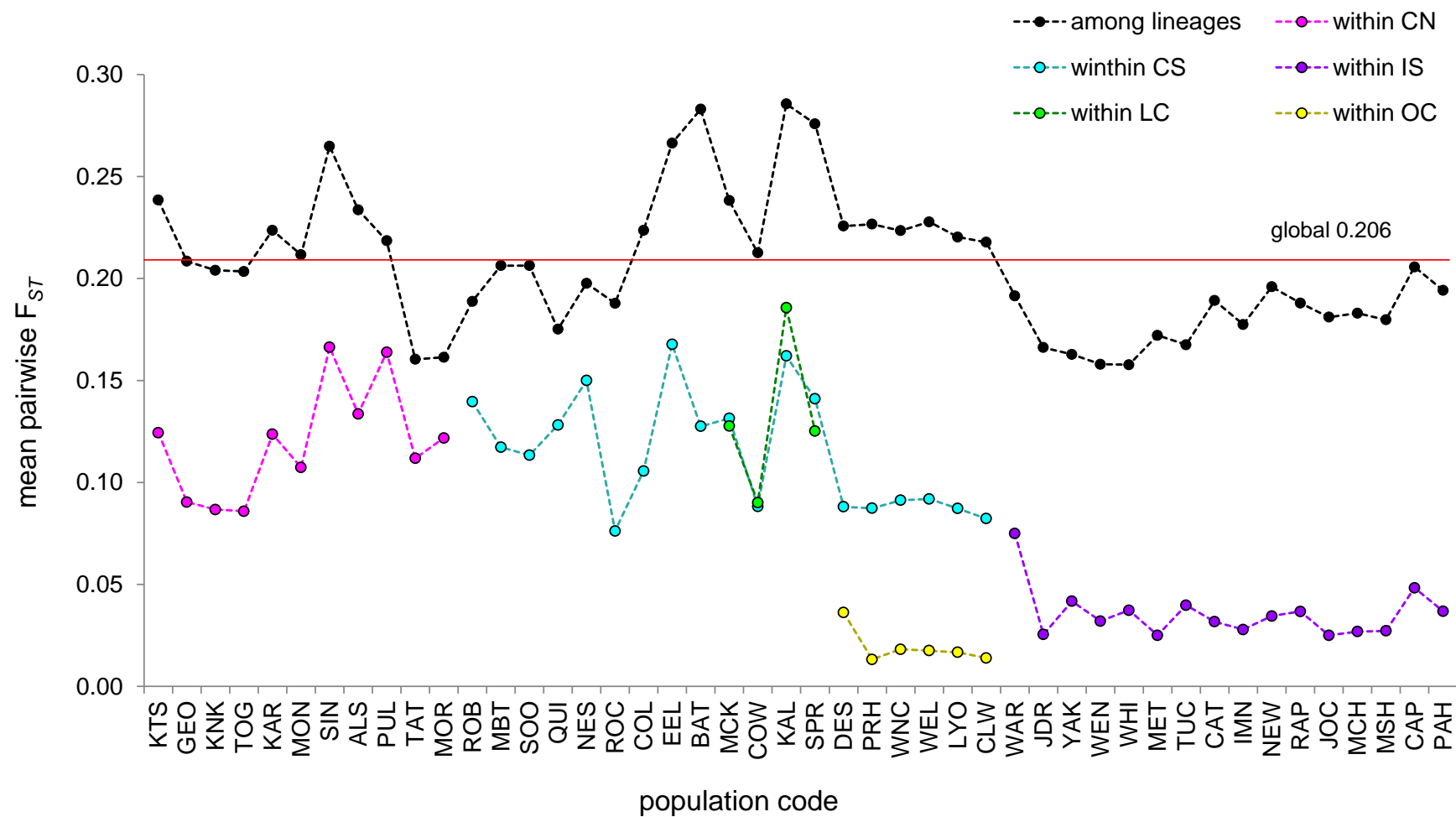
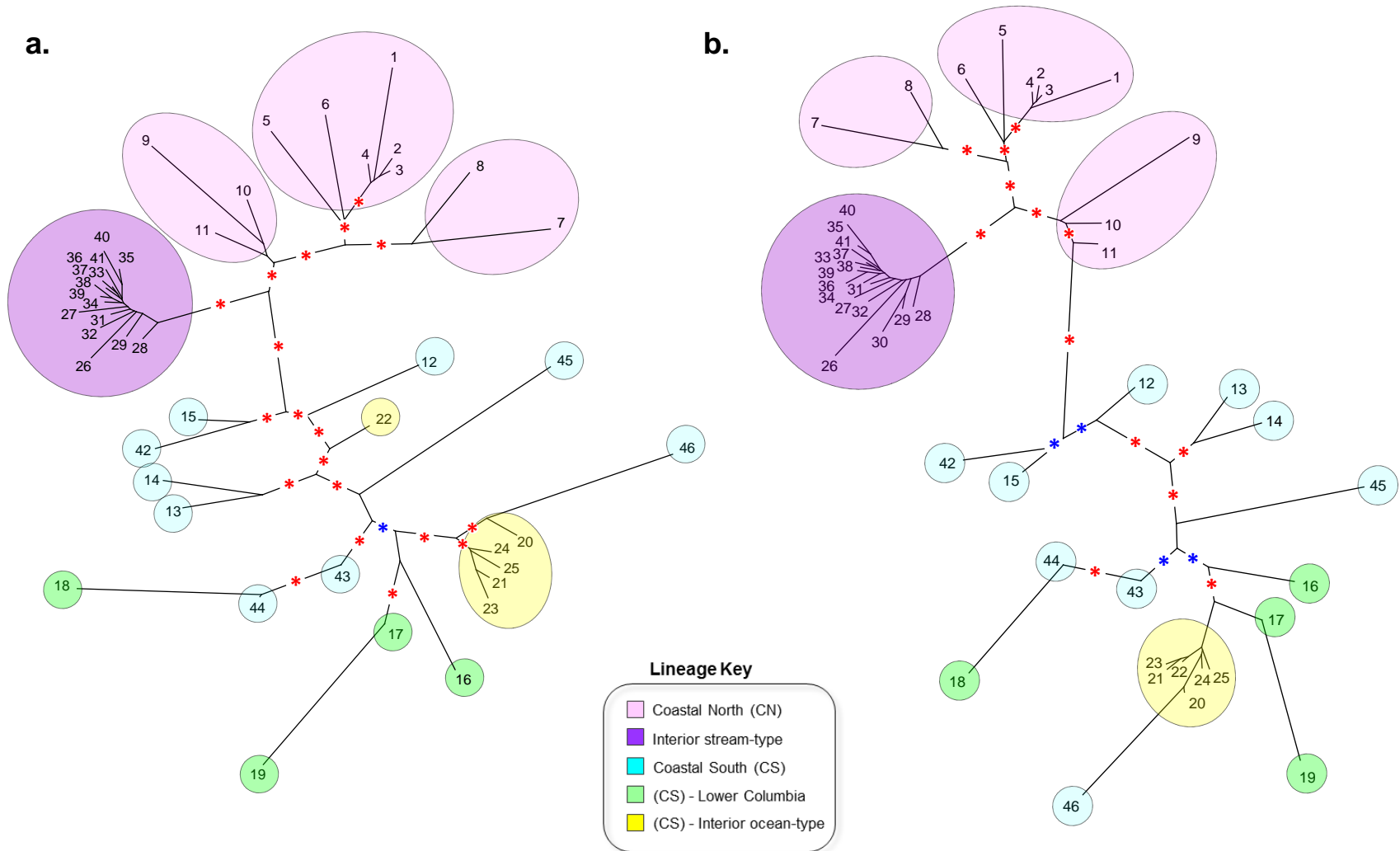


Figure 3. Chinook RAD neighbor-joining trees for a) the set of all neutral loci, and b) the set of all loci. Collection reference numbers correspond with Table 1. Asterisks indicate 100% bootstrap support (red) and >95% bootstrap support (blue) based on 1000 replicate data sets.





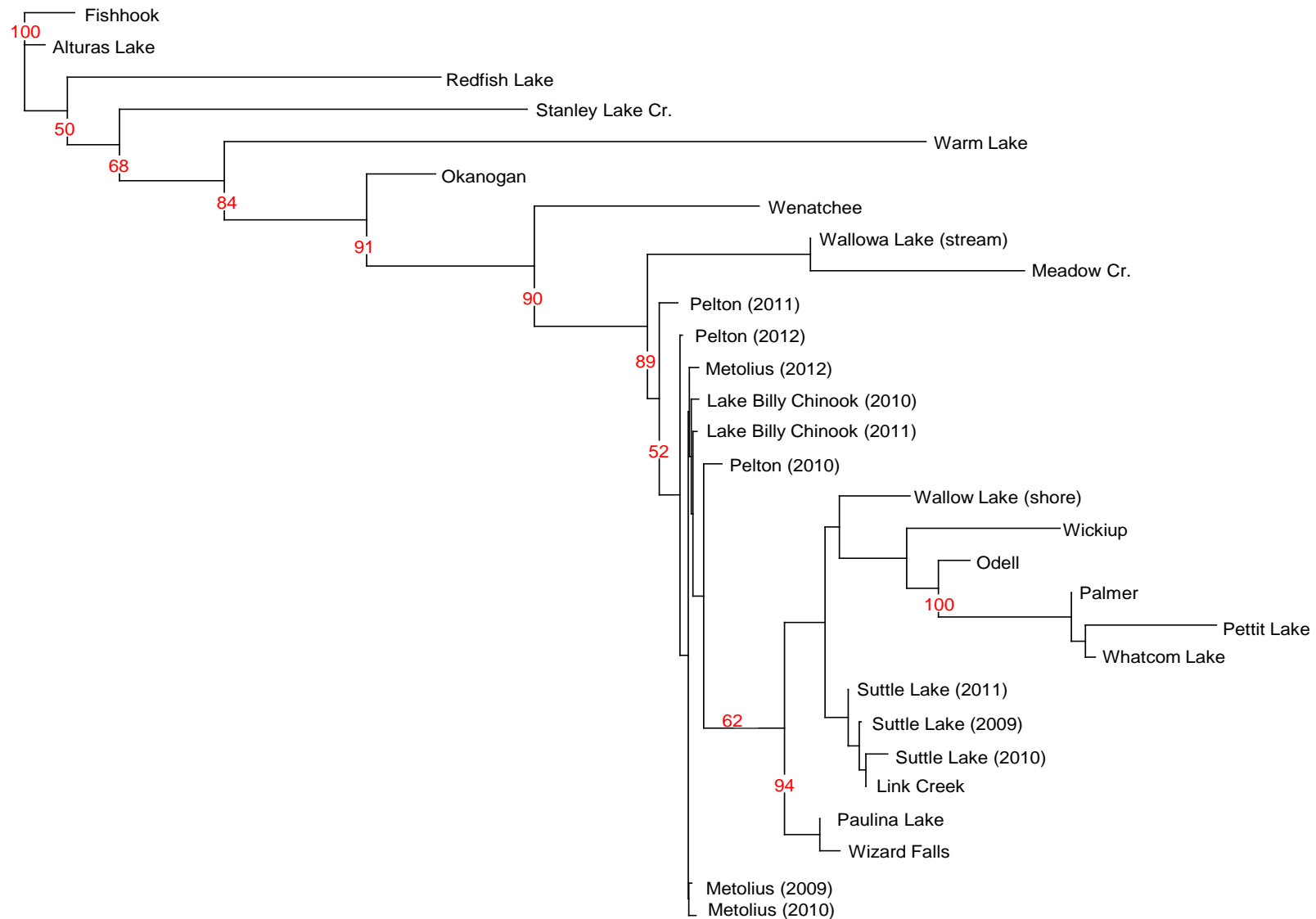
### *O. nerka*

The mean observed heterozygosity across 96 loci and among 29 collections ranged from 0.154 in Redfish Lake captive broodstock to 0.329 in the Lake Billy Chinook 2011 collections. There were no fixed allele frequencies across loci and baseline collections, and no loci appear diagnostic for either life history type in the data set (kokanee or sockeye salmon). The mean observed pairwise  $F_{ST}$  across loci ranged from 0.004 in the Wizard Falls collection to 0.323 in the Redfish Lake collection (Figure 4). In general, all comparisons indicate significant variation with the exception of Deschutes River collections: no difference among temporal Suttle Lake collections and no difference between Temporal LBC/MET collections, or LBC/MET and Pelton trap samples (see Table 2). We demonstrated genetic similarity among populations within each region or major subbasin through phylogenetic relationships in the topology of an unrooted NJ phylogram (Figure 5). The confidence or concordance (>50%) of the NJ topology is indicated with bootstrap values at the nodes. Results revealed defined clustering of the most genetically similar collections for *O. nerka*. Collections within each region, particularly temporally stratified collections, exhibited substantial similarity while populations between regions (i.e., Wenatchee River, Deschutes River, Osoyos Lakes, and Snake River) were highly divergent (Figures 5 & 6). Results were complimentary to those from pairwise  $F_{ST}$ , indicating no distinction between temporal Suttle Lake Samples, or temporal samples from Lake Billy Chinook and the Metolius River in the Deschutes River system.

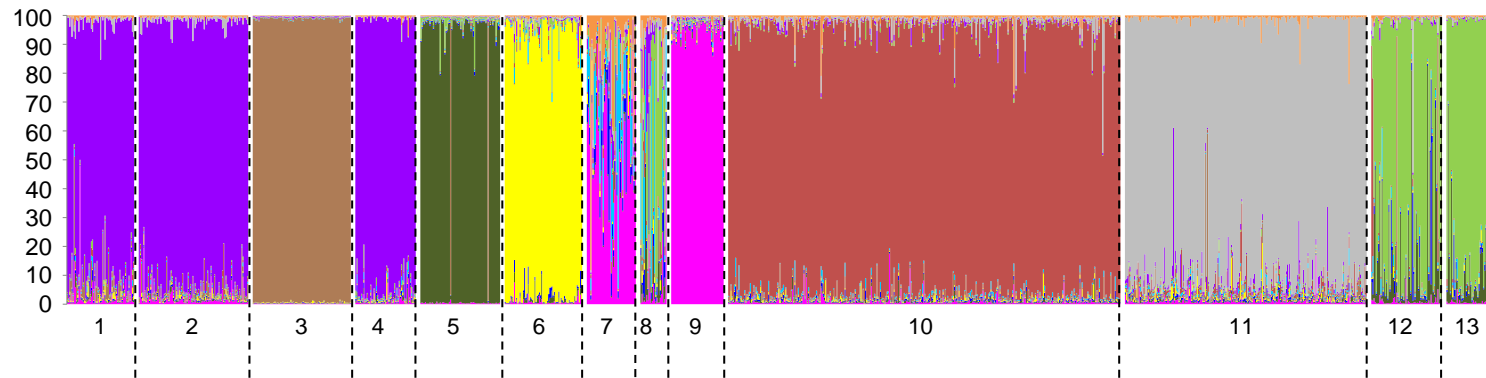
Figure 4. Pairwise among-group variation for *O. nerka*. Numbered collections are identified in Table 2. The  $F_{ST}$  values appear in the lower half matrix while the upper half matrix is the corresponding probability values. Most comparisons are statistically significant ( $P=0$ );  $F_{ST}$  values that are not significantly different between collections appear in red text.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0.024			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0.341	0.357			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0.201	0.221	0.479			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0.169	0.198	0.421	0.373			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0.321	0.340	0.464	0.487	0.430			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0.174	0.179	0.183	0.333	0.272	0.365			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0.204	0.225	0.102	0.370	0.291	0.396	0.058			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.003	0	0	0	0	0
9	0.256	0.275	0.088	0.375	0.317	0.370	0.073	0.030		0.063	0.709	0.072	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0.270	0.289	0.075	0.390	0.330	0.382	0.085	0.030	0.001		0.002	0.073	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0.253	0.271	0.095	0.375	0.313	0.365	0.071	0.031	0.000	0.003		0.288	0	0	0	0	0	0	0	0	0	0	0	0.001	0	0	0	0	0
12	0.260	0.279	0.089	0.385	0.322	0.373	0.072	0.032	0.001	0.002	0.001		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0.195	0.211	0.165	0.299	0.275	0.337	0.042	0.054	0.050	0.061	0.042	0.050	0.924	0.839	0.270	0.127	0	0	0.684	0.092	0.575	0	0	0	0	0	0	0	0
14	0.195	0.210	0.169	0.300	0.270	0.332	0.038	0.054	0.048	0.060	0.042	0.050	-0.001	0.930	0.276	0.381	0	0	0.593	0.123	0.216	0	0	0	0	0	0	0	0
15	0.196	0.211	0.173	0.305	0.274	0.335	0.040	0.057	0.049	0.061	0.041	0.050	-0.001	-0.001	0.989	0.733	0	0	0.660	0.237	0.711	0	0	0	0	0	0	0	0
16	0.203	0.219	0.183	0.311	0.277	0.340	0.042	0.062	0.056	0.069	0.048	0.057	0.001	0.000	-0.002	0.297	0	0	0.499	0.020	0.446	0	0	0	0	0	0	0	0
17	0.205	0.218	0.178	0.312	0.281	0.341	0.040	0.061	0.053	0.065	0.047	0.054	0.001	0.000	-0.001	0.000	0	0	0.707	0.015	0.078	0	0	0	0	0	0	0	0
18	0.234	0.253	0.098	0.390	0.316	0.392	0.055	0.048	0.047	0.045	0.050	0.044	0.083	0.084	0.084	0.089	0.087		0	0	0	0	0.073	0	0	0	0	0	0
19	0.248	0.265	0.138	0.365	0.307	0.367	0.062	0.052	0.013	0.022	0.011	0.015	0.032	0.029	0.030	0.035	0.034	0.067		0	0	0	0.001	0.122	0	0	0	0	0
20	0.224	0.243	0.174	0.369	0.317	0.402	0.035	0.050	0.038	0.052	0.036	0.042	-0.001	-0.001	-0.001	0.000	-0.001	0.073	0.027		0.638	0.717	0	0	0	0	0	0	0
21	0.200	0.216	0.180	0.350	0.293	0.394	0.032	0.047	0.053	0.064	0.047	0.054	0.004	0.003	0.002	0.005	0.006	0.080	0.039	-0.001		0.140	0	0	0	0	0	0	0
22	0.191	0.205	0.178	0.295	0.270	0.335	0.037	0.057	0.058	0.069	0.050	0.059	0.000	0.001	0.000	0.000	0.001	0.087	0.038	-0.001	0.003		0	0	0	0	0	0	0
23	0.269	0.288	0.111	0.470	0.363	0.478	0.044	0.043	0.039	0.044	0.040	0.037	0.056	0.056	0.056	0.063	0.059	0.014	0.044	0.045	0.045	0.062		0	0	0	0	0.002	0
24	0.265	0.281	0.157	0.378	0.325	0.377	0.077	0.068	0.021	0.032	0.018	0.023	0.036	0.033	0.034	0.040	0.040	0.088	0.001	0.034	0.046	0.043	0.072		0	0	0	0	0
25	0.207	0.213	0.254	0.412	0.321	0.429	0.047	0.142	0.157	0.169	0.159	0.155	0.127	0.122	0.127	0.127	0.123	0.096	0.148	0.131	0.119	0.120	0.112	0.170		0	0	0	0
26	0.266	0.268	0.304	0.308	0.351	0.385	0.166	0.188	0.218	0.226	0.205	0.218	0.111	0.116	0.109	0.105	0.113	0.216	0.205	0.118	0.101	0.100	0.200	0.214	0.245		0	0	0
27	0.120	0.127	0.245	0.185	0.226	0.276	0.125	0.132	0.181	0.190	0.177	0.187	0.116	0.116	0.112	0.117	0.121	0.167	0.174	0.120	0.106	0.105	0.164	0.185	0.178	0.113		0	0
28	0.297	0.320	0.042	0.436	0.368	0.435	0.136	0.044	0.042	0.027	0.048	0.042	0.123	0.125	0.126	0.135	0.130	0.049	0.087	0.120	0.126	0.133	0.065	0.108	0.204	0.262	0.213		0.386
29	0.325	0.347	0.049	0.478	0.395	0.453	0.147	0.056	0.047	0.031	0.053	0.047	0.135	0.135	0.136	0.144	0.139	0.054	0.094	0.132	0.140	0.143	0.071	0.115	0.215	0.274	0.230	0.001	

Figure 5. Neighbor-joining trees based on Nei's genetic distance (Nei 1972) among *O. nerka* collections. Bootstrap support greater than 50% is identified in red text at nodes.



Based on Bayesian clustering analysis using STRUCTURE, the most likely number (inferred) of populations for the data set is  $k=11$ . The resulting membership coefficients and clustering indicate that Lake Billy Chinook (LBC) and Metolius River (MET) collections are genetically indistinct, as are Suttle Lake (SUT) and Link Creek (LIN) collections. However, the LBC/MET population is significantly differentiated from the SUT/LIN population. Note that one population component is generally unique to LBC/MET from among all 29 collections evaluated. Wentachee River sockeye and Okanagan sockeye populations remain distinct from each other and from all other reference collections in the baseline. The Sawtooth valley lakes collections from the Snake River Basin include at least four distinct groups (Figure 6). Kokanee hatchery stocks from Meadow Creek and Whatcom Lake appear to cluster relatively tightly with populations which have likely experienced significant outplanting in the past. For example, Both LBC/MET and SUT/LIN include a proportion of individuals that appear most similar to Paulina Lake *O. nerka* (including Wizard Falls Hatchery), but among the two, only SUT/LIN shows a significant presence of Lake Whatcom hatchery stock. The genetic characterizations of Palmer Lake, Wallowa Lake shore spawners, and Odell Lake are clearly influenced by the presence of Lake Whatcom hatchery stock based on Bayesian cluster analysis.



**Snake River**

- 01. Alturas Lake
- 02. Fishhook Creek
- 03. Redfish Lake
- 04. Stanley Lake
- 05. Pettit Lake
- 06. Warm Lake
- 07. Wallowa Lake (stream spawner)
- 08. Wallowa Lake (shore spawner)

**Washington/B.C.**

- 09. Meadow Creek
- 10. Lake Wenatchee
- 11. Okanogan River/ Osoyoos Lake
- 12. Palmer Lake
- 13. Lake Whatcom

**Deschutes River Subbasin**

- 14. Suttle Lake
- 15. Link Creek
- 16. Lake Billy Chinook
- 17. Metolius River
- 18. Odell Lake
- 19. Paulina Lake
- 20. Wizard Falls Hatchery
- 21. Pelton Adult Trap (2010)
- 22. Pelton Adult Trap (2011)
- 23. Pelton Adult Trap (2012)

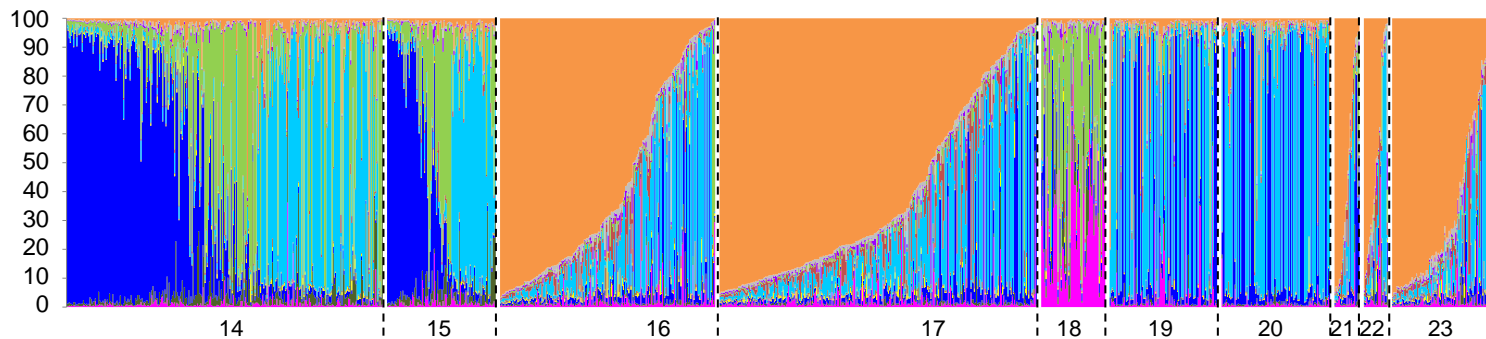


Figure 6.

Bayesian cluster analysis for *O. nerka* collections using  $k=11$ . Individuals by collection are on the x-axis (vertical lines) and the y-axis provides the percent membership by inferred cluster.

## Discussion

We have compiled extensive data sets of SNP genotypes for Chinook salmon and *O. nerka* covering diverse regions in the Columbia River Basin (including the Snake River Basin). The steelhead trout data set is the most extensive but has not changed since our 2012 report. Our goal was to construct SNP baselines of genotypes that will be expanded annually to provide continued evaluation of these species that is both spatially and temporally stratified when and where possible. 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) will play a major role in how genetic divergence and differentiation is distributed geographically, and it will be important to evaluate such effects on the ability to differentiate populations both qualitatively (phenotypes; landscapes) and quantitatively (e.g., genetic stock identification)

The results presented in this report substantiate and complement differentiation of groups of Chinook salmon (Waples et al. 2004; Narum 2008b; Narum et al. 2010b). Results further suggest SNPs are a class of markers that perform well in terms of their potential for monitoring population distinctions and composition during fish migrations and fisheries harvests. We have currently identified outlier loci for adaptive divergence, and in the next report year we will investigate the Chinook salmon and steelhead RAD datasets in order to demonstrate that SNPs offer an opportunity to characterize adaptive variation. Environmental/climate related variables 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 expansion efforts reported here complement previously reported results. The continued expansion of SNP panels and updating of baseline collections will help us achieve a greater level of resolution (or statistical power to identify population distinctions), at least among the major tributaries and subbasins throughout the CRB. Such results will be most beneficial to the larger application of the baselines, namely genetic stock ID. Annual implementations of each baseline expansion have proven valuable for frequently increasing resolution on a regional scale (but also locally). In comparisons among species-specific SNP panels, the panel for *O. nerka* has been extremely powerful owing to the biological nature of the species and its distribution within the CRB. In the past, our ability to differentiate among regions and in some cases within regions has exceeded what we have been able to ascertain with Chinook salmon and steelhead. However the application of next generation sequencing techniques (i.e. 21,619 Chinook loci) has proven powerful as portrayed in this report. Locus associations with environment may be highly informative for GSI and other applications, particularly with regard to run timing. A similar outcome is anticipated for steelhead. Applications of this nature have already been shown to be useful identifying population differences distributed across landscapes (e.g., Narum et al. 2010a; Matala et al. 2011). Our data will be evaluated in current and ongoing application of PBT and GSI methods for each species. PBT can be used to validate assignment origins based on GSI. Future efforts include adding collections to the baseline to increase basin-wide coverage of all species, particularly those that account for stock transfer history of *O. nerka* throughout the basin. We also intend to continually strive to increase the numbers of markers (SNP loci)

employed for genetic applications, as evident by our updating effort in this report and with new RAD sequencing capabilities.

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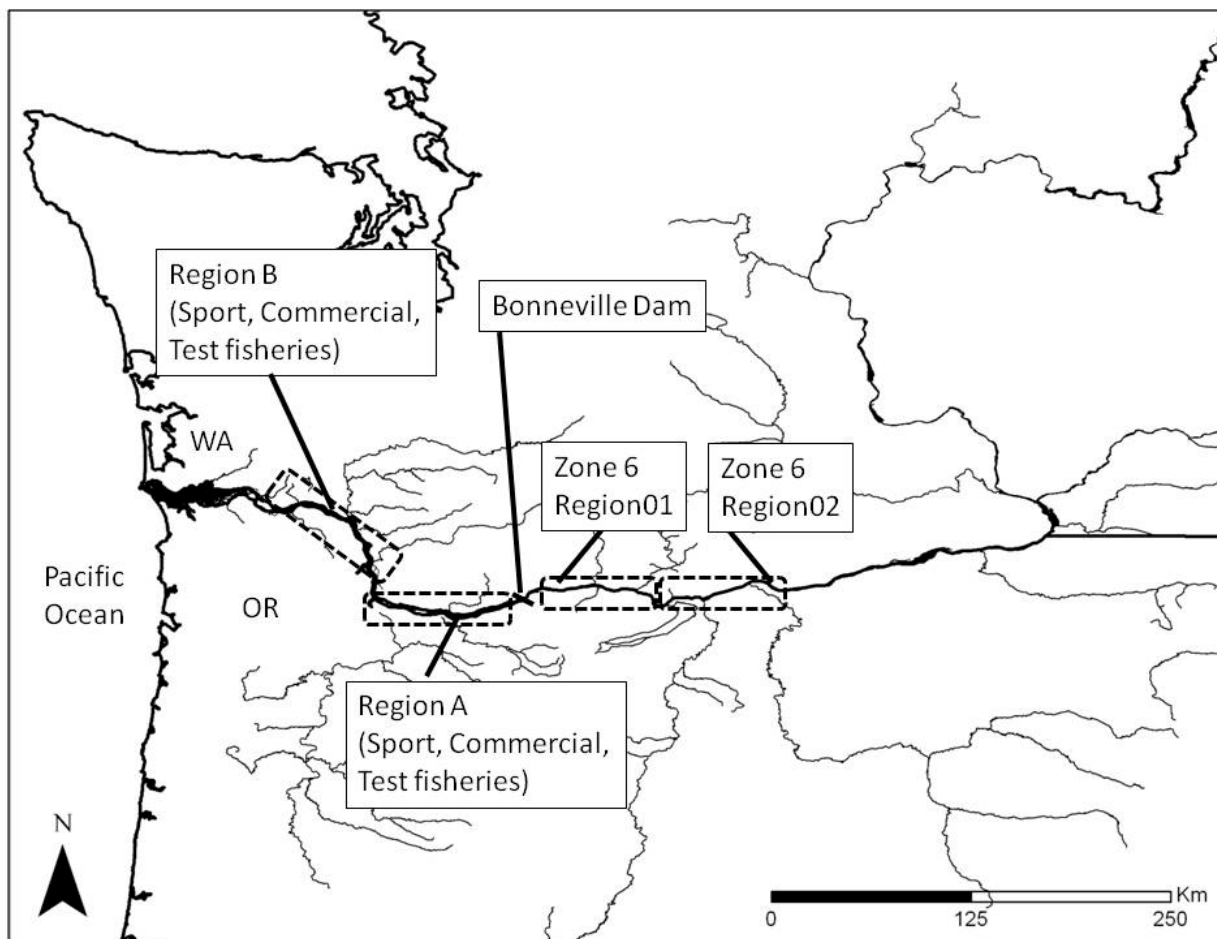
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### Section 3: Genetic Stock Identification of Chinook Salmon, Sockeye Salmon, and Steelhead Trout Harvest Mixtures in the Mainstem Columbia River

#### 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, and lineages of both species can be further broken into populations that are genetically structured on a finer spatial scale (e.g., Waples et al. 2004; Narum et al. 2010; Blankenship et al. 2011). In this study we use 192 SNP markers to discriminate 19 reporting groups for Chinook salmon, 4 reporting groups for sockeye salmon, and 14 reporting groups for steelhead.



**Figure 1. Project scope showing sources of Chinook salmon, sockeye salmon, and steelhead trout mixtures that were analyzed using GSI.**

Despite continuous improvements of the power of our Chinook salmon and steelhead baselines in GSI applications (Hess et al. 2013), we have determined that further improvement in the detail of data and accuracy of stock assignments could be made by utilizing a recently developed genetic technology, parentage based tagging (PBT), in combination with GSI, in a tiered approach for stock identification. PBT is an efficient approach for mass tagging of fish. The method is carried out by first genotyping a set of potential parents which then provides the opportunity to assign a set of genotyped offspring to their true parent pair. PBT is currently being utilized on a broad scale in the Columbia River Basin to tag all Snake River Chinook salmon and steelhead trout hatchery broodstock (Steele et al. 2011). This application has effectively tagged all Snake River hatchery Chinook salmon and steelhead starting with the 2008 brood years. When parent pairs of a Snake River hatchery fish are identified with PBT, we can provide accurate information including age of the fish and the source hatchery in which its parents were spawned. We use PBT in this Chinook salmon and steelhead harvest study to identify all Snake River hatchery-origin fish, and then we use GSI to estimate stock-of-origin of all other hatchery fish that were not assigned with PBT (i.e. non-Snake River hatchery-origin) and all wild fish. PBT can assign the 3- and 4-year old Snake River hatchery-origin spring Chinook salmon (i.e. brood years 2008-2009) from the 2012 spring Chinook salmon harvest and the 1-, 2-, and 3-ocean age Snake River hatchery-origin steelhead (i.e. brood years 2008-2012) from the 2012 steelhead harvest. Eventually the PBT baseline will be expanded beyond the Snake River, and this year we continue with our inclusion of the first middle Columbia River hatchery, Klickitat spring Chinook salmon hatchery.

Fisheries conducted in the mainstem of the lower and middle Columbia River provide an ideal and important application of genetic stock analyses because the fish harvested consist of mixtures of stocks from a large extent of the CRB. Further, Chinook salmon fisheries in this location represent a majority of the CRB harvest of this species taken by the commercial, sport, and tribal fishermen. In order to help support sustainable fisheries, PBT and GSI can be used to address two primary questions: 1) how are Chinook salmon and steelhead stocks temporally and spatially distributed in the mainstem 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 four primary objectives: 1) utilize a combination of PBT and GSI analyses to estimate stock composition of Chinook salmon passing Bonneville Dam; 2) determine stock composition of Chinook salmon harvested in sport, commercial, and tribal fisheries in the mainstem Columbia River; 3) utilize a combination of PBT and GSI to estimate stock composition of unclipped steelhead harvested above Bonneville Dam in the zone 6 fishery; and 4) 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. In 2012, 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 2013. For steelhead, we have again sampled a group of fish that were non-adipose clipped and captured in the tribal fishery harvest above Bonneville Dam in Zone 6. This latter project was in collaboration with Alan Byrne (Idaho Department of Fish and Game) and Stuart Ellis (Columbia River Inter-Tribal Fish Commission) and is currently being written into an IDFG Technical report. Finally, we include in this report our first analysis of sockeye salmon fisheries in the Columbia River mainstem. Differences in relative abundance of the three main stocks (Okanagan, Wenatchee, and Snake) provide challenges to managing lower river harvest because 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 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.

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?**

## **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 trout*

Tissues were sampled from Chinook salmon in 2013 from a total of ten different mixture sources: 1) Bonneville Dam (results discussed in section 4), and the spring-run seasons of the following fisheries: 2) lower river commercial, 3) lower river sport, and 4) Wind R. sport, 5) tribal spring, and the summer management period harvests of the following fisheries: 6) lower river commercial, 7) lower river sport, and 8) Zone 6 tribal, and the fall-run harvests from 9) the lower river mark-selective sport fishery of 2012 and 10) fall mark-selective sport fishery of 2013. 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. In addition, we analyzed a test fishery from the lower

river that was conducted during the spring-run fishing season. A portion of the tribal ceremonial harvest was conducted in the spring and sampled by Bubba Holliday and Megan Begay as part of the Warm Springs and Yakama Nation fishery programs, respectively. The results may or may not be applicable to other portions of the ceremonial harvest and would not be applicable to the overall spring season zone 6 tribal fishery which occurs over a much more extended time period. These other harvest tissues were collected in coordination with existing monitoring programs led by Washington Department of Fish and Wildlife (WDFW) and Oregon Department of Fish and Wildlife (ODFW) and 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 region 01 as part of the Wind River sport fishery and Warm Springs and Yakama Nation tribal ceremonial fisheries (Fig. 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. All samples obtained from the sport and commercial fisheries below Bonneville Dam were analyzed, but due to the large number of samples from zone 6, we analyzed a subset at a consistent 7% sample rate that was applied temporally across the season, and spatially across reservoirs, and between origin classifications (adipose clipped versus intact fish).

One part of the fall management period Chinook fishery was sampled only below Bonneville Dam. 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 (Fig. 1): Region A corresponds to our grouping of pre-existing Oregon and Washington state sport fishing zones 1-4 (or commercial zones 4-5), Region B corresponds to our grouping of sport zones 5-10 (or commercial zones 1-3), and Region 01 and Region 02 in the Zone 6 fishery correspond to pre-existing Oregon and Washington state fishing Zone 61 and a grouping of Zones 62 and 63, respectively. These sets of groupings were established for this study in order to achieve balanced sampling for analysis of these fishery datasets, as well as to set an appropriate spatial scale of analysis to minimize variance of our estimates of stock proportions over temporal strata.

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

[illegible]

Note: The lower river sport fishery was closed during Stat week 18, and so the 13 samples listed here must have been mislabeled. Statistical week 8 equals 2/18/2013-2/24/2013, week 24 equals 6/10/2013-6/16/2013, week 30 equals 7/22/2013-

7/28/2013, week 36 equals 9/2/2013-9/8/2013, and week 39 equals 9/23/2013-9/29/2013. Regions are shown in Fig. 1. Adipose clip is indicated by “Y” and fin-intact is “N”. In the case of the fall sport fisheries, we analyzed both 2012 and 2013.



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 unclipped hatchery and natural origin fish from Bonneville Dam and the tribal subsistence and ceremonial harvests above and below Bonneville Dam, respectively. Not the entire tribal spring season fishery was sampled.

Tissues were sampled from sockeye salmon in 2013 from four fishery mixture sources: 1) lower river commercial, 2) lower river sport, 3) Bonneville Dam (results discussed in section 4), 4) the tribal fishery in Zone 6. All samples obtained from the sport and commercial fisheries below Bonneville Dam were analyzed, but due to the large number of samples from Zone 6, we analyzed a subset at a consistent 15% sample rate that was applied temporally across the season. Harvest prior to week 25 and after week 30 was not sampled. Genetic samples were run on 14% of the total tribal catch.

Tissues were sampled from steelhead in 2013 from two fishery mixture sources: 1) Bonneville Dam (results discussed in section 4) and 2) the summer and fall season tribal fishery in Zone 6 (Fig. 1). The tribal fishery was conducted during the A and B Index accounting period (July 1<sup>st</sup>-October 31<sup>st</sup>) and was sampled as part of the Yakama Nation fishery monitoring program. This fishery is not mark-selective, and we conducted genetic stock analyses on the subset of samples that were not adipose-clipped (putatively natural-origin). However, there are a few steelhead hatcheries in the Snake River and in the upper Columbia River that release unmarked smolts as part of supplementation efforts and so a proportion of the samples in our dataset were expected to be unmarked hatchery-origin. Fish with adipose clips were analyzed separately by IDFG and a report of those results has been generated with input from IDFG and CRITFC staff.

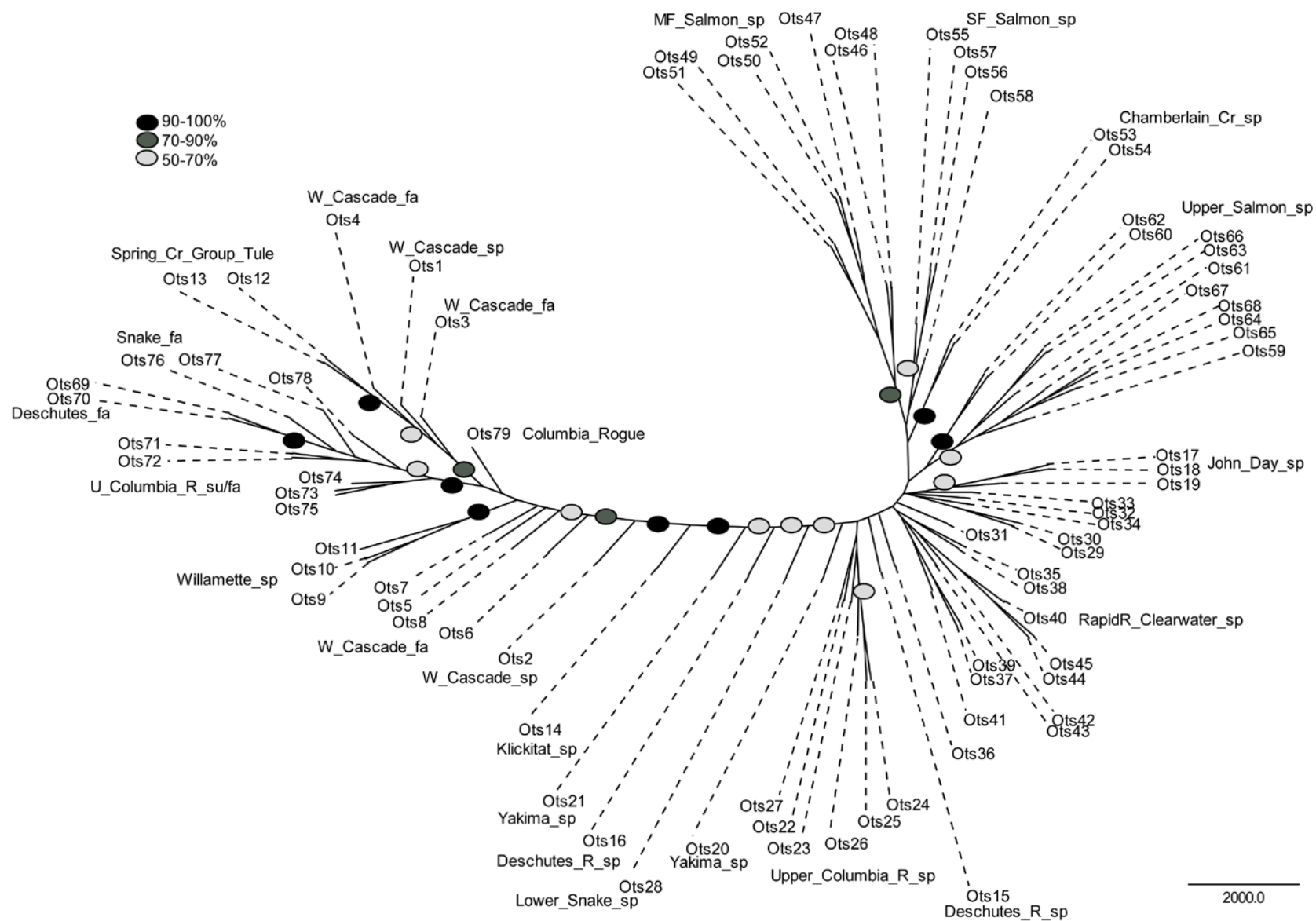
### *Molecular data*

Methods for DNA extraction, DNA amplification, microfluidics, and genotyping of SNP assays are available at (<https://www.monitoringmethods.org/Protocol/Details/230>). Additional details regarding how 192 SNPs were pared down to 186 SNPs can be found in Hess et al. (2012, 2013). We used 186 total SNP markers for GSI and for PBT analyses, we used 95 of the 192. We used 180 total SNP markers for GSI of steelhead mixtures and 95 of these were used for PBT (Hess et al. 2013).

### *GSI baselines for Chinook salmon, sockeye salmon, and steelhead trout*

Chinook salmon GSI analyses were performed using the updated baseline referred to as “OtsSNP186baseJan28\_2014v2”. This baseline deviates in a few minor ways from the baseline described in Hess et al. (2013, Section 2). Seventy-nine collections were delineated into the following 19 reporting groups: W\_Cascade\_sp, W\_Cascade\_fa, Willamette\_sp, Spring\_Cr\_Group\_Tule, Klickitat\_sp, Deschutes\_R\_sp, John\_Day\_sp, Yakima\_sp, Upper\_Columbia\_R\_sp, Lower\_Snake\_sp, RapidR\_Clearwater\_sp, SF\_Salmon\_sp,

MF\_Salmon\_sp, Chamberlain\_Cr\_sp, Upper\_Salmon\_sp, Deschutes\_fa, Upper\_Columbia\_R\_su/fa, Snake\_fa, and Columbia\_Rogue (Table 2). Additional collections include three that represent Wenatchee River spring-run Chinook salmon (Ots24 – 26), one from Spring Creek Hatchery (Ots13), and one from the upper Deschutes R above Sherars Falls (Ots70). Reporting groups were primarily determined by the relative genetic similarity among populations according to a phylogenetic analysis, and preliminary results demonstrated sufficient power to discern three reporting groups (Deschutes\_fa, Upper\_Columbia\_R\_su/fa, and Snake\_fa) among the interior ocean-type collections. Previously we had grouped all interior ocean-type collections into a single reporting group “Interior\_Columbia\_R\_su/fa” (Hess et al. 2013). STRUCTURE v2.3.2 (Pritchard et al. 2000) was used to identify strays (>80% assignment to a different lineage) in each collection by setting a K value of 3 or higher; 30 strays were swapped into a more appropriate collection (e.g. in Entiat R., interior ocean-type and stream-type lineages co-occur and have slightly overlapping spring and summer run-timing and so must be genetically identified to make a pure collection) and 30 strays were removed entirely from the dataset (results not shown). Genetic distances were computed from allele frequencies based on Nei’s (1972) genetic distance, with the PHYLIP v 3.69 (Felsenstein 1989) and 1000 bootstrap replicates were performed. Distances were clustered using the Neighbor – Joining method (Saitou and Nei, 1987), and a consensus tree was constructed (<http://evolution.genetics.washington.edu/phylip/>).



**Figure 2. Neighbor-joining tree of Chinook salmon baseline populations using Nei's 1972 genetic distance of 186 SNP loci.** The clusters are labeled with names of reporting groups used to aggregate the collections based on a combination of factors including

genetic similarity, life history, and geographic proximity. Bootstrap support is shown with shaded ovals. We used a final set of 19 reporting groups for all GSI analyses (Table 2).

The Upper\_Columbia\_R\_sp 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 Upper\_Columbia\_R\_sp reporting group. The Columbia\_Rogue reporting group represents an out-of-basin genetic stock (originating from the Rogue R., OR) that is reared within the Columbia R. at Youngs Bay. Basic QAQC was performed to remove duplicate individuals and strays from the reference populations in the baseline. The baseline and reporting groups file is available on request.

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

ID	Collection	BPA Subbasin	(n)	Lineage	Run	Origin	Age	Year	Reporting Groups
Ots1	Cowlitz R.	Cowlitz	90	LC	SP	Hat.	A	2004	W_Cascade_sp
Ots2	Kalama R.	Kalama	83	LC	SP	Hat.	A	2004	W_Cascade_sp
Ots3	Cowlitz R.	Cowlitz	82	LC	F	Hat.	A	2004	W_Cascade_fa
Ots4	Elochoman R.	Elochoman	86	LC	F	N	A	1995-97	W_Cascade_fa
Ots5	Lewis R.	Lewis	93	LC	F	N	A	2003	W_Cascade_fa
Ots6	North Fork Lewis R.	Lewis	84	LC	*(LFB)	N	A	2004	W_Cascade_fa
Ots7	North Fork Lewis R.	Lewis	94	LC	*(EFB)	N	A	2004	W_Cascade_fa
Ots8	Sandy R.	Sandy	83	LC	F	N	A	2002	W_Cascade_fa
Ots9	McKenzie R.	Willamette	79	LC	SP	Hat.	A	2004	Willamette_sp
Ots10	North Santiam R.	Willamette	79	LC	SP	Hat.	A	2004	Willamette_sp
Ots11	Sandy R.	Sandy	48	LC	SP	N	A	2006	Willamette_sp
Ots12	White Salmon R.	Big White Salmon	77	LC	F	N	J	2008	Spring_Cr_Group_Tule
Ots13	Spring Creek Hatchery	Big White Salmon	49	LC	F	Hat.	A	2011-12	Spring_Cr_Group_Tule
Ots14	Klickitat R.	Klickitat	85	ST	SP	Hat.	A	2002	Klickitat_sp
Ots15	Shitike Cr.	Deschutes	93	ST	SP	N	J	2004	Deschutes_R_sp
Ots16	Warm Springs R.	Deschutes	90	ST	SP	Hat.	A	2004	Deschutes_R_sp
Ots17	John Day R.	John Day	78	ST	SP	N	J/A	2000	John_Day_sp
Ots18	Middle Fork John Day r.	John Day	47	ST	SP	N	A	2006	John_Day_sp
Ots19	North Fork John Day r.	John Day	42	ST	SP	N	A	2006	John_Day_sp
Ots20	American R.	Yakima	76	ST	SP	N	A	2003	Yakima_sp
Ots21	Cle Elum R.	Yakima	88	ST	SP	Hat.	U	1997	Yakima_sp
Ots22	Winthrop NFH (Carson stock)	Methow	82	ST	SP	Hat.	A	2001	Upper_Columbia_R_sp
Ots23	Little White Salmon R.	Little White Salmon	93	ST	SP	Hat.	J	2007	Upper_Columbia_R_sp
Ots24	Chiwawa R.	Wenatchee	29	ST	SP	N	A	2007-09	Upper_Columbia_R_sp
Ots25	Nason Cr.	Wenatchee	40	ST	SP	N	A	2005,2007-09	Upper_Columbia_R_sp
Ots26	White R.	Wenatchee	40	ST	SP	N	A	2005,2007-09	Upper_Columbia_R_sp
Ots27	Entiat R.	Entiat	98	ST	SP	N	J	2006	Upper_Columbia_R_sp

Ots28	Tucannon R.	Tucannon	85	ST	SP	N	A	2003	Lower_Snake_sp
Ots29	Imnaha R.	Imnaha	92	ST	SP	N	A	1998	RapidR_Clearwater_sp
Ots30	Imnaha R	Imnaha	43	ST	SP	N	J	08	RapidR_Clearwater_sp
Ots31	Catherine Cr	Grande Ronde	93	ST	SP	N	A	04, 06	RapidR_Clearwater_sp
Ots32	Upper Grande Ronde R.	Grande Ronde	43	ST	SP	N	J	08	RapidR_Clearwater_sp
Ots33	Lostine R	Grande Ronde	176	ST	SP	N	J	03, 05, 09	RapidR_Clearwater_sp
Ots34	Minam R	Grande Ronde	80	ST	SP	N	J	94, 02	RapidR_Clearwater_sp
Ots35	Wenaha R.	Grande Ronde	45	ST	SP	N	J	2006	RapidR_Clearwater_sp
Ots36	Dworshak Hatchery	Low. Clearwater	88	ST	SP	U	U	2005	RapidR_Clearwater_sp
Ots37	Lochsa R. (Powell)	M. F. Clearwater	77	ST	SP	Hat.	A	2005	RapidR_Clearwater_sp
Ots38	Crooked F Lochsa R	M. F. Clearwater	27	ST	SP	N	C	07, 08, 09, 10	RapidR_Clearwater_sp
Ots39	Powell Weir	M. F. Clearwater	31	ST	SP	N	A	09	RapidR_Clearwater_sp
Ots40	Newsome Cr.	S. F. Clearwater	82	ST	SP	N	A	2001	RapidR_Clearwater_sp
Ots41	Crooked R. Weir	S. F. Clearwater	67	ST	SP	N	A	09, 10	RapidR_Clearwater_sp
Ots42	Lolo Cr	S. F. Clearwater	89	ST	SP	N	J	01, 02	RapidR_Clearwater_sp
Ots43	Red R.	S. F. Clearwater	72	ST	SP	N	C	07, 08, 09, 10	RapidR_Clearwater_sp
Ots44	Rapid R.	Little Salmon	91	ST	SP	N	A	06	RapidR_Clearwater_sp
Ots45	Rapid R.	Salmon	93	ST	SP	Hat.	A	1999	RapidR_Clearwater_sp
Ots46	Big Cr.	M. F. Salmon	89	ST	SP	N	A	2001	MF_Salmon_sp
Ots47	Bear Valley Cr.	M. F. Salmon	80	ST	SP	N	C	07, 08, 09, 10	MF_Salmon_sp
Ots48	Camas Cr.	M. F. Salmon	58	ST	SP	N	J	06, 09	MF_Salmon_sp
Ots49	Capehorn Cr.	M. F. Salmon	112	ST	SP	N	C, J	05, 06, 07, 09, 10	MF_Salmon_sp
Ots50	Elk Cr.	M. F. Salmon	84	ST	SP	N	C, J	07, 08, 09, 10	MF_Salmon_sp
Ots51	Marsh Cr.	M. F. Salmon	66	ST	SP	N	C	07, 08, 09, 10	MF_Salmon_sp
Ots52	Sulphur Cr.	M. F. Salmon	35	ST	SP	N	C, J	08, 09, 10	MF_Salmon_sp
Ots53	Chamberlain Cr.	Salmon	70	ST	SP	N	C, J	03, 04, 06, 07	Chamberlain_Cr_sp
Ots54	Chamberlain Cr.	Salmon	45	ST	SP	N	J	2009	Chamberlain_Cr_sp
Ots55	Johnson Cr.	S. F. Salmon	92	ST	SP/SU	N	A	2002	SF_Salmon_sp
Ots56	Lake Cr, Summit Cr.	S. F. Salmon	74	ST	SP	N	C	07, 08, 09, 10	SF_Salmon_sp

Ots57	Secesh R.	S. F. Salmon	131	ST	SP	N	C, J A,	01, 07, 08, 09, 10	SF_Salmon_sp
Ots58	SF Salmon R.	S. F. Salmon	140	ST	SP	N	C	09, 10	SF_Salmon_sp
Ots59	East Fork Salmon R.	Up. Salmon	94	ST	SP	N	A	Unk	Upper_Salmon_sp
Ots60	Hayden Cr.	Up. Salmon	79	ST	SP	N	C, J	09, 10	Upper_Salmon_sp
Ots61	Lemhi R. (upper)	Up. Salmon	95	ST	SP	N	C, J	09, 10	Upper_Salmon_sp
Ots62	Lemhi R. (lower)	Up. Salmon	90	ST	SP	N	J A,	09, 10	Upper_Salmon_sp
Ots63	Pahsimeroi R.	Up. Salmon	92	ST	SP	N	C	07, 08, 09, 10	Upper_Salmon_sp
Ots64	Sawtooth Weir	Up. Salmon	90	ST	SP	N	A	09, 10	Upper_Salmon_sp
Ots65	Valley Cr.	Up. Salmon	56	ST	SP	N	C	07, 08, 09, 10	Upper_Salmon_sp
Ots66	Pahsimeroi R.	Up. Salmon	92	ST	SP	U	U	2004	Upper_Salmon_sp
Ots67	Sawtooth Hatchery	Up. Salmon	90	ST	SP	Hat.	A	2003	Upper_Salmon_sp
Ots68	W. F. Yankee Fork R.	Up. Salmon	75	ST	SP	N	J	2005	Upper_Salmon_sp
Ots69	Upper Deschutes R.	Deschutes	89	OT	F	N	J	1998	Deschutes_fa
Ots70	Upper Deschutes R.	Deschutes	163	OT	F	N	A	2011	Deschutes_fa
Ots71	Lower Yakima R.	Yakima	62	OT	F	N	A	1998	U_Columbia_R_su/fa
Ots72	Hanford Reach	Columbia Lower Mid.	93	OT	F	N	A	2000	U_Columbia_R_su/fa
Ots73	Tumwater & Dryden	Wenatchee	92	OT	SU	N	A	1993	U_Columbia_R_su/fa
Ots74	Entiat R.	Entiat	52	OT	SU	N	A	2008	U_Columbia_R_su/fa
Ots75	Methow R.	Methow	87	OT	SU	N	J	1993	U_Columbia_R_su/fa
Ots76	Lyons Ferry Hatchery	Tucannon	90	OT	F	Hat.	A	2000	Snake_fa
Ots77	Nez Perce Tribal Hatchery	Clearwater	85	OT	F	Hat.	A	2003	Snake_fa
Ots78	Clearwater R.	Clearwater	143	OT	F	Hat.	A	08	Snake_fa
Ots79	Youngs Bay	Youngs Bay	91	Rogue	F	Hat.	A		Columbia_Rogue

Note: Chinook salmon baseline collections (n=6358). Lineages are: ST- stream type, OT – ocean type, and LC – Lower Columbia. “Sp” , “su”, and “fa” notation designate spring-, summer-, and fall-run-timing, respectively.



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, Okanogan, and Snake River sockeye salmon.

Steelhead GSI analyses utilized the same baseline “OmySNPbase180JEHonor.txt” described in Hess et al. (2013). The 131 baseline collections were delineated into the following 13 Columbia River Basin reporting groups: LOWCOL, SKAMAN, WILLAM, BWSALM, KLINKR, MGILCS, YAKIMA, UPPCOL, SFCLWR, UPCLWR, SFSALM, MFSALM, and UPSALM. The MGILCS reporting group includes the following Snake River stocks: George Cr, Asotin Cr, Alpowa Cr, and Tucannon R as well as the reporting groups from the Grande Ronde, Imnaha, Lower Clearwater and Lower Salmon R.; because they are genetically indistinguishable from other middle Columbia River stocks. It is notable that this group contains part of the Snake River DPS and part of the Mid-Columbia DPS. Other studies (e.g. PIT-tags show high levels of straying of natural-origin fish, J. Bumgarner WDFW pers. comm.) appear to support this lack of distinction among populations that occur in the middle Columbia River and Snake River DPS. We created a map of the geographic distribution of the baseline collections and reporting groups (Fig. 3).

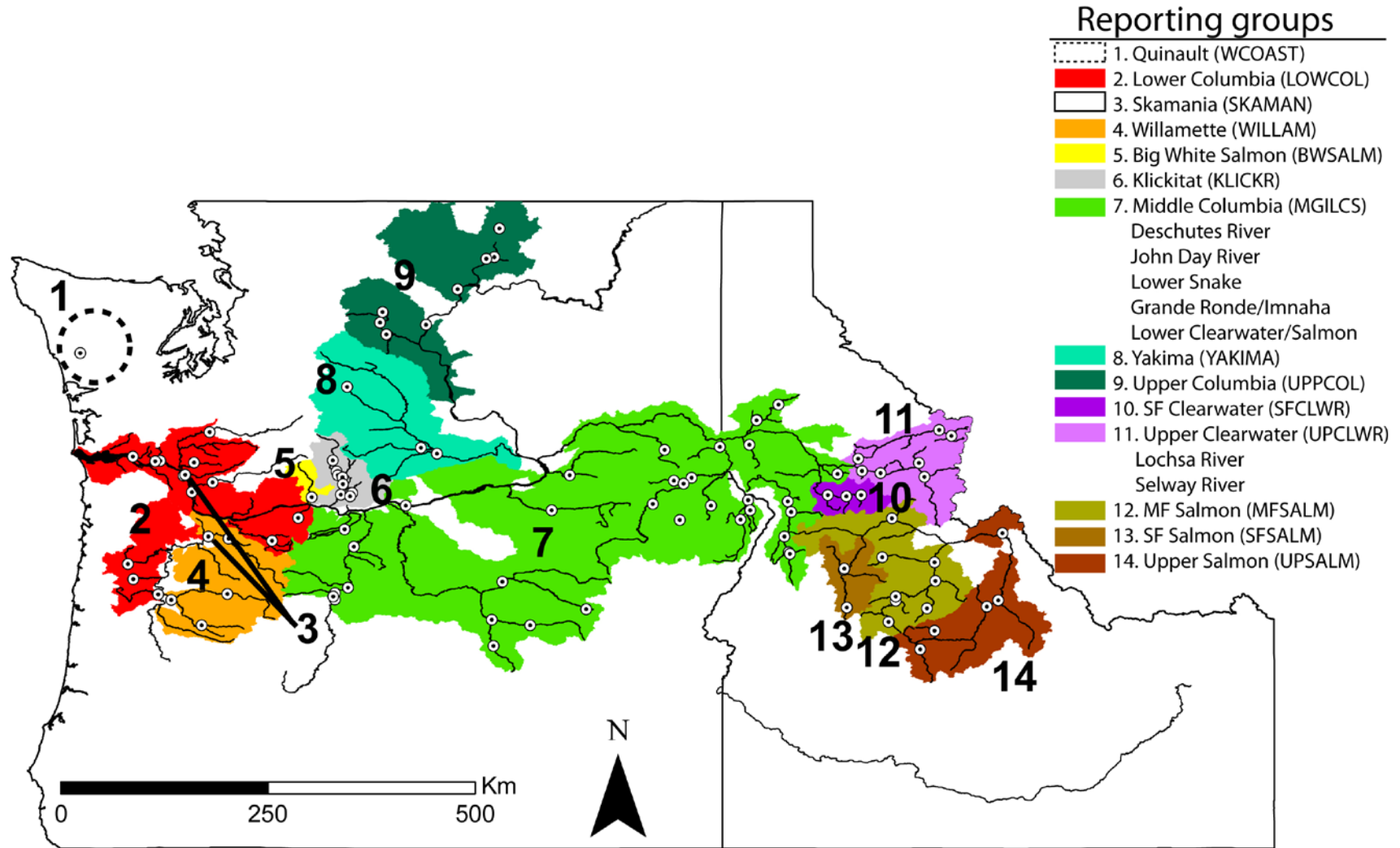


Figure 3. Map of steelhead baseline collections and reporting groups.

### *Baseline power analyses*

The accuracy of the Chinook and sockeye salmon and steelhead baselines was characterized using mixture simulations performed by the program ONCOR v1.0 (available at <http://www.montana.edu/kalinowski>). [Method: Predicting the accuracy of genetic stock identification v1.0](https://www.monitoringmethods.org/Method/Details/1346) (<https://www.monitoringmethods.org/Method/Details/1346>).

### *Combined application of PBT and GSI*

We combined PBT and GSI results together by first accepting all confident PBT assignments to hatchery broodstock for the Chinook salmon and steelhead individuals and then for the remaining individuals, we used the best estimate GSI assignments to provide likely population of origin. Current PBT baselines allowed us to identify the source hatcheries of 3- and 4-year old Snake River and Klickitat River spring-run Chinook salmon (2008-2009 broodyears) and the 1-, 2-, and 3-ocean age steelhead from the Snake River (2008-2010 broodyears). For Snake River steelhead the SNPPIT file included brood years 2008-2011 (See methods for [Parentage assignments using SNPPIT software v1.0](#), ID: 1341).

The program ONCOR was used to estimate the most likely population-of-origin for the Chinook salmon and steelhead harvest samples. Individuals were assigned using a “best estimate” approach (Method: [Assigning individual samples using Individual Assignment \(IA\) genetic methods v1.0](#), ID: 1334). We also used GSIsim for “[Mixture modeling to estimate stock proportions v1.0](#)” (ID: 1333).

## Results

### *Accuracy testing of 186 SNP and 94 SNP Chinook salmon baselines*

The 79 collections were grouped into 19 reporting groups based on the clustering we observed in the phylogenetic analysis (Fig. 2). Results from the leave-one-out analysis showed less than half of the baseline collections displayed greater than 90% correct individual assignment to the reporting group level, however nearly 2/3 of the collections displayed greater than 80% correct assignment. The spring-run (interior stream-type lineage) baseline collections displayed the lowest correct assignment and the collections that were below 60% included John Day R. (56%), North Fork John Day (36%), Winthrop Carson stock (50%), Entiat R. (57%), Wenaha R. (59%), and SF Salmon R. (47%). The average correct assignment based on this leave-one-out analysis is provided for each reporting group (Table 3).

We grouped all collections into the three major genetic lineages (i.e. Lower Columbia LC, interior stream-type ST, and interior ocean-type OT) and made the Columbia Rogue (CR) into a fourth group, and then tested accuracy of the baseline using only the 94 SNPs that are included in the PBT SNP panel. Average accuracies of collections within each reporting group for assigning to the “Lineage-level” were quite high, and only 1 of 79 collections had less than 90% correct assignment. The single collection was Kalama R. spring run Chinook salmon, which is known to have some level of introgression with the interior stream-type lineage (probably due to mixing with the ubiquitous Carson stock). This high level of accuracy attained using only 1 panel of PBT SNPs will allow coarse level GSI for mark-selective hatcheries in which we want to know primarily the composition of Snake River hatcheries (via PBT) and the composition of lower River stocks versus upriver stocks for all non-Snake River hatchery-origin fish.

Table 3. Average GSI accuracy for Chinook salmon at the reporting group level using 186 SNPs and at the lineage level using 94 SNPs.

Reporting groups	Leave-1-out % correct assignment		
	186 SNP	Lineage	94SNP
W_Cascade_sp	81.9%	LC	90.6%
W_Cascade_fa	93.5%	LC	97.3%
Willamette_sp	100.0%	LC	100.0%
Spring_Cr_Group_Tule	93.1%	LC	97.1%
Klickitat_sp	94.9%	ST	97.9%
Deschutes_R_sp	90.4%	ST	100.0%
John_Day_sp	57.3%	ST	99.4%
Yakima_sp	100.0%	ST	100.0%
Upper_Columbia_R_sp	69.0%	ST	100.0%
Lower_Snake_sp	73.0%	ST	98.1%
RapidR_Clearwater_sp	76.7%	ST	99.9%
MF_Salmon_sp	89.7%	ST	100.0%
Chamberlain_Cr_sp	91.6%	ST	100.0%
SF_Salmon_sp	73.9%	ST	100.0%
Upper_Salmon_sp	85.1%	ST	100.0%
Deschutes_fa	94.7%	OT	97.3%

U_Columbia_R_su/fa	86.0%	OT	99.2%
Snake_fa	71.9%	OT	100.0%
Columbia_Rogue	100.0%	CR	97.4%
Total	82.6%		99.2%

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

*Parentage based tagging assignments of spring-run Chinook salmon in harvest and at Bonneville Dam*

The PBT analysis resulted in 1,309 hatchery-origin salmon that could be assigned back to Klickitat and Snake River hatchery broodstock parents that were spawned in 2008 – 2010 (Table 4). These salmon were found to have originated from the following fifteen sources: Klickitat, S.F. Clearwater, Catherine Creek, Dworshak, Grande Ronde, Imnaha, Johnson Cr., Lookingglass Cr., Lostine, McCall, Nez Perce Tribal Hatchery, Pahsimeroi, Clearwater (Powell), Rapid R., Sawtooth, and Tucannon hatcheries. Among the 4-year-old fish, the broodstock from Rapid R. 2009 yielded the highest number of assignments (N=342), and the Catherine Cr. and Lostine 2009 broodstock yielded the least (N=1). There were seven 4-year-olds and 3 jacks sampled from the Wind R. fishery that assigned to the following PBT source hatcheries: Dworshak, McCall, Powell, and Rapid R. hatcheries.

Table. 4. Summary information on numbers and origins of Chinook salmon that assigned to the PBT baseline.

PBT source hatchery	PBTpop	Tag Rate	Region:	Lower R.				Bonneville		Zone 6		Wind	Total
			Fishery:	Comm- ercial	Test	Sport	Sub- sistence	Dam		Ceremonial		R.	
			Origin:	HOR	HOR	HOR	HOR	HOR	NOR	HOR	NOR	HOR	
Klickitat Hatchery	OtsKLKH08S			1		3				4			8
	OtsKLKH09S			6		1	1	12					20
	OtsKLKH10S			6	3	3		10			1		23
LSRCP/IDFG - Clearwater (SF)	OtsCLWH08S	96.7%			1	8		3		1			13
	OtsCLWH09S	84.6%		3	2	8	2	5	2	12	4		38
	OtsCLWH10S	99.5%		4	9	1		17	3	1			35
LSRCP/ODFW - Catherine Creek	cath.conv.09	95.1%						1					1
	OtsCTHW10S	89.5%		1	1			3					5
LSRCP/USFWS - Dworshak	OtsDWOR08S	97.5%		1	2	35	1	4		2	1		46
	OtsDWOR09S	99.0%		11	5	51	7	28	5	33	3	1	144
	OtsDWOR10S	98.5%		13	1	3	1	30	1	3		2	54
LSRCP/ODFW - Grande Ronde	grnd.capt.09	95.4%			1			1					2
	grnd.conv.09	94.7%							2		1		3
	gruw.conv.10	98.7%						3	5				8
LSRCP/ODFW -	OtsIMNW08S	99.2%		2				2		1			5

Imnaha	OtsIMNW09S	97.4%	7	1	2		6		1		17
	OtsIMNW10S	97.6%			6		15	2			23
	OtsJHNW08S	100.0%						1			1
Johnson Cr.	OtsJHNW09S	100.0%						2		1	3
	OtsJHNW10S	94.2%						2			2
	OtsLOOK08S	98.7%			1						1
LSRCP/ODFW - Lookingglass Creek	OtsLOOK09S	93.9%		1		1	2		2		6
	OtsLOOK10S	98.7%	3		1		14			1	19
	lost.conv.08	94.6%					2				2
LSRCP/ODFW/NPT – Lostine	lost.conv.09	94.4%						1			1
	OtsLSTW10S	95.4%	1		7		11				19
	OtsMCCA08S	97.6%	5	1			2		1		9
LSRCP/IDFG - McCall (SFSR)	OtsMCCA09S	96.4%	16	1	24		35	1	3		81
	OtsMCCA09S_2	95.9%						2		1	3
	OtsMCCA10S	99.4%	2		2		12	16			32
Nez Perce Tribal Hatchery (NPTFH)	OtsNPFH09S	96.3%						2	1		3
	OtsPAHH08S	97.8%	1		1						2
Idaho Power/IDFG - Pahsimeroi	OtsPAHH09S	96.8%	5		2		6				13
	pahs.ibs.10	94.9%	2		2		3	1			8
	OtsPOWP08S	98.7%	1		2			1			4
LSRCP/IDFG - Clearwater (Powell)	OtsPOWP09S	47.1%	1	2	5	7	6	1	13		36
	OtsPOWP10S	97.7%	4	2			3	1			10
	OtsRAPH08S	98.3%	2	3	49	1	4		9		68
Idaho Power/IDFG - Rapid River	OtsRAPH09S	90.6%	52	20	132	16	78	3	37		342
	OtsRAPH10S	99.1%	25	9	6	3	62	3	4		113
	OtsSAWT08S	99.0%	5		3	2	4				14
LSRCP/IDFG - Sawtooth	OtsSAWT09S	99.6%	5	1	4		9	1	7	1	28
	OtsSAWT10S	99.4%	8		3		19	11			41
LSRCP/WDFW - L.F. (Tucannon)	OtsTUCW09S	91.1%						2			2
	OtsTUCW10S	96.3%								1	1

Snake PBT avg:	95.4%	Snake PBT N	180	63	358	41	390	71	131	14	10	1258
		Total PBT N	193	66	365	42	412	71	135	15	10	1309
		Sample Total	426	191	796	76	618	597	247	74	138	3163
		%Snake of Sample Total	44.3%	34.6%	47.2%	56.6%	66.2%	12.5%	55.6%	19.8%	7.6%	

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Note: PBTpop is the abbreviated hatchery source and the digits indicate the spawn year, e.g. 08S is spawn year 2008. Adipose-clipped (HOR) and adipose unclipped (NOR) categories of fish could be separated for the Bonneville Dam and Ceremonial mixtures. All other fishery mixtures were mark-selective (HOR only). The “Snake PBT N” indicates only parentage assignments to Snake River hatcheries, and “PBT N” is the total number of assignments that were identified for Klickitat and Snake River hatcheries. The “%Snake of Sample Total” indicates our estimate of the composition of Snake River hatcheries in each fishery mixture using an expansion factor based on average tagging rate across all Snake River hatcheries. Bonneville Dam includes only fish sampled during statistical weeks 17-25, which is the timeframe of sampling within the spring-run harvest period.

#### *Stock composition strategies*

Analyses of Chinook salmon utilized the following two approaches: “Strategy 1” – data analysis was conducted on 94 SNPs only, which comprise the PBT panel and therefore could assign individuals to Snake River (and Klickitat River) hatcheries as well as could perform GSI for three main groups (lower Columbia River, interior stream-type, and interior ocean-type stocks). “Strategy 2” – data analysis utilized a complete set of 192 SNPs which could discriminate a larger number of stocks with GSI (19 reporting groups), and includes PBT capability. “Strategy 1” was employed with adipose-clipped fishery mixtures and “Strategy 2” was employed primarily with adipose-intact fishery mixtures, but was also applied in cases where higher resolution of hatchery stocks was required (e.g. see Wind River harvest analysis below).

#### *Wind River spring-run Chinook salmon sport harvest*

Both “strategy 1” and “strategy 2” 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 (Carson Hatchery) Chinook salmon that are harvested by using the context of stock proportions in other spring management period fisheries and at Bonneville Dam. Using strategy 1 to compare fishery mixtures, the Wind River sport mark selective Chinook salmon mixture had the lowest proportion of Snake River hatchery fish (7.6%,



Table 5). The lowest proportion of Snake River hatchery fish that was observed in the other adipose-clipped fishery mixtures was found in a test fishery (34.6%), which was evaluating a new off-channel area in the lower Columbia River. The natural origin mixtures (adipose-intact fish) from Bonneville Dam and the zone 6 Ceremonial harvest also had higher proportions of Snake River hatchery fish (12.5% and 19.8%, respectively) as compared to the Wind River harvest.

We also applied “strategy 2” to the Wind River analysis, and found that the Upper\_Columbia\_R\_sp (includes upper Columbia River hatcheries as well as Carson Hatchery, Walla Walla Hatchery, and Umatilla Hatcheries) was the genetic stock representing the largest proportion of the Wind River harvest (57%, Fig. 4). PBT would be better suited than GSI for identifying the target stock (Carson Hatchery), and in future years this approach will be possible. From the Snake River, there was 7% assignment to PBT hatcheries. Using GSI, 8% of the harvest in this fishery was identified from the RapidR\_Clearwater\_sp, and 1% from MF\_Salmon\_sp. Assignment of the RapidR\_Clearwater\_sp genetic stock can be the result of misassignment that occurs between the two genetically similar stocks: Carson\_H/U\_Col\_R\_sp versus RapidR\_Clearwater\_sp. This misassignment is known to occur based on comparisons of GSI results and coded wire tags. For example, 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, 9 of them were concordant with the GSI assignment estimates (75% concordance rate). The 3 misassignments occurred between Carson\_H/U\_Col\_R\_sp versus RapidR\_Clearwater\_sp stocks. In 2012, the Wind River sport harvest was analyzed with PBT and GSI methods, and there were 10 Snake River PBT assignments observed among 349 fish (3%). The GSI method assigned 5% of fish to Snake River stocks. Our estimate in this current report would therefore represent a two-fold increase, but is still lower than the proportion of natural-origin Snake River fish in collections taken from Bonneville Dam.

To be clear, this analysis cannot precisely quantify Carson Hatchery fish that are harvested in the bubble fishery nor can it tell 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.

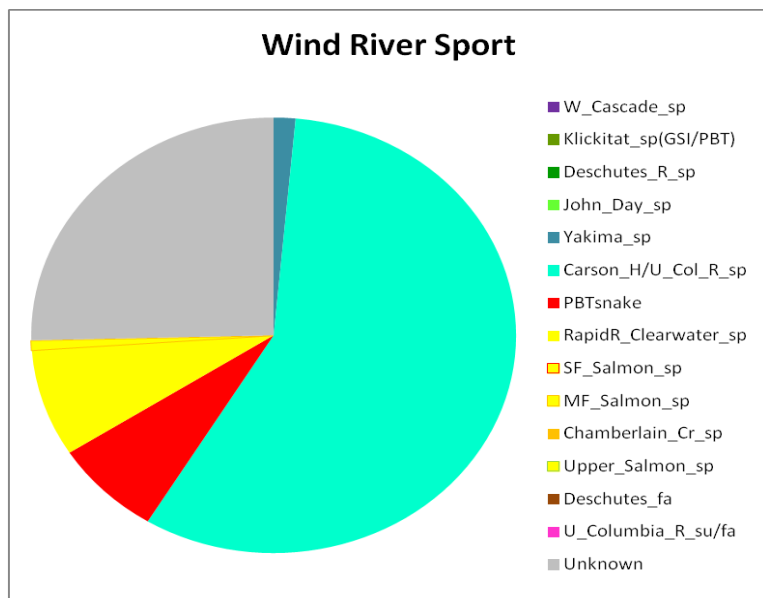


Figure 4. Genetic stock composition of the Wind River sport harvest in 2013

*Comparison of stock composition among spring management period Chinook salmon fisheries*

“Strategy 1” analysis of spring-run Chinook salmon from adipose-clipped fishery mixtures identified relatively larger proportions of lower Columbia River stocks (likely composed of mostly Willamette River fish based on previous analyses) near the mouth of the Columbia River, such that region B was comprised of a range of 37–39% and region A had a range of 8–17% of lower Columbia River stocks in commercial and sport fisheries (Fig. 5). PBT-assignments made it possible to discriminate fish by their hatchery-of-origin (16 total hatcheries represented), which is a much finer scale than the scale that GSI assignments can resolve. Stream-type (upriver) stocks were dominant upstream of region B. Analysis of adipose-intact fishery mixtures showed similar patterns, however the Snake River PBT-assigned stock (hatchery-of-origin) was found in smaller proportions than the adipose-clipped samples. The tribal bank fishery data and the tribal ceremonial fishery data would not necessarily represent the total tribal spring season harvest which occurred since samples were not collected throughout the fishery.

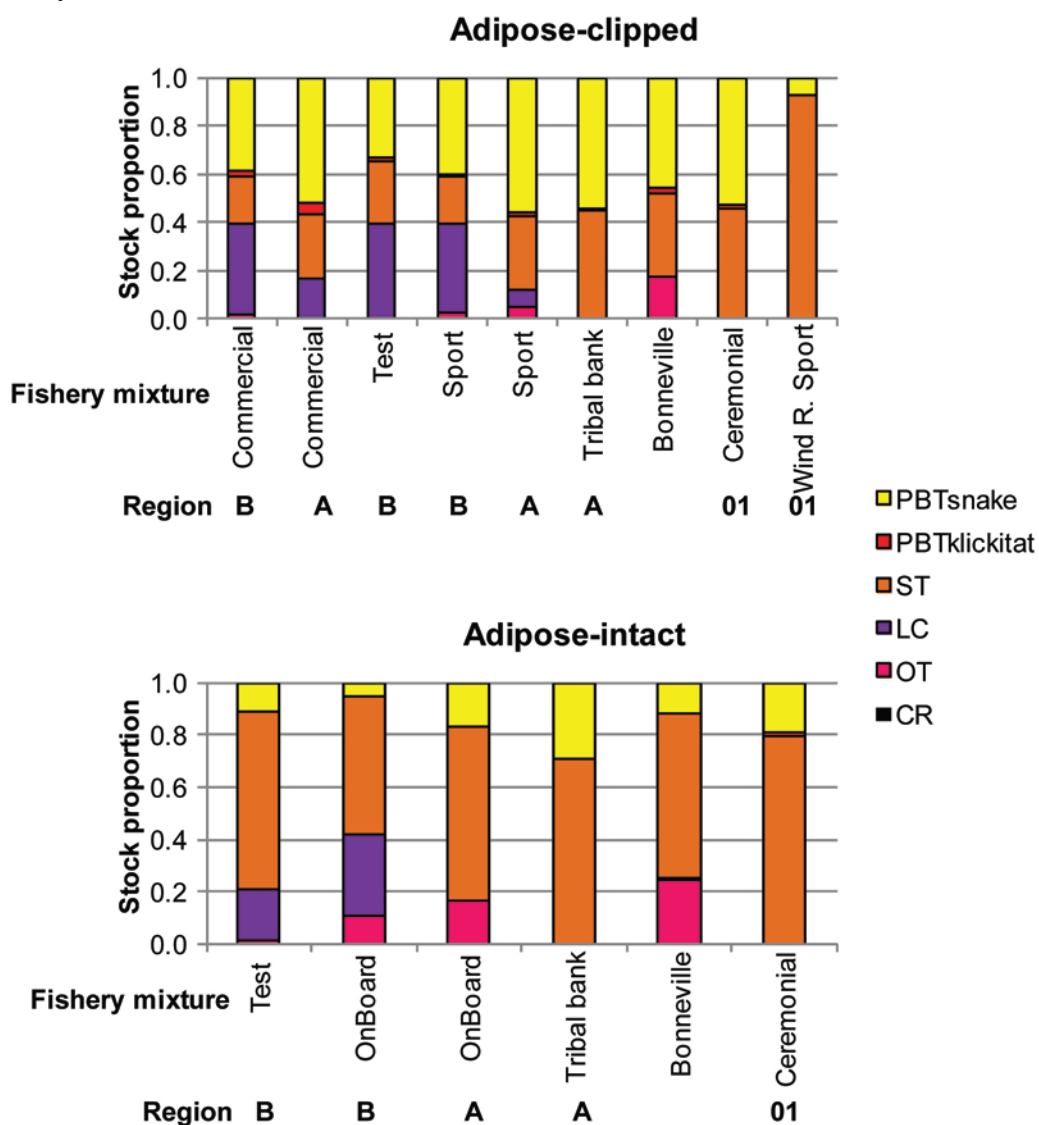


Figure 5.

Stock composition of spring management period Chinook salmon harvest mixtures. Note: “ST” includes upriver spring run and Snake River spring/summer run Chinook salmon stocks, “LC”

includes all lower river stocks (e.g. Willamette, Cowlitz, Lewis, and Sandy stocks), “OT” includes upper Columbia summer and upriver fall Chinook salmon stocks, and “CR” denotes Columbia River Rogue stock reared in Youngs Bay net pens. PBT was used to assign Klickitat and Snake River hatchery spring-run Chinook salmon.

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

Analysis of summer management period Chinook salmon fisheries utilized “Strategy 1” to execute the following objectives: 1) estimate % stock composition of ST (i.e. upriver spring Chinook salmon stocks) for the mark selective sport fishery below Bonneville Dam, 2) compare % stock composition of ST of adipose-clipped versus adipose-intact fish from the commercial harvest below Bonneville Dam, 3) characterize % stock composition of ST by pool and statistical week in zone 6, and 4) compare changes in % stock composition of ST of adipose-clipped versus adipose-intact fish through time in Zone 6. While jack Chinook salmon are not harvested at high rates in fisheries and there are no specific harvest limits for jacks, jack Chinook salmon if sampled could have been incorporated into this analysis.

Table 5. Comparison of stock composition of summer management period Chinook salmon fisheries.

Method	Genetic stock	Lower R.						Bonneville		Zone 6	
		Sport		Commercial		Dam		Tribal			
		Ad-clip		Ad-clip	Ad-intact	Ad-clip	Ad-intact	Ad-clip	Ad-intact		
		B	A	B	A	B	A				
GSI	CR	0.0%	0.0%	0.0%	0.0%	2.2%	0.0%	0.0%	0.0%	0.2%	0.6%
	LC	15.9%	11.1%	49.2%	23.1%	19.8%	12.8%	0.0%	0.0%	0.0%	1.0%
	OT	50.0%	50.0%	41.0%	74.4%	71.4%	80.9%	86.3%	85.8%	92.6%	87.1%
	ST	2.3%	5.6%	0.0%	2.6%	3.3%	4.3%	3.7%	9.7%	1.4%	9.6%
PBT	Klickitat	4.5%	5.6%	0.0%	0.0%	0.0%	0.0%	1.3%	0.0%	1.5%	0.0%
	Snake	27.3%	27.8%	9.8%	0.0%	3.3%	2.1%	8.6%	4.6%	4.3%	1.7%
		100.0	100.0	100.0	100.0	100.0	100.0	100.0		100.0	
GSI/PBT	Total	%	%	%	%	%	%	%	100.0%	%	100.0%
	Total PBT	31.8%	33.3%	9.8%	0.0%	3.3%	2.1%	9.9%	4.6%	5.8%	1.7%
	Total ST (GSI/PBT)	34.1%	38.9%	9.8%	2.6%	6.6%	6.4%	13.7%	14.2%	7.2%	11.3%

Note: Each fishery mixture is categorized by whether fish had clipped or intact adipose fins. Also, the lower river fisheries indicate which region (B or A, Fig. 1) from which the harvest mixture originated. “Total ST” indicates the total proportion of upriver spring and Snake River spring/summer stocks and represents combined analyses of GSI and PBT.

The lower river sport and commercial fisheries below Bonneville Dam were composed of very different proportions of ST stock. “Total ST” stock includes proportions of upriver spring and Snake River spring/summer stocks measured using both GSI and PBT. In the sport fishery,

this proportion was greater than three times the Total ST stock proportions observed in all other fishery mixtures (Table 5). This high proportion of the Total ST stock likely owes to the fact that the sport fishery occurred in the first few weeks of the summer management period (Table 1), which is when the ST stock is in highest abundance. Below Bonneville Dam, stock proportions did not vary much between regions nor between adipose categories within a particular fishery, however, the Region B adipose-clipped mixture from the commercial fishery was unique among the commercial mixtures because of the relatively high proportion of lower Columbia stock (LC).

We characterized the % stock composition of ST stock by pool and statistical week in Zone 6 (Fig. 6). This percent decreases through time in the Dalles and John Day pools, however the Bonneville pool maintains greater than 10% across weeks.

We compared changes in % stock composition of ST stocks of adipose-clipped versus adipose-intact fish through time in Zone 6, and found that the percentage of ST stocks declined in both harvest and Bonneville Dam mixtures over time, but the harvest may have generally lower representation of this group than what passes the dam in any given week (Fig. 7). There is no obvious explanation of why there would be a significant difference in the composition of the run at Bonneville and the composition in the Zone 6 fishery. There were no large differences observed between adipose-clipped and adipose-intact fish, except for week 26 in which we observed higher proportions of adipose-intact fish.

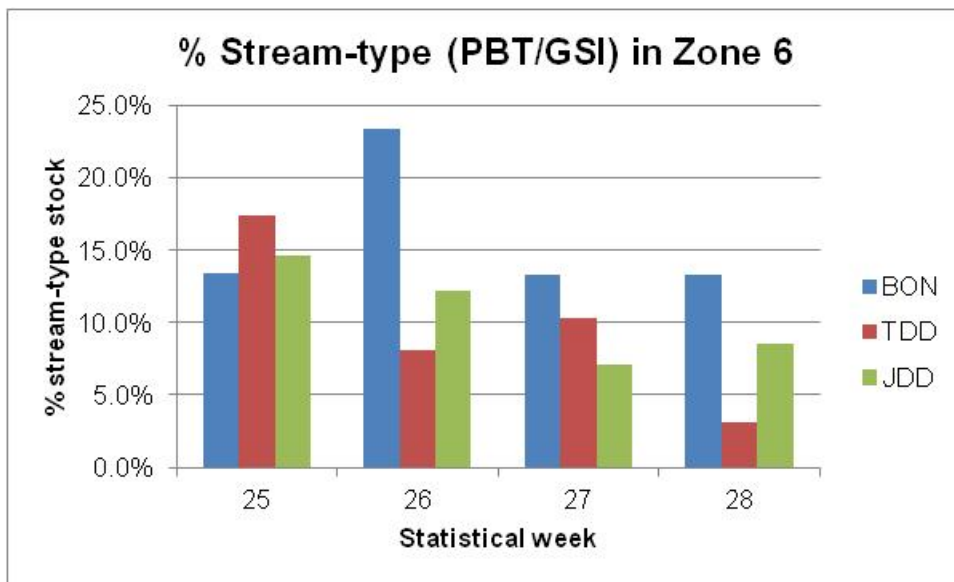


Figure 6. The temporal and spatial patterns in percent of ST stock (“stream-type”) that comprise the tribal zone 6 summer-run Chinook salmon harvest. Four statistical weeks are shown on the x-axis and the reservoir from which the salmon were harvested is indicated as Bonneville (BON), The Dalles (TDD), and John Day (JDD) pools. There is a level of uncertainty around pool specific harvest data because pool of origin is not always correct. There is no obvious reason why there would be differences in the composition of the catch in any of the three pools, but any difference could be attributable to sampling effects.

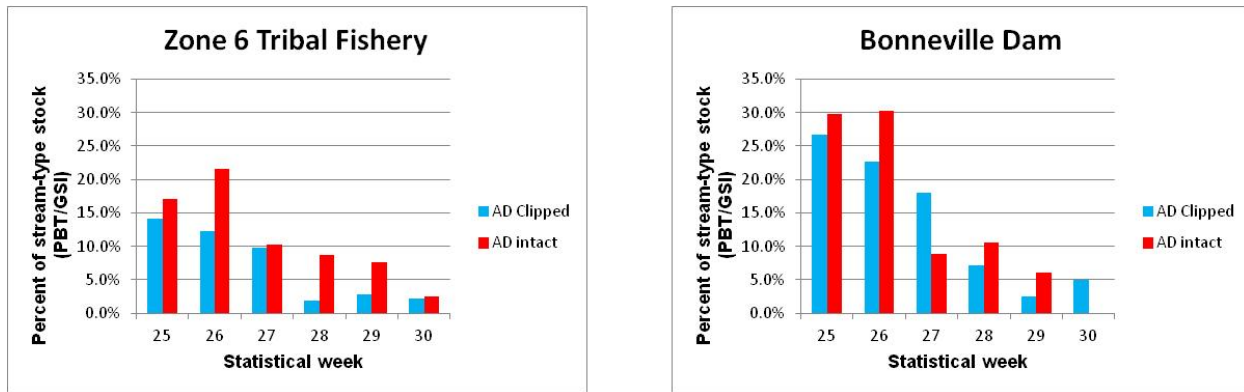


Figure 7. Temporal patterns of the percent of ST stock (upriver spring and Snake River spring/summer stocks) in adipose-clipped and adipose-intact mixtures of summer management period Chinook salmon sampled in Zone 6 tribal fishery and Bonneville Dam in 2013.

*Parentage based tagging assignments of harvested summer management period Chinook salmon*

The PBT analysis resulted in 94 hatchery-origin adult and jack salmon that could be assigned back to Klickitat and Snake River hatchery broodstock parents that were spawned in 2008 – 2010 (Table 6). These salmon were found to have originated from the following eight sources: Klickitat, Dworshak, Imnaha, Lostine, McCall, Pahsimeroi, Rapid River, and Sawtooth hatcheries. Among the 4-year-old fish, the broodstock from McCall 2009 yielded the highest number of assignments (N=8), however across several broodstock (e.g. Klickitat, Lostine, McCall, and Sawtooth), jacks were more numerous than 4-year-olds. Comparisons of the PBT stock composition between the spring-run (Table 5) and summer-run harvests (Table 6) show a clear run-timing effect in which typically late-running stocks predominate in the summer-run harvest. Jack age classes typically run later than 4-year-olds (Hess et al. 2013), which also explains why jacks comprised larger proportions than 4-year-olds in the summer-run harvest versus the spring-run harvest. The broodstock with the highest number of assignments were the jacks from McCall 2010 (N=16), which were twice in number of that stock's 4-year-olds.

Table 6. Parentage assignments of summer management period Chinook salmon fisheries.

PBT source hatchery	PBTpop	Tag Rate	Region:	Lower R.		Zone 6		Total	
			Fishery:	Commercial	Sport	Tribal			
			Origin:	HOR	NOR	HOR	HOR	NOR	
Klickitat Hatchery	OtsKLKH08S						2		2
	OtsKLKH09S					1	6		7
	OtsKLKH10S					3	5		8
LSRCP/USFWS - Dworshak	OtsDWOR08S	97.5%					2		2
LSRCP/ODFW - Imnaha	OtsIMNW08S	99.2%					2		2
	OtsIMNW09S	97.4%		2			5		7
	OtsIMNW10S	97.6%		1		1	1		3
	lost.conv.09	94.4%					1	1	2
LSRCP/IDFG -	OtsLSTW10S	95.4%				4	3	1	8
	OtsMCCA08S	97.6%					1		1

McCall (SFSR)	OtsMCCA09S	96.4%		2		2	3		7
	OtsMCCA09S_2	95.9%			1				1
	OtsMCCA10S	99.4%		1	1	8	3	3	16
	OtsPAHH09S	96.8%				2	8		10
	pahs.ibs.10	94.9%			1	1	2	1	5
	OtsRAPH10S	99.1%				1	1		2
	OtsSAWT08S	99.0%					2		2
	OtsSAWT09S	99.6%					2		2
	OtsSAWT10S	99.4%			1	3	2	1	7
	Snake PBT avg:	95.4%	PBT N	6	4	26	51	7	94
LSRCP/IDFG - Sawtooth	Snake Total			6	4	22	38	7	77
	%Snake			6.3%	3.0%	28.8%	5.9%	1.8%	
			Total	100	138	80	678	401	1397

Note: PBTpop is the abbreviated hatchery source and the digits indicate the spawn year, e.g. 08S is spawn year 2008. Adipose-clipped (HOR) and adipose unclipped (NOR) categories of fish could be separated for the Commercial and Tribal fishery mixtures. The sport fishery is mark-selective (HOR only). The “%Snake” indicates our estimate of the composition of Snake River hatcheries in each fishery mixture using an expansion factor based on average tagging rate across all Snake River hatcheries.

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

For the lower river fall-run Chinook salmon sport fishery, we used “Strategy 2” to estimate stock composition in 2012 and 2013. In neither year PBT assignments were observed, and in 2013 the major GSI stocks from greatest to least were U\_Columbia\_R\_su/fa (73%), W\_Cascade\_fa (11%), Snake\_fa (3%), Spring\_Cr\_Group\_Tule (2%). Compared to the 2012 harvest, the 2013 harvest appears to have increased its proportion of upriver stocks relative to the targeted stock (W\_Cascade\_fa), which had previously comprised 24% of the harvest (Fig. 8). The fall season mark selective fishery was for a short period within the overall fall season fisheries which were mostly full retention fisheries. Results from these fisheries would not be applicable to any other portion of the fall fisheries.

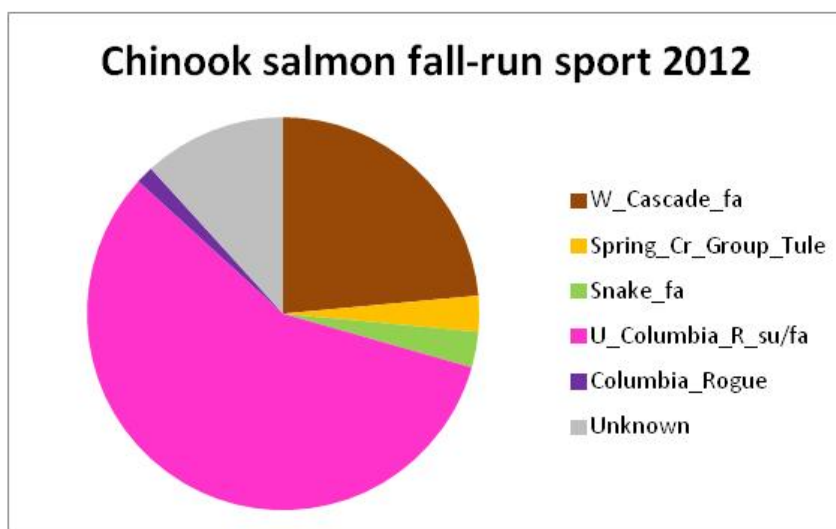
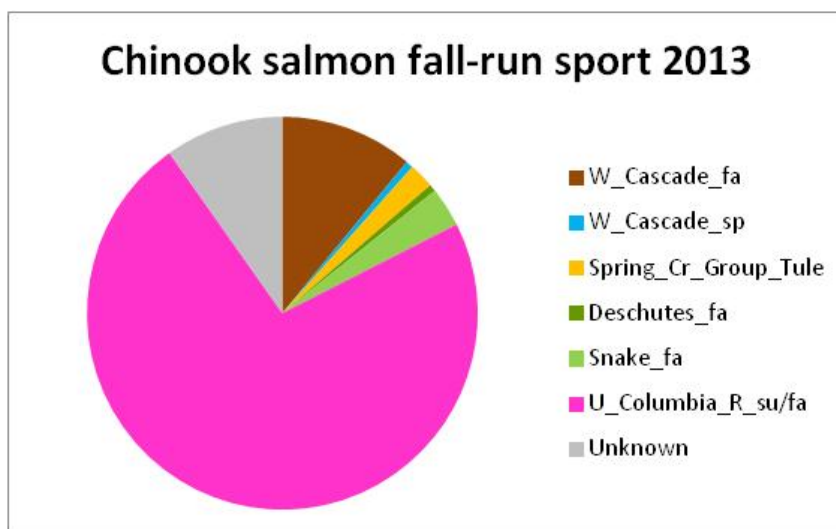


Figure 8. Genetic stock

composition of the lower Columbia River fall-run Chinook salmon sport harvest in 2012 and 2013. Note: the difference between assignment of West Cascade spring stocks in 2013 and 2012 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 each fish was assigned to three Columbia River genetic stocks (Table 7). 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. For the tribal fishery, sockeye were sampled at a higher rate for the gillnet fishery compared to the platform fishery even though the platform fishery generally catches larger numbers of fish. It is not possible to be sure if the proportion of Snake River sockeye in any of the harvests was significantly different than the proportion of Snake River sockeye at Bonneville (Table 8). The lower confidence limit for the Snake River stock in the



Zone 6 tribal fishery was 1% of that harvest, and the commercial fishery below Bonneville Dam had a lower confidence limit of less than 1%.

The Zone 6 tribal harvest was not sampled prior to week 25 or after week 30. The lower river sport fishery was only sampled for a small portion of the entire period it was open.

Table 7. Summary of sample numbers and stock assignments for each sockeye salmon fishery by weekly strata.

Fishery	Stock	Statistical week										Total
		22	23	24	25	26	27	28	29	30		
Commercial	Okanagan	1		4	10	12			4		31	
	Snake					2					2	
	Wenatchee				13	2					15	
Sport	Okanagan				7						7	
	Snake											
	Wenatchee				4						4	
Zone6	Okanagan				161	175	133	116	44	16	645	
	Snake				2	4	4	7		2	19	
	Wenatchee				145	134	127	73	16	5	500	

Table 8. Comparison of stock-specific abundance and percent composition among sockeye salmon fisheries.

Mixture source	Mean (95% C.I.)					Stock proportion				
	Wenatchee	Okanagan	Snake	Whatcom	Other	Wenatchee	Okanagan	Snake	Whatcom	Other
Commercial	47 (28 – 66)	96 (76 – 114)	6 (1 – 18)	0 (0 – 5)	NA	31.2%	64.6%	4.2%	0.0%	NA
Sport	183 (61 – 309)	319 (177 – 430)	0 (0 – 62)	0 (0 – 71)	NA	36.4%	63.6%	0.0%	0.0%	NA
Zone 6	3436 (3205 – 3667)	4466 (4223 – 4679)	131 (88 – 204)	0 (1 – 32)	NA	42.8%	55.6%	1.6%	0.0%	NA
Total Harvest	3665 (3390 – 3920)	4882 (4591 – 5107)	137 (97 – 232)	0 (2 – 82)	NA	42.2%	56.2%	1.6%	0.0%	NA
Bonneville Dam*	47434	109510	1401	NA	2317	29.5%	68.2%	0.9%	NA	1.4%

Note: The mean stock abundance estimate is provided for each fishery harvest and includes 95% confidence intervals.

\*Bonneville Dam abundance estimates shown here differ from those in Table 13 since they only include the interval of weeks that coincide with sockeye salmon fisheries (statistical weeks 25-30), and were calculated based on a combination of PIT-tag and genetic data, and include. The total harvest estimates are a summation across all fisheries sampled here.

The timing of the sockeye salmon fisheries may influence the harvested proportion of each stock, and is consistent with run-timing distributions we observed in our previous report, particularly that the Wenatchee stock has relatively early run-timing compared to other stocks. The Snake River stock was only represented by 19 fish in the Zone 6 fishery samples (Table 7) making run-timing estimates imprecise for this stock. Of the 19 Snake River sockeye salmon identified with GSI, the largest number (7) were sampled in week 28 and they were estimated to be in highest relative stock proportion in week 30 (Figure 9). However, these GSI results are contradicted by Snake River sockeye PIT tagged as juveniles which do not show this late timing. Stock composition in weekly strata show variability of Snake River stock with no Snake River sockeye estimated in weeks 25 and 30 at Bonneville and no fish estimated in the tribal catch in week 29. This compares to 20 PIT tagged Snake River sockeye which passed Bonneville in week 25 and 8 PIT tagged Snake River sockeye which passed Bonneville in week 30. The first three weeks also had the highest proportions of Wenatchee stock (Fig. 9). Differential stock representation between Bonneville Dam and the Zone 6 tribal harvest was also evident for the Wenatchee stock which was 42% versus 30% in the harvest versus dam, respectively (Table 8).

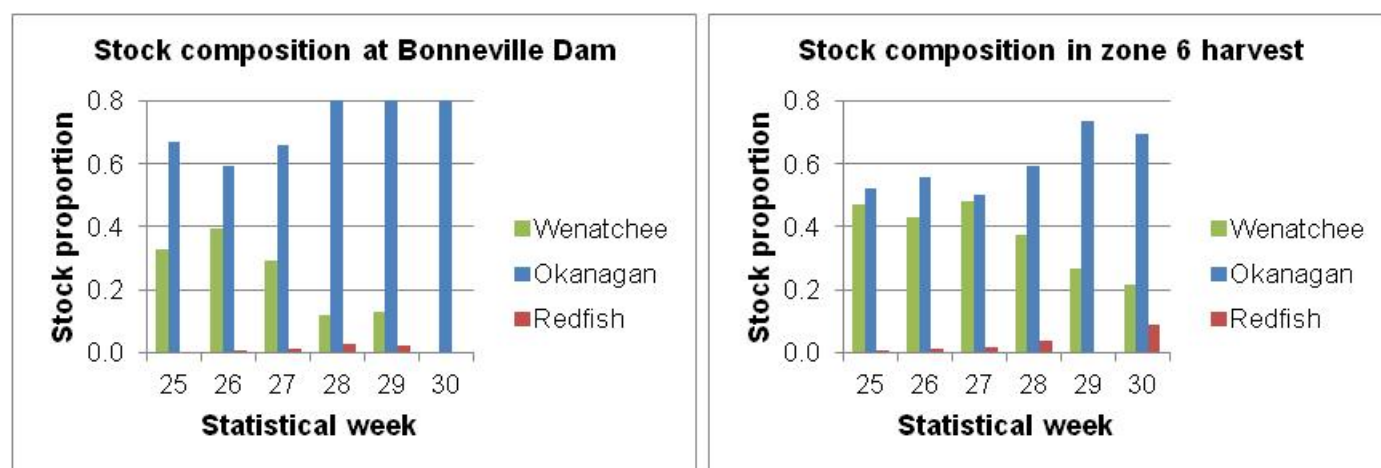


Figure 9. Stock composition at Bonneville Dam and the Zone 6 sockeye harvest on a weekly basis.

#### *Stock composition and PBT of the adipose-intact steelhead from the Zone 6 tribal fishery*

There were 654 tissues for unclipped steelhead in the Zone 6 harvest mixture, however 17 tissues failed to genotype with less than 10% missing data, 2 tissues were identified as cutthroat hybrids (determined by positive genotypes for at least 2 of the 3 species diagnostic loci), and 2 tissues were found to be duplicate pairs of genotypes. We excluded 21 samples because of these issues. These exclusions left 633 Zone 6 harvest samples for analysis. Of these 633 total samples, 68 fish assigned to PBT source hatcheries (Table 9). The twelve hatcheries were Touchet, Tucannon, Lyons Ferry, Wallowa, Grande Ronde Cottonwood, Little Sheep Cr. , Squaw, Dworshak, Oxbow, E.F. Salmon R., Sawtooth, and Pahsimeroi hatcheries. This hatchery component made up 11% of the stock composition of these unclipped steelhead (Fig. 10). There are many Snake River hatcheries that release unclipped smolts, and so this proportion is expected. The largest proportion of this harvest was derived from the MGILCS reporting group (63%), which is also the most broadly geographically distributed and includes a large area of the mid-Columbia R. and the lower Snake River basin. All other stocks were less than 10%, and only the Upper Columbia R. (6%) and upper Salmon R. (9%) were above 5% proportion.

The PBT assigned fish from the Zone 6 unclipped steelhead harvest were aged according to the year in which their parents were spawned. The 2-ocean age fish were on average higher in abundance (average 0.8% of total harvest) among all PBT source hatcheries as compared to 1-ocean age fish (average 0.4% of total harvest). Among 2-ocean age fish, Dworshak hatchery was the most abundant (3.8% of total harvest) and followed by E.F. Salmon R (0.9%). Among 1-ocean age fish, the Sawtooth was most abundant (1.3% of total harvest), followed by E.F. Salmon R (0.8%).

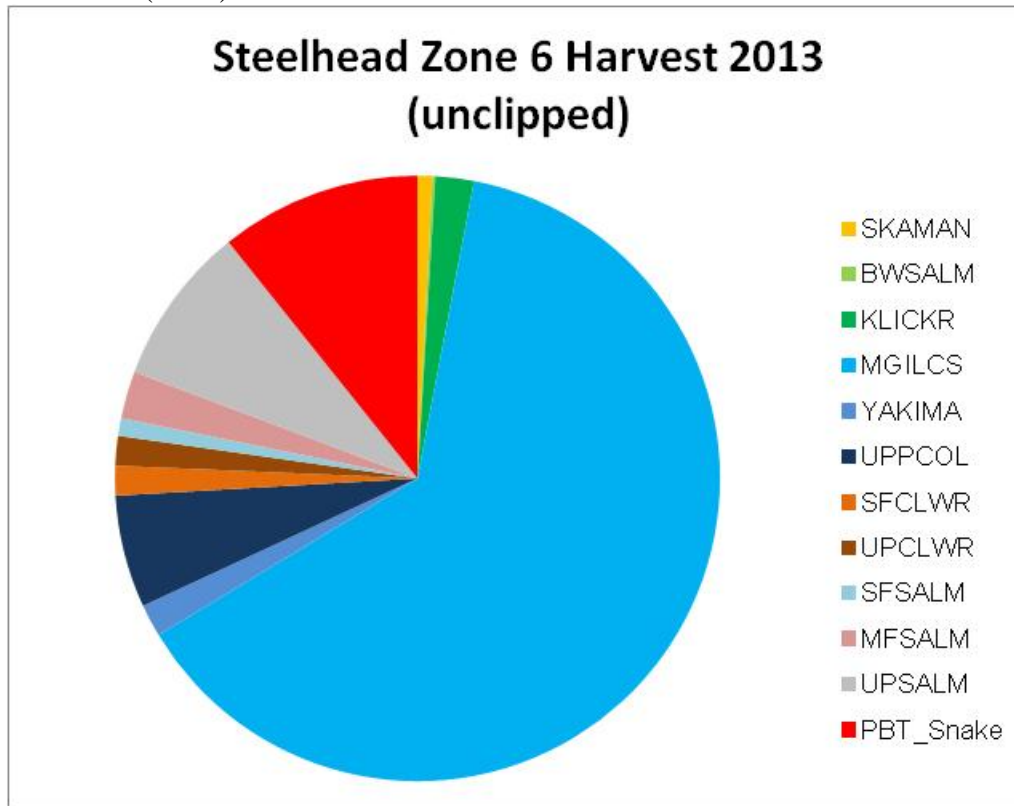


Figure 10. Stock composition of adipose-intact steelhead harvested in 2013 from Zone 6. The following eleven of 13 total stocks in the Columbia River Basin GSI baseline were represented: Skamania “SKAMAN”, Big White Salmon “BWSALM”, Klickitat “KCLICKR”, MGILCS (see below), Yakima “YAKIMA”, upper Columbia “UPPCOL”, S.F. Clearwater “SFCLWR”, upper Clearwater “UPCLWR”, S.F. Salmon “SFSALM”, M.F. Salmon “MFSALM”, and upper Salmon “UPSALM”. The MGILCS stock includes the following Snake River stocks: George Cr, Asotin Cr, Alpowa Cr, and Tucannon R as well as the stocks from the Grande Ronde, Imnaha, Lower Clearwater and Lower Salmon R.; and all other middle Columbia River stocks that were not listed above, because this large group of stocks is genetically highly similar.

Table 9. Summary information on numbers and origins of steelhead that were assigned using a combination of GSI and PBT.

Hatchery	Origin	Genetic stock	Statistical week																	Total
			25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		
		SKAMAN	2												1		1	1	5	
		BWSALM														1			1	
		KLICKR			1			2	1			5					2	2	13	
		MGILCS	1	3	1	32	1	88	11	2		51	20	36	29	25	30	71	401	
		YAKIMA				2		3							1	1		4	11	
		UPPCOL	1		2	5	1	8	2			2	6	5	2	1		3	38	
		SFCLWR										1		1			1	7	10	
		UPCLWR										1		2	3	1	2	1	10	
		SFSALM											1	2	2	1			6	
		MFSALM						2	2				3	3	2		2	2	16	
		UPSALM				2		12	1		1	10	1	5	8	2	7	5	54	
LSRCP/WDFW-L.F. (Touchet)	Snake R. hatchery brookstock	OmyTOUW11S											1						1	
LSRCP/WDFW-L.F. (Tucannon)		OmyTUCW10S															2		2	
LSRCP/WDFW-Lyons Ferry		OmyLYON11S						1											1	
		OmyLYON12S						1											1	
LSRCP/ODFW-Wallowa F.H.		OmyWALL10S																1	1	
		OmyWALL11S										1	1	1					3	
LSRCP/WDFW-L.F. (G.R. cottonwood)		OmyCGRW11S																1	1	
LSRCP/ODFT - Little Sheep Cr. FH		OmyLSCR10S																1	1	
		OmyLSCR11S														1			1	

LSRCP/IDFG Sawtooth (USB/Squaw)	OmySQUW10S												1		1	2	4
LSRCP/IDFG/USFWS Dworshak/C.W.	OmyDWOR10S									2	3	2	9	8			24
	OmyDWOR11S										1						1
Idaho Power/IDFG,Oxbow F.H.	OmyOXBO11S												1				1
LSRCP/IDFG Sawtooth (EFSR)	OmyEFSW10S								2					1		3	6
	OmyEFSW11S				1						1	1		1	1		5
LSRCP/IDFG Sawtooth (IDFG & SBT)	OmySAWT10S								2				1				3
	OmySAWT11S				1				1		3	2	1				8
Idaho Power/IDFG, Pahsimeroi F.H.	OmyPAHH10S									1							1
	OmyPAHH11S										1	2					3
	cutthroat				1		1										2
	duplicate										1					1	2
	failed				1		1			1		1	3	3	3	4	17
	Grand Total	4	3	4	43	2	121	17	2	1	78	34	63	63	42	61	654

Note: The Zone 6 fishery samples could be broken into weekly strata, and statistical week 25 begins 6/17/2013 and week 40 ends on 10/06/2013. Natural-origin individuals were assigned using GSI to the stock with the highest probability (best estimate). Snake River hatchery stocks were assigned using PBT and include broodyears from 2008 to 2012. The hatchery sources are abbreviated and the digits indicate the spawn year, e.g. 08S is spawn year 2008.

## 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 steelhead fisheries. For the first time, we were able to assign all three major age classes of spring Chinook from Snake River hatcheries and the Klickitat hatchery, which is the only hatchery outside of the Snake River included in the current PBT baseline. Expansion of the PBT baseline will make it possible to assign returning adult Snake River fall-run Chinook salmon (~2015), and our 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 needed. This report includes the first 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 likely 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 tribal mainstem summer-run Chinook salmon harvests above Bonneville and non-treaty fisheries below Bonneville Dam which are part of the total non-treaty summer fisheries, part of the fall-run Chinook salmon sport fishery below Bonneville Dam, the mainstem treaty sockeye salmon harvests above and non-treaty harvest below Bonneville Dam, and the summer and fall tribal harvest of steelhead above 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 versus summer management period adult and jack 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 stocks 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 summer Chinook salmon are almost all past Bonneville by June 15 in most years. However, specific conclusions relative to the harvest impacts on spring or summer Chinook salmon cannot be made from this genetic analysis as jacks are included in the current analysis.

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 natural-origin samples from Bonneville Dam and tribal harvests during the same set of weeks. However, 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 mark selective fall-run Chinook salmon sport fishery in the lower Columbia River mainstem was also analyzed and tested for its effectiveness in avoiding upriver stocks. It appears that there were some differences in stock proportion of the W\_Cascade\_fa stock between years, indicating that more upriver stocks were captured in 2013 as compared to 2012. More years of analysis may be needed before a possible trend and its causes can be characterized.

The sockeye salmon tribal fishery is managed in a way that attempts to harvest as many harvestable sockeye as possible under the allowed harvest rate schedule in the U.S. v. Oregon Management Agreement. Our first analysis of sockeye salmon harvest indicates 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 our previous report; 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. Further analysis will be needed to examine these patterns for consistency and delve into explanations.

We analyzed a second year of the unclipped steelhead harvested in the Zone 6 fishery. These steelhead were found to be composed of 11% unmarked hatchery-origin fish from the Snake River based on PBT results. Most of these fish were from Snake River hatcheries that share genetic similarity with the large GSI reporting group that spans much of the middle Columbia River and lower Snake River tributaries (MGILSC) which comprised 63% of the harvested unclipped steelhead.



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

### Introduction

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

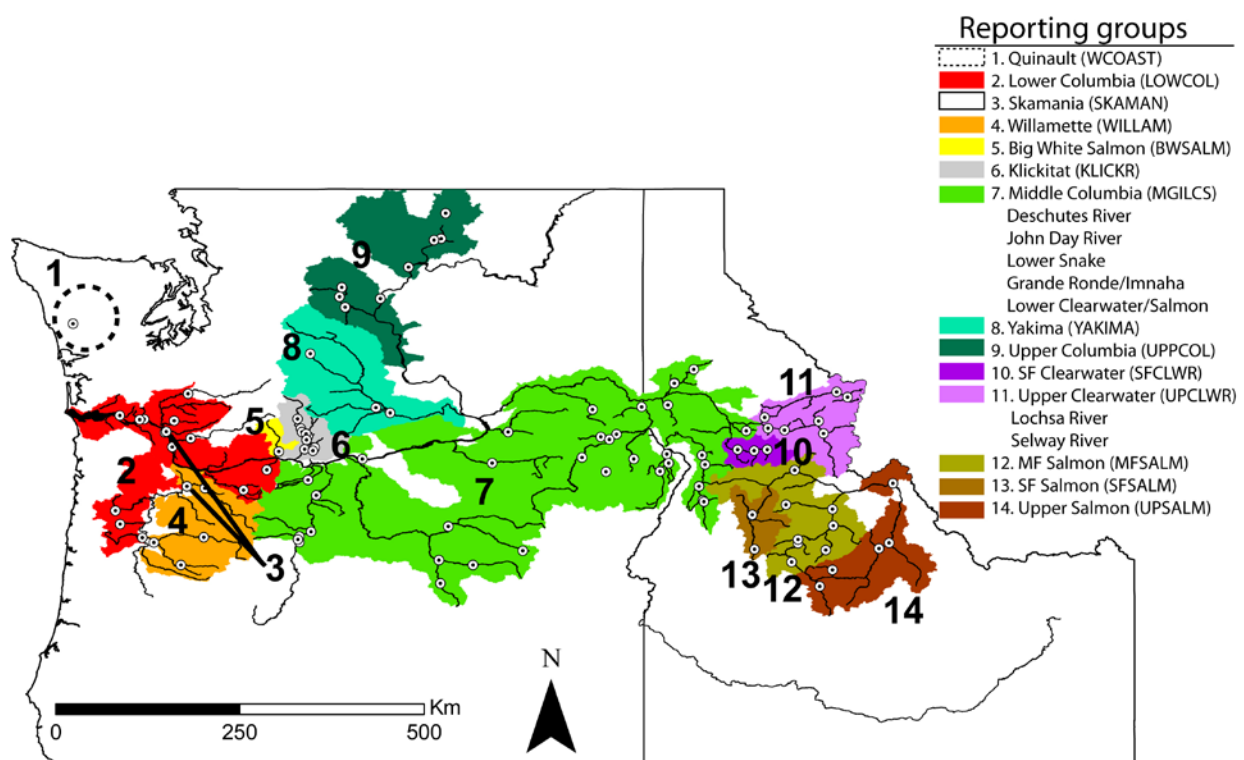


Figure 1. Scope of project includes all stocks of Chinook salmon, sockeye salmon, and steelhead trout distributed within the Columbia River basin. Shown here are the steelhead reporting groups (i.e. stocks) and collections that comprise the steelhead GSI baseline.

As evident from the genetic stock identification (GSI) analyses of Chinook and sockeye salmon and steelhead fisheries harvests in Section 3, certain stocks seem to have strong spatial and temporal associations. However, because the type of fishery gear, harvest regulations, and locations targeted varies considerably among fisheries, 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. However, sampling at Bonneville

Dam remains a challenge due to trapping limitations at this location. In addition to information on abundance and run-timing, biological data including fork length and age can be examined with estimated stock of origin in order to characterize life history differences among stocks. This type of examination is especially important for steelhead, which has been managed using two life-history categories, A- and B-run. These life-history categories have been observed to be differentially characterized by run-timing at Bonneville Dam (e.g. B-run typically arrives after Aug. 25<sup>th</sup>), 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 and Chinook and sockeye salmon from April to October to estimate temporally stratified proportions of stocks and extrapolate abundance using a daily census that is conducted at the Bonneville Dam fish counting window. We examine steelhead and Chinook salmon using two sets of species-specific SNP assays for a combined total of 192 loci per species. GSI of sockeye salmon requires fewer markers than for other species in this study, and a set of 96 SNP loci can accurately resolve the fewer number of sockeye stocks that are present in the basin relative to other salmonids. Although there are some methodological differences among these species-specific applications (e.g. different temporal strata, 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 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 and 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:**

**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?**

**Can selective fisheries targeting hatchery fish or healthy populations reduce impacts on ESA-listed populations? (Not applicable to this project)**

Analysis of the 2011 dataset by Hess et al. (2012) was the first year we were able to apply an additional genetic tool, referred to as Parentage Based Tagging (PBT), to assign a

portion of Snake River hatchery-origin spring-run Chinook salmon and summer-run steelhead back to their hatchery parents (Steele et al. 2011). This new and powerful genetic tool provides the opportunity to obtain additional types of data including accurate age of fish, quantification of the number of unmarked (non-adipose clipped) hatchery fish, and precise assignments of fish to their source hatchery (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. However, these tools can provide the greatest benefit when applied in combination, as GSI has the ability to provide information on wild fish throughout the Columbia River basin, while PBT is most effective for hatchery origin fish. The current PBT baseline only includes Snake River hatcheries but will be expanded to others above Bonneville Dam in the near future. Therefore, GSI is still a necessary tool for both hatchery and wild fish that originate from outside the Snake River basin. This year's report marks the first 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. This study integrates PBT and GSI results to provide the greatest amount of stock information ever available for hatchery and wild steelhead and Chinook salmon passing Bonneville Dam.

Aside from these analyses on salmonids, PBT technology has made it possible to monitor conservation efforts in a non-salmonid species, Pacific lamprey (*Entosphenus tridentatus*). Pacific lamprey (populations are in decline (Close et al. 2002) and are relatively understudied. Research to answer critical biological and management related uncertainties is undertaken by CRITFC and its member tribes. Translocation (i.e. transporting individuals from a source population to a recipient site), artificial propagation, and habitat improvement are all being used to rebuild the Pacific lamprey abundance in the interior Columbia River Basin, where declines have been most severe. Lamprey from the lower Columbia River are a key source for translocation and artificial propagation strategies. We used 94 SNPs (described in Hess et al. 2013) to perform a parentage analysis as a direct method to validate the reproductive success of translocated adults in Newsome Creek, a tributary of the Clearwater River in the Snake River Basin. Pacific lamprey appear to have been extirpated from this creek and its adjacent tributaries since 2003 (Cochnauer & Claire 2009). Starting in 2007, 50 adults (which had been captured at one or more mainstem dams on the Columbia River and held for at least one winter) were translocated to Newsome Creek, and subsequently (between 2008 and 2010), 22–25 adults were translocated per year (Ward et al. 2012). We genotyped 100% of the total number of translocated adults from 2007. A screw trap located at the mouth of Newsome Creek was used to collect outmigrating ammocoetes in 2012, and parentage assignments showed that this group was 99% composed of 5-year-old offspring from the 2007 translocation event (Hess et al. in review). Our objective in this study was to test whether any of this 2007 cohort could be identified as juvenile mortalities on the turbine screen of Lower Granite Dam, nearly 250 km downstream of Newsome Creek. This information would help fill a critical data gap on outmigration timing of juvenile life stages of lamprey.

#### *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=1,280), Chinook (n = 2,205) and sockeye salmon (n=223, and PIT-tag n=576) adults in 2013 during migration runs at Bonneville Dam. This sampling effort is covered under Scientific Research Permit #1379 under Section 10 of the ESA (permit included in PISCES attachments).

Sampling for Chinook salmon at Bonneville Dam began during statistical week 17 (April 22, 2013). 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 under 4 percent of the total Spring management period adult Chinook salmon count had passed Bonneville by the sampling start date (April 22). While samples were taken from the large majority of the total spring Chinook salmon run, some early timed stocks may be slightly underestimated 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 very low. The total sample rate for the adult and jack spring Chinook run in 2013 was 0.9%. The total sample rate for adult and jack summer Chinook was 0.7%.

Based on numbers of fish collected, samples were pooled into weekly strata for Chinook (Table 1) and sockeye salmon (Table 2) and monthly strata for steelhead (Table 4) 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"](#). In addition, at Lower Granite Dam we processed 188 juvenile mortalities of Pacific lamprey that had been frozen after retrieval from turbine units and after defrosting we dried a piece of tissue for DNA extraction and analysis.

**Table 1. Sample numbers by weekly strata for Chinook salmon that were DNA sampled or tallied for abundance at Bonneville Dam in 2013.**

	Statistical week	Fish count	Sample					
			Ad-clipped(HOR)		unclipped(NOR)		Total	rate
			Non-PBT	PBT	Non-PBT	PBT		
Management period Spring	17	21931	35	46	23	5	109	0.5%
	18	32177	53	77	46	8	184	0.6%
	19	28551	45	56	40	5	146	0.5%
	20	15415	73	75	80	17	245	1.6%
	21	9839	44	74	86	10	214	2.2%
	22	7986	27	25	53	9	114	1.4%
	23	19023	70	33	78	9	190	1.0%
	24	17170	72	22	87	4	185	1.1%

Summer	24	3463	0	0	0	0	0	0.0%
	25	21835	26	4	33	4	67	0.3%
	26	15513	35	9	40	3	87	0.6%
	27	15682	50	11	43	2	106	0.7%
	28	10289	53	3	90	5	151	1.5%
	29	6381	40	1	49	0	90	1.4%
	30	5324	20	0	28	0	48	0.9%
	31	2232	1	1	12	0	14	0.9%
Fall	31	2572	0	0	5	0	5	-
	32	11587	5	0	17	0	22	0.2%
	33	17183						0.0%
	34	56323	16	0	34	0	50	0.1%
	35	161481						0.0%
	36	208345	13	0	37	0	50	0.0%
	37	290491						0.0%
	38	142972	12	0	38	0	50	0.0%
	39	81817	-		-			0.0%
	40	35911	17	0	33	0	50	0.1%
	41	31804	-		-			0.0%
	42	14156	9	0	19	0	28	0.2%
Total		1287453	716	437	971	81	2205	0.2%

Note: For reference, statistical week 17 is 4/22/13-4/28/13 and 42 is 10/14/13-10/20/13. “Fish count” is based on tallies of Chinook salmon adults and jacks provided by the Fish Passage Center (<http://www.fpc.org>) as observed by the Corps of Engineers at their fish counting window. Genetic sample numbers (N) above 10 were used to estimate stock abundance with GSI. This year we did not sample every week in the fall and instead lumped all samples from even-numbered weeks together from 32 – 42. 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 15<sup>th</sup>, June 16<sup>th</sup> to July 31<sup>st</sup>, and August 1<sup>st</sup> to December 31 which are used to categorize spring-, summer-, and fall-run Chinook salmon.

**Table 2. Sample numbers and PIT-tag/genetic stock assignments of sockeye salmon that passed Bonneville Dam in 2013.**

Statistical week	Fish count	Sample								
		Combined PIT-tag and GSI results							Total	rate
		DES	ENT	OKA	RED	WEN	YAK			
1-22	100							0	0.0%	
23	3393			14		2		16	0.5%	
24	20439			107	2	39		148	0.7%	
25	43301			94		46		140	0.3%	
26	47120	1		138	1	91	1	232	0.5%	

27	37274	1	2	56	1	25		85	0.2%
28	23209		1	97	3	14	2	117	0.5%
29	7712			40	1	6		47	0.6%
30	2046			14				14	0.7%
31-39	911							0	0.0
Total	185505	2	3	560	8	223	3	799	0.4%

Note: For reference, statistical week 23 is 6/03/13-6/09/13 and 30 is 7/22/13-7/28/13. "Fish count" is based on tallies of sockeye salmon adults provided by the Fish Passage Center (<http://www.fpc.org>) as observed by the Corps of Engineers at their fish counting window. PIT-tag and GSI stocks are Deschutes (DES), Entiat (ENT), Okanagan (OKA), Snake River (RED), Wenatchee (WEN), and Yakima (YAK). The # of samples for a given week was used to calculate sample rate.

Very few sockeye salmon were sampled from three stocks, Snake River (n=8), Deschutes R. (n=2), and Entiat R. (n=3), which greatly limited inferences regarding run-timing and abundance of these stocks. For example, of the 8 Snake River sockeye salmon sampled, return numbers for weeks 24-29 were 2, 0, 1, 1, 3, 1, respectively. Thus, run timing of Snake River Sockeye based on genetic sampling was irregular and no obvious trends could be determined, but generally late at the beginning and then compressed with nearly 50% of the fish passing Bonneville in weeks 28 and 29. These estimates are inconsistent with known origin Snake River Sockeye PIT tagged as juveniles which had a regular and somewhat later timing than the run at large, but showed little similarity to the genetic data (Table 3).

**Table 3. Comparison of run-timing results between GSI- versus PIT-tag-based (tagged as juveniles) estimates.**

Week	Proportion of Sample Estimated to Be Snake R.	Estimated Number of Snake R. Sockeye	Cumulative Proportion of Snake R. Identified Genetically	Number of Snake River PIT Tagged Fish	Cumulative Proportion of PIT Tagged fish	Cumulative Proportion Bon Count	Cumulative Count
21-22	0.00%	0	0.0%	2	1.0%	0.1%	100
23	0.0%	0	0.0%	0	1.0%	1.9%	3493
24	1.4%	276	16.5%	4	2.9%	12.9%	23932
25	0.0%	0	16.5%	20	12.6%	36.2%	67233
26	0.4%	203	28.6%	75	48.8%	61.6%	114353
27	1.2%	439	54.7%	62	78.7%	81.7%	151627
28	2.6%	595	90.2%	26	91.3%	94.3%	174836
29	2.1%	164	100.0%	7	94.7%	98.4%	182548
30	0.0%	0	100.0%	8	98.6%	99.5%	184594
31+	0.0%	0	100.0%	3	100.0%	100.0%	185505



**Table 4. Sample numbers by monthly strata for steelhead that were DNA sampled or tallied for abundance at Bonneville Dam in 2013.**

		Fish count				Sample					
		Statistical week	Statistical month	Ad- clipped	unclipped	Ad-clipped(HOR)		unclipped(NOR)		Total	rate
						Non-PBT	PBT	Non-PBT	PBT		
Management period	Skamania Summer	14-16		496	281					0	0.0%
		17		110	45	6	1	1		8	5.2%
		18		119	14	3	2			5	3.8%
		19		110	10	7	1			8	6.7%
		20		144	5	6	2			8	5.4%
		21		177	30	13	1	2		16	7.7%
		22		231	66	5	4	2		11	3.7%
		23		363	88	3	4			7	1.6%
		24		394	156	2	1	2		5	0.9%
		25		868	350	2	2	3		7	0.6%
		26	19_26	1065	616	1	5	2		8	0.5%
	A and B Index Summer	27		1503	1423		3	4		7	0.2%
		28		2510	3636	6	14	28	1	49	0.8%
		29		5316	8900	11	56	108	1	176	1.2%
		30	27_30	9142	14130	13	42	119	3	177	0.8%
		31		12347	14235	12	43	49		104	0.4%
		32		29651	20844	8	43	38		89	0.2%
		33		12608	8005	18	63	74	4	159	0.8%
		34	31_34	12145	6127	4	38	16		58	0.3%
		35		10282	4886	14	60	32	1	107	0.7%
		36		7415	3417	3	17	6	2	28	0.3%
		37		9896	3965	1	9	2	2	14	0.1%
		38	35_38	5283	2185	4	37	11	4	56	0.7%

	39	5161	2241	9	30	11	11	61	0.8%	
	40	2949	1252	2	34	10	7	53	1.3%	
	41	1697	800	1	38	4	6	49	2.0%	
	42	39_42	716	310	1	5	1	3	10	1.0%
	43-44	424	185					0	0.0	
	Total	133122	98202	155	555	525	45	1280	1.0%	

Note: For reference, statistical week 17 is 4/22/13-4/28/13 and 42 is 10/14/13-10/20/13. “Fish count” is based on tallies of adipose-clipped and unclipped adult steelhead provided by the Fish Passage Center (<http://www.fpc.org>) as observed by the Corps of Engineers at their fish counting window. Genetic sample numbers (N) above 10 per stratum were used to estimate stock abundance with GSI. The total sum of all samples for a given week was used to calculate sample rate. The management periods approximate the date ranges from April 1<sup>st</sup> to June 30<sup>th</sup> and July 1<sup>st</sup> to October 31<sup>st</sup> 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 unclipped, and then further split according to the number of samples that were either assigned (PBT) or not assigned (non-PBT) to Snake River hatcheries using PBT.

### *Molecular markers*

Genetic markers are provided in Hess et al. 2013.

### *Statistical analyses*

Snake River Chinook salmon and steelhead and Pacific lamprey 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 GSIsm 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. The juvenile lamprey at Lower Granite Dam

were used to estimate effective population size. [Inbreeding effective population size estimated using the software program Colony v1.0](#) (ID: 1342).

## Results

### *Estimated relative abundance of Chinook salmon stocks in 2013*

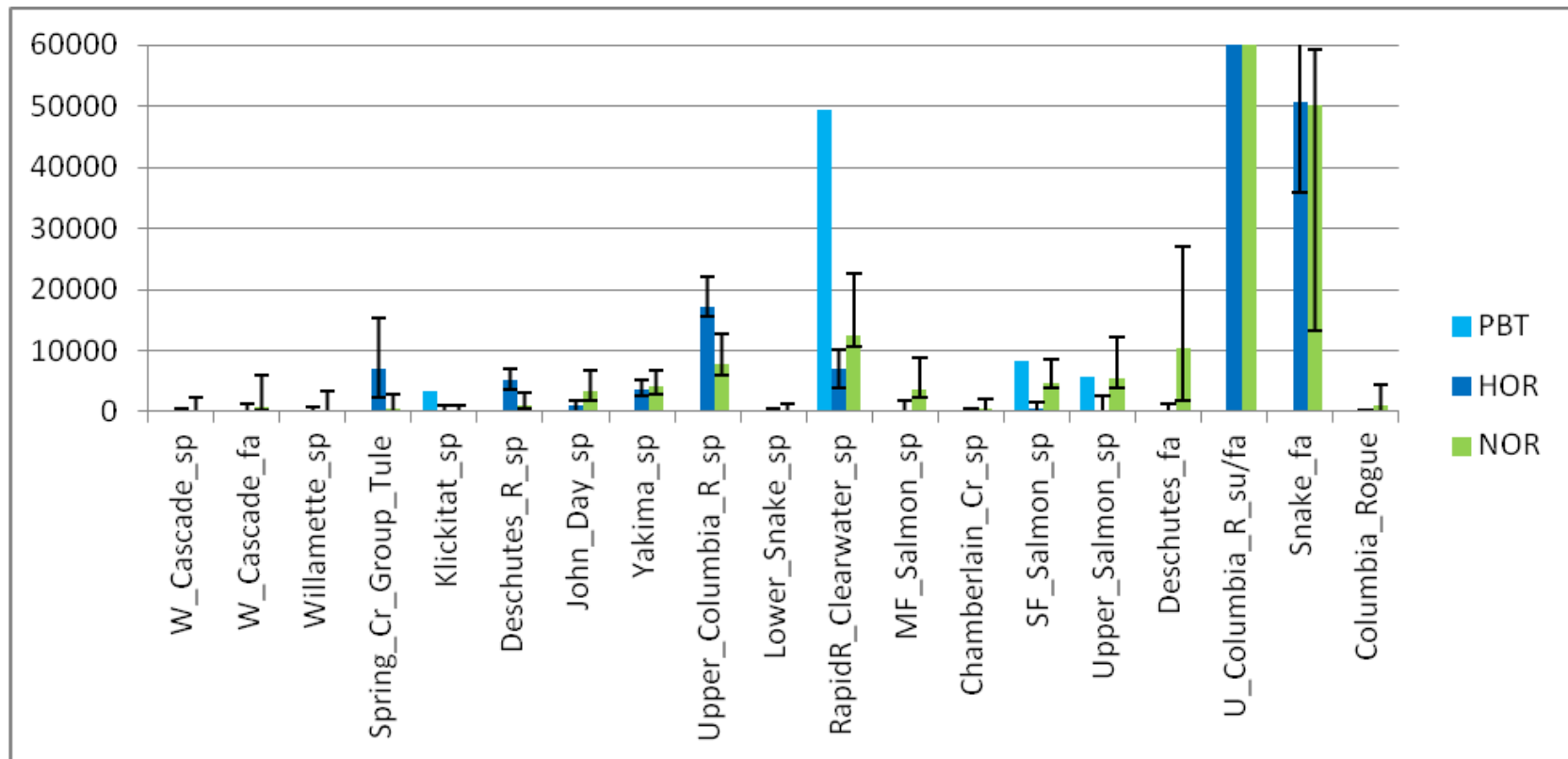
There were ten adipose clipped Chinook salmon stocks passing Bonneville Dam that we estimated relative abundance greater than 1,000 fish in the season (Table 5, Fig. 2). The ten major stocks in order of increasing magnitude were Yakima R. spring (3,586), Klickitat R. spring (3,758), Deschutes R. spring (5,273), upper Salmon R. spring/summer (5,732), Spring Creek group tule fall (6,994), South Fork Salmon R. spring/summer (8,724), upper Columbia R. spring (17,076), Snake R. fall (50,804), Rapid R./Clearwater R. spring (56,343), and upper Columbia R. summer/fall (109,808). 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 have their adipose intact. Therefore PBT assignments improved our ability to accurately identify hatchery-origin fish and estimate total stock abundance (Fig. 2). Further, using PBT assignments we can now provide abundance, run-timing, and size and age information at a spatial scale of a particular hatchery (Table 6). At the level of hatchery, we estimated less than 1,000 fish for Tucannon TUCW, Nez Perce Tribal Fish Hatchery NPFH, Catherine CTHW, and Johnson Creek JHNW hatchery stocks. The following twelve hatchery stocks produced greater than 1,000 fish: Pahsimeroi PAHH (1,079), Grande Ronde GRUW (1,445), Lookingglass LOOK (1,753), Powell POWP (2,898), Lostine LSTW (3,094), Imnaha IMNW (3,204), Klickitat KCLKH (3,412), Clearwater CLWH (3,635), Sawtooth SAWT (4,509), McCall MCCA (7,447), Dworshak DWOR (9,478), and Rapid RAPH (22,915).

**Table 5. Stock-specific relative abundance and run-timing distributions of hatchery-origin Chinook salmon passing Bonneville Dam.**

Reporting group	mean	Estimated abundance				Run-timing distribution						
		Total	Before June 16 -		After	1st	3rd	5th	95th	Median	Interquartile	
		95% C.I.	15-Jun	July 31	August 1	Median	quartile	quartile	percentile	percentile	date	range (d)
W_Cascade_sp	0	0 – 480	0	0	0	-	-	-	-	-	-	-
W_Cascade_fa‡	103	5 – 1410	0	103	0	184	183	186	182	188	3-Jul	3
Willamette_sp	0	0 – 815	0	0	0	-	-	-	-	-	-	-
Spring_Cr_Group_Tule‡	6994	2209 – 15395	0	0	6994	-	-	-	-	-	-	-
Klickitat_sp†	3758	3469 – 4484	3043	716	0	132	126	158	116	184	12-May	32
Deschutes_R_sp	5273	3654 – 6898	4753	520	0	134	126	152	116	173	14-May	26
John_Day_sp	949	1 – 1910	949	0	0	129	127	132	118	159	9-May	5
Yakima_sp	3586	2506 – 5152	3549	37	0	133	126	155	116	162	13-May	29
Upper_Columbia_R_sp	17076	15592 – 22080	16203	873	0	124	119	132	115	167	4-May	13
Lower_Snake_sp†	237	237 – 685	237	0	0	123	122	134	119	138	3-May	-
RapidR_Clearwater_sp†	56343	53348 – 59573	52425	3918	0	126	120	135	115	171	6-May	15
MF_Salmon_sp	139	15 – 1706	139	0	0	137	135	141	133	145	17-May	6
Chamberlain_Cr_sp	0	0 – 474	0	0	0	-	-	-	-	-	-	-
SF_Salmon_sp†	8724	8313 – 9749	5874	2709	142	158	144	171	127	190	7-Jun	27
Upper_Salmon_sp†	5732	5653 – 8281	4456	1276	0	148	135	165	122	190	28-May	30
Deschutes_fa‡	0	0 – 1249	0	0	0	-	-	-	-	-	-	-
U_Columbia_R_su/fa‡	109808	86227 – 122863	11694	29112	69002	175	165	185	155	201	24-Jun	-
Snake_fa‡	50804	35940 – 72759	21	880	49903	204	196	207	175	209	23-Jul	11
Columbia_Rogue	0	0 – 271	0	0	0	-	-	-	-	-	-	-
Total	269528		103342	40144	126042							

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. †Combined with PBT estimated abundance. ‡Abundance and run-timing of these groups is inaccurate due to data gaps in the fall portion of the run. The run-timing distributions the ordinal days for the median date, inter-quartile dates, and 5th and 95th percentile dates. The distributions were based on the weekly estimated reporting group proportions that were applied to the total

abundance of Chinook salmon tallied at the Bonneville Dam fish counting window. This method for estimating run-timing distributions minimizes bias imposed by uneven sampling. Hatchery-origin run-timing distributions include stock abundance estimated from PBT and GSI assigned fish. Note that although the John Day R. and Middle Fork Salmon R. reporting groups are listed here, there are no hatchery programs that exist in these tributaries and their estimated abundance (less than 1000 fish) was likely due to misassignment error in GSI.



**Figure 2. Estimated relative abundance of reporting groups for Chinook salmon passing Bonneville Dam in 2013.** This data was generated by splitting the daily tallies of Chinook salmon at the Bonneville Dam fish counting window into three categories: adipose clipped fish and non-adipose clipped fish and all fish that were PBT-assigned (combined adipose and non-adipose clipped

fish). Reporting group proportions for each of these three categories were estimated on weekly pooled mixtures of Chinook salmon passing Bonneville Dam and then these proportions were multiplied with weekly tallies of Chinook salmon at the Bonneville Dam fish counting window. Abundance that was observed during weeks 32 to 42 only accounted for even-numbered weeks because did not have complete sampling in this timeframe.  $U_{\text{Columbia\_R\_su/fa}}$  is off the chart with estimates of ~110,000 and ~330,000 for adipose-clipped and intact fish, respectively.

**Table 6. Hatchery broodstock-specific relative abundance and run-timing distributions of unclipped and clipped PBT-assigned Chinook salmon passing Bonneville Dam.**

Broodstock collection	Tag rate	Estimated abundance				Run-timing distribution							Sex and size					
		Total	Before	Jun. 16 -	After	Median	1st	3rd	5th	95th	Median	Inter- quartile range (d)	Female		Male		Total	
			15-Jun	Jul. 31	Aug. 1		Quar- tile	Quar- tile	%	%	date		N	Length	N	Length	N	Length
OtsKLKH08S	95.4%	190	0	190	0	178	176	180	175	181	27-Jun	4	1	71.5	-	-	1	71.5
OtsKLKH09S	95.4%	1657	1637	20	0	128	123	136	116	163	8-May	13	4	71.5	8	64.6	12	66.9
OtsKLKH10S	95.4%	1565	1059	506	0	155	132	179	116	186	4-Jun	47	1	66.0	12	52.8	13	53.8
OtsCLWH08S	96.7%	540	540	0	0	121	117	123	114	125	1-May	6	2	78.0	1	79.0	3	78.3
OtsCLWH09S	84.6%	1305	1305	0	0	119	116	125	114	133	29-Apr	9	6	68.5	1	70.5	7	68.8
OtsCLWH10S	99.5%	1790	1770	19	0	134	128	141	123	162	14-May	13	-	-	20	52.4	20	52.4
cath.conv.09	95.1%	189	189	0	0	116	115	117	113	118	26-Apr	2	1	73.0	-	-	1	73.0
OtsCTHW10S	89.5%	335	335	0	0	125	123	135	120	138	5-May	12	-	-	3	47.5	3	47.5
OtsDWOR08S	97.5%	739	739	0	0	123	119	126	115	130	3-May	7	3	82.7	1	80.0	4	82.0
OtsDWOR09S	99.0%	4815	4795	19	0	123	121	127	116	158	3-May	6	19	70.7	14	71.1	33	70.9
OtsDWOR10S	98.5%	3924	3924	0	0	129	127	132	123	142	9-May	5	1	75.5	29	51.1	31	51.9
grnd.capt.09/ conv.09	95.4%/ 94.7%	470	470	0	0	120	116	124	114	151	30-Apr	8	3	71.3	-	-	3	71.3
gruw.conv.10	98.7%	975	975	0	0	125	120	132	115	139	5-May	12	-	-	8	51.0	8	51.0
OtsIMNW08S	99.2%	111	111	0	0	138	135	142	133	146	18-May	7	2	86.5	-	-	2	86.5
OtsIMNW09S	97.4%	513	454	59	0	163	144	165	135	167	12-Jun	21	3	68.7	3	77.2	6	72.9
OtsIMNW10S	97.6%	2580	1402	1178	0	165	157	182	142	188	14-Jun	25	-	-	24	54.9	24	54.9
OtsJHNW08S	100.0%	104	104	0	0	157	156	159	154	160	6-Jun	3	1	87.5	-	-	1	87.5
OtsJHNW09S	100.0%	119	119	0	0	148	144	151	140	153	28-May	7	1	74.0	1	69.0	2	71.5
OtsJHNW10S	94.2%	540	160	380	0	176	159	179	143	181	25-Jun	20	-	-	4	54.1	4	54.1
OtsLOOK09S	93.9%	251	251	0	0	123	122	133	119	138	3-May	11	1	74.0	1	64.5	2	69.3
OtsLOOK10S	98.7%	1502	1502	0	0	132	128	139	126	158	12-May	11	-	-	14	54.3	14	54.3
lost.conv.08	94.6%	98	98	0	0	143	141	144	140	146	23-May	3	2	87.5	-	-	2	87.5
lost.conv.09	94.4%	355	0	355	0	171	169	173	168	174	20-Jun	4	-	-	1	84.0	1	84.0
OtsLSTW10S	95.4%	2641	701	1940	0	171	164	177	150	185	20-Jun	13	-	-	16	52.4	16	52.4



OtsMCCA08S	97.6%	95	95	0	0	143	141	144	140	146	23-May	3	2	86.8	-	-	2	86.8
OtsMCCA09S/ 09S_2	96.4%/ 95.9%	2893	2695	198	0	146	140	155	127	182	26-May	15	21	72.4	18	70.5	39	71.7
OtsMCCA10S	99.4%	4460	2207	2111	142	167	156	175	134	210	16-Jun	19	1	72.0	35	55.6	36	56.1
OtsNPFH09S	96.3%	409	409	0	0	128	127	130	126	132	8-May	3	-	-	2	72.8	2	72.8
OtsPAHH09S	96.8%	665	590	74	0	139	130	155	126	199	19-May	25	3	73.3	4	67.5	7	70.0
pahs.ibs.10	94.9%	415	247	167	0	164	144	190	140	194	13-Jun	46	-	-	6	55.8	6	55.8
OtsPOWP08S	98.7%	206	206	0	0	116	115	117	113	118	26-Apr	2	-	-	1	91.0	1	91.0
OtsPOWP09S	47.1%	2310	2310	0	0	117	116	122	113	125	27-Apr	6	3	70.2	4	78.0	7	74.6
OtsPOWP10S	97.7%	383	383	0	0	132	128	137	126	143	12-May	9	-	-	4	54.1	4	54.1
OtsRAPH08S	98.3%	723	723	0	0	117	116	118	113	124	27-Apr	2	2	83.0	2	84.3	4	83.6
OtsRAPH09S	90.6%	14492	14492	0	0	122	117	125	114	132	2-May	8	37	68.9	44	70.1	81	69.6
OtsRAPH10S	99.1%	7700	7681	19	0	128	123	134	117	146	8-May	11	-	-	63	49.8	65	49.9
OtsSAWT08S	99.0%	287	287	0	0	150	140	156	134	159	30-May	16	4	86.4	-	-	4	86.4
OtsSAWT09S	99.6%	1010	991	19	0	125	123	150	120	165	5-May	27	2	77.0	8	69.9	10	71.3
OtsSAWT10S	99.4%	3211	2196	1015	0	156	137	170	130	188	5-Jun	33	-	-	35	50.8	35	50.8
OtsTUCW09S	91.1%	237	237	0	0	123	122	134	119	138	3-May	12	1	67.5	1	64.0	2	65.8
Total		66804	58391	8271	142								127	75.5	388	64.5	518	68.1

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.

There were twelve non-adipose-clipped Chinook salmon stocks estimated with relative abundance greater than 1,000 fish (Table 7, Fig. 2). These non-adipose clipped stocks in order of increasing magnitude were Columbia R. Rogue fall (1,126), Deschutes R. spring (1,137), John Day R. spring (3,357), Middle Fork Salmon R. spring/summer (3,728), Yakima R. spring (4,021), South Fork Salmon R. spring/summer (4,795), upper Salmon R. spring/summer (5,319), upper Columbia R. spring (7,820), Deschutes R. fall (10,283), Rapid R./Clearwater R. spring (12,406), Snake R. fall (50,058), and upper Columbia R. summer/fall (328,827). 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). The fall run stocks should be considered a subtotal abundance, because we only analyzed even-numbered statistical weeks during the 32 – 42 week interval.

**Table 7. Relative abundance and run-timing distributions of unclipped (natural origin) Chinook salmon stocks passing Bonneville Dam in 2013.**

Reporting group	mean	Estimated abundance				Run-timing distribution						
		Total	Before	June 16 -	After	1st	3rd	5th	95th	Median	Interquartile	
		95% C.I.	15-Jun	July 31	August 1	Median	quartile	quartile	percentile	percentile	date	range (d)
W_Cascade_sp	62	11 – 2244	62	0	0	136	134	137	133	139	16-May	3
W_Cascade_fa‡	716	143 – 5950	0	0	716	274	273	276	273	279	1-Oct	3
Willamette_sp	253	40 – 3281	0	117	135	213	211	215	210	216	1-Aug	4
Spring_Cr_Group_Tule‡	527	41 – 2855	0	0	527	220	218	222	217	223	8-Aug	4
Klickitat_sp	0	0 – 1002	0	0	0	-	-	-	-	-	-	-
Deschutes_R_sp	1137	468 – 2997	1137	0	0	125	117	130	114	155	5-May	13
John_Day_sp	3357	1813 – 6789	3178	180	0	129	123	139	115	175	9-May	16
Yakima_sp	4021	2855 – 6691	3630	391	0	134	126	150	121	170	14-May	24
Upper_Columbia_R_sp	7820	6034 – 12714	7732	88	0	126	122	135	116	156	6-May	13
Lower_Snake_sp	0	0 – 1316	0	0	0	-	-	-	-	-	-	-
RapidR_Clearwater_sp	12406	10559 – 22510	10916	1490	0	128	121	151	115	176	8-May	30
MF_Salmon_sp	3728	2316 – 8893	3074	655	0	136	127	156	122	177	16-May	29
Chamberlain_Cr_sp	473	103 – 2112	473	0	0	142	129	152	126	159	22-May	23
SF_Salmon_sp	4795	3978 – 8559	4045	751	0	157	145	164	133	188	6-Jun	19
Upper_Salmon_sp	5319	3933 – 12156	3551	1768	0	154	136	170	123	180	3-Jun	34
Deschutes_fa‡	10283	1833 – 27096	100	182	10001	250	246	261	233	287	7-Sep	15
U_Columbia_R_su/fa‡	328827	306590 – 359648	10717	33256	284853	250	245	261	171	275	7-Sep	16
Snake_fa‡	50058	13334 – 59243	136	1586	48336	250	235	261	217	273	7-Sep	26
Columbia_Rogue‡	1126	63 – 4443	0	0	1126	235	233	236	231	237	23-Aug	3
Total	434909		48750	40463	345696							

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.

‡ Abundance and run-timing of these groups is inaccurate due to data gaps in the fall portion of the run.

### *Run-timing of Chinook salmon stocks in 2013*

We were able to obtain sufficient sample sizes to characterize the run-timing distributions of ten hatchery-origin adipose-clipped adult and jack Chinook salmon spring-run stocks (Table 5). We included all PBT-assigned Chinook salmon in this abundance estimate regardless of whether they were adipose-clipped or not. The Chinook salmon management periods divide the run into the following three date ranges: April to June 15<sup>th</sup> (Spring-run), June 16<sup>th</sup> – July 31<sup>st</sup> (Summer-run), and August 1<sup>st</sup> - October (Fall-run). The following four hatchery-origin stocks were found to terminate within the Spring management period (i.e. the 95<sup>th</sup> percentile of their run distribution occurred on or before June 15<sup>th</sup>): lower Snake R., Middle Fork Salmon R., John Day R., and Yakima R. Note that although the John Day R. and Middle Fork Salmon R. reporting groups are listed here, there are no hatchery programs that exist in these tributaries and their estimated abundance (less than 1000 fish) was likely due to misassignment error in GSI. The following six hatchery-origin stocks were found to terminate within the summer management period (i.e. the 95<sup>th</sup> percentile of their run distribution occurred after June 15<sup>th</sup> and on or before July 31<sup>st</sup>): upper Columbia R., Rapid R./Clearwater R., Deschutes R., Klickitat R., South Fork and upper Salmon R. The run-timing of the remaining summer/fall-run hatchery-origin stocks were not characterized due to the way in which odd-numbered weeks were omitted from the analysis in statistical weeks 32 – 42 : Spring Cr. group tule, upper Columbia R. summer/fall, West Cascade.

The run distributions of 11 natural-origin non-adipose-clipped adult and jack Chinook salmon spring-run stocks were similarly characterized (Table 7). The following four natural-origin stocks were found to terminate within the Spring management period (i.e. the 95<sup>th</sup> percentile of their run distribution occurred on or before June 15<sup>th</sup>): West Cascade, Deschutes R., upper Columbia R., and Chamberlain Cr. The following six natural-origin stocks were found to terminate within the summer management period (i.e. the 95<sup>th</sup> percentile of their run distribution occurred after June 15<sup>th</sup> and on or before July 31<sup>st</sup>): Yakima R., John Day R., Rapid R./Clearwater R., Middle Fork Salmon R., and upper Salmon and South Fork Salmon R. The run-timing of the remaining summer/fall-run hatchery-origin stocks were not characterized due to the way in which odd-numbered weeks were omitted from the analysis in statistical weeks 32 – 42. However, one spring-run stock, Willamette R., was found to terminate its run after the July 31<sup>st</sup> date, and August 1<sup>st</sup> was its median run date. Although these categories differ in composition of stocks depending whether we examine hatchery- or natural-origin fish, comparison to previous analyses of these stocks demonstrates some consistent patterns, e.g. combined adult and jack Salmon R. stocks have relatively late runs. It may be of interest to managers that we observed more than 5,000 natural-origin and 10,000 hatchery-origin fish from spring-run Chinook salmon reporting groups (mostly Snake R. origin) that are estimated to return during the summer management (Tables 5, 7). Data from known origin PIT tagged fish which were PIT tagged as juveniles shows similar patterns. In 2013, while PIT tags for adult spring and Snake River spring/summer fish were approximately 96% percent complete at Bonneville by June 15, the combined group of adult and jack fish were only 88% complete by June 15 (PITAGIS).

Using the PBT-assigned adult and jack Chinook salmon, we also characterized the run-distributions at a very fine scale according to hatchery and spawn year (Table 6). This analysis showed that the following 12 broodstock sources were found to terminate within the summer

management period (i.e. the 95<sup>th</sup> percentile of their run distribution occurred after June 15<sup>th</sup> and on or before July 31<sup>st</sup>): Imnaha (IMNW09S and IMNW10S), Lostine (LSTW09S and LSTW10S), Klickitat (KLKH08S and KLKH10S), Johnson Cr. (JHNW10S), McCall (MCCA09S and MCCA10S), Sawtooth (SAWT10S), and Pahsimeroi (PAHH09S and PAHH10S). The fish that assigned to the remaining 28 broodstock sources terminated within the spring management period. One pattern worth noting is that the 3-year-old jack Chinook salmon (fish that assigned to broodstock that were spawned in 2010), were found to arrive later than the 4-year-old salmon from the same hatchery source which is the same pattern seen with PIT tag data (PITAGIS). There were 14 hatcheries which had assignments for both age classes (3- and 4- year old), and for most of these hatcheries (13 of 14), the 3-year old fish had a later median day relative to the 4-year olds (Table 6). The one exception was the Lostine, but in this case, the 4-year old abundance was estimated from a single fish.

### *Parentage based tagging analyses of Chinook salmon in 2013*

We were able to assign 518 adult and jack Chinook salmon sampled at Bonneville Dam in 2013 to the 2008 - 2010 spring-run Chinook salmon broodstock from sixteen different Snake River hatcheries and the Klickitat Hatchery (Table 8). The fifteen Snake River hatchery sources identified by PBT were aggregated into the appropriate GSI reporting group in order to integrate the relative abundance estimates from this analysis with relative abundance estimates from GSI analyses. Tucannon hatchery was placed in the lower Snake R. reporting group. Johnson Cr. and McCall hatcheries were placed in the South Fork Salmon R. reporting group. Pahsimeroi and Sawtooth were placed in the Upper Salmon R. reporting group, and all other hatcheries were grouped into the Rapid R./Clearwater R. reporting group. Adipose marking rates vary across source hatcheries, for the nine hatcheries with sample sizes above 15 fish assignments, the adipose marking rate was below 10% unmarked. The three exceptions were Clearwater, McCall, and Sawtooth hatcheries which had a sample sizes above 30 fish but were 16.7% , 32.5%, and 26.5% unmarked, respectively. Sex (determined by a genetic marker) and length information were available for these PBT-assigned fish and were used to characterize the broodstock sources (Table 6).

Table 8. Summary information on the PBT Chinook salmon source hatcheries and numbers of assignments

PBT source hatchery	PBTpop	Tag Rate	Age	Adipose clip		
				Y	N	Total
Klickitat Hatchery	OtsKLKH08S		5	1		1
	OtsKLKH09S		4	12		12
	OtsKLKH10S		3	13		13
LSRCP/IDFG - Clearwater (SF)	OtsCLWH08S	96.7%	5	3		3
	OtsCLWH09S	84.6%	4	5	2	7
	OtsCLWH10S	99.5%	3	17	3	20
LSRCP/ODFW - Catherine Creek	cath.conv.09	95.1%	4	1		1
	OtsCTHW10S	89.5%	3	3		3
LSRCP/USFWS - Dworshak	OtsDWOR08S	97.5%	5	4		4
	OtsDWOR09S	99.0%	4	28	5	33

	OtsDWOR10S	98.5%	3	30	1	31
	grnd.capt.09	95.4%	4	1	2	3
LSRCP/ODFW - Grande Ronde	grnd.conv.09	94.7%	4			0
	gruw.conv.10	98.7%	3	3	5	8
	OtsIMNW08S	99.2%	5	2		2
LSRCP/ODFW - Imnaha	OtsIMNW09S	97.4%	4	6		6
	OtsIMNW10S	97.6%	3	22	2	24
	OtsJHNW08S	100.0%	5		1	1
Johnson Cr.	OtsJHNW09S	100.0%	4		2	2
	OtsJHNW10S	94.2%	3		4	4
	OtsLOOK08S	98.7%	5			0
LSRCP/ODFW - Lookingglass Creek	OtsLOOK09S	93.9%	4	2		2
	OtsLOOK10S	98.7%	3	14		14
	lost.conv.08	94.6%	5	2		2
LSRCP/ODFW/NPT – Lostine	lost.conv.09	94.4%	4		1	1
	OtsLSTW10S	95.4%	3	16		16
	OtsMCCA08S	97.6%	5	2	1	3
	OtsMCCA09S	96.4%	4	35		35
LSRCP/IDFG - McCall (SFSR)	OtsMCCA09S_2	95.9%	4		3	3
	OtsMCCA10S	99.4%	3	15	21	36
Nez Perce Tribal Hatchery (NPTFH)	OtsNPFH09S	96.3%	4		2	2
	OtsPAHH08S	97.8%	5			0
Idaho Power/IDFG - Pahsimeroi	OtsPAHH09S	96.8%	4	7		7
	pahs.ibs.10	94.9%	3	4	2	6
	OtsPOWP08S	98.7%	5		1	1
LSRCP/IDFG - Clearwater (Powell)	OtsPOWP09S	47.1%	4	6	1	7
	OtsPOWP10S	97.7%	3	3	1	4
	OtsRAPH08S	98.3%	5	4		4
Idaho Power/IDFG - Rapid River	OtsRAPH09S	90.6%	4	78	3	81
	OtsRAPH10S	99.1%	3	62	3	65
	OtsSAWT08S	99.0%	5	4		4
LSRCP/IDFG - Sawtooth	OtsSAWT09S	99.6%	4	9	1	10
	OtsSAWT10S	99.4%	3	23	12	35
LSRCP/WDFW - L.F. (Tucannon)	OtsTUCW09S	91.1%	4		2	2
	OtsTUCW10S	96.3%	3			0
				437	81	518

*Estimated relative abundance of steelhead stocks in 2013*

There were five major stocks (abundance > 1000) represented in the total estimated relative abundance (N=138,269) of hatchery steelhead passing Bonneville Dam in 2013. These stocks in

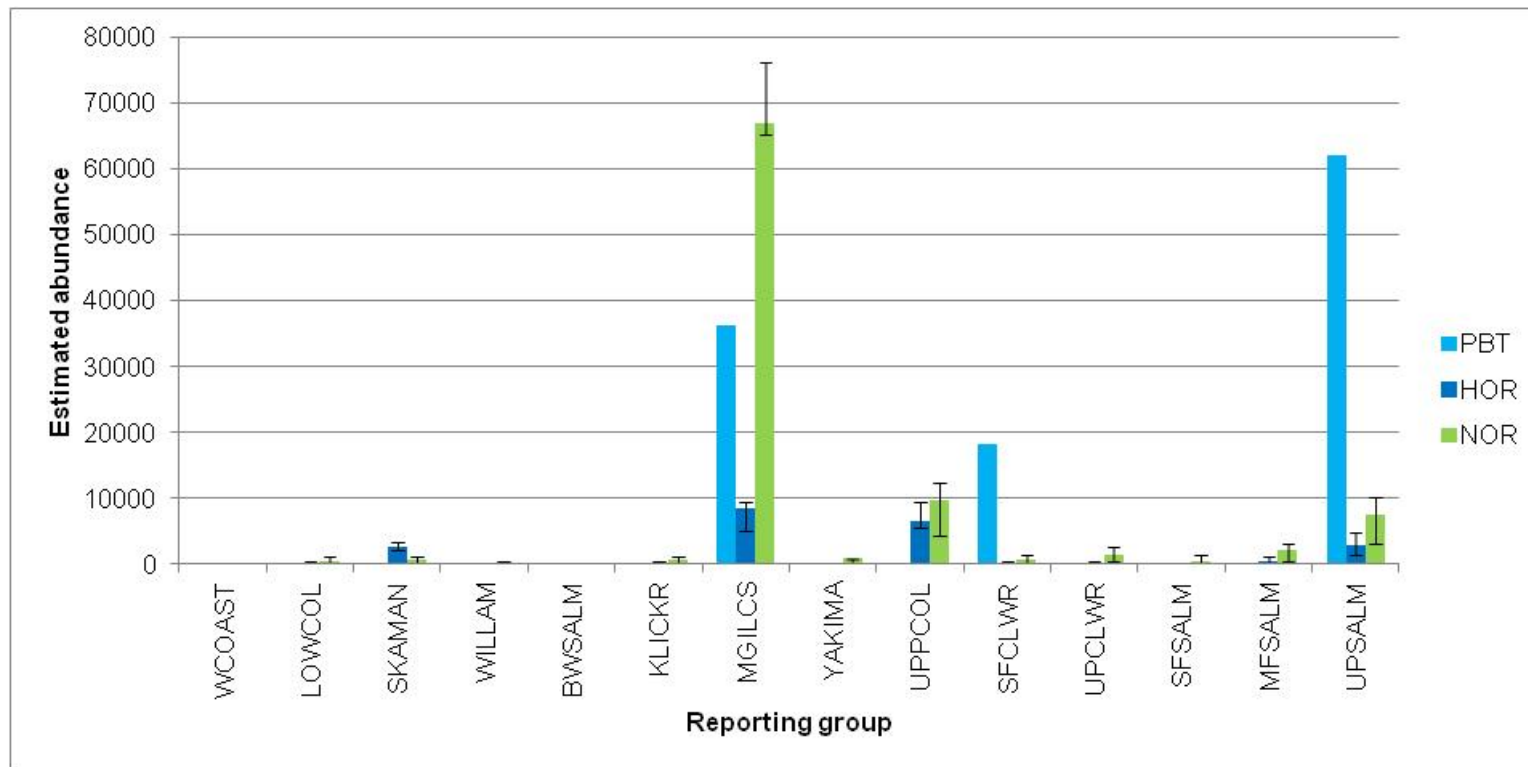
order of increasing magnitude were Skamania summer-run (2666), Upper Columbia R. (6568), South Fork Clearwater R. (18,579), MGILCS-mega complex (44,858), and Upper Salmon R. (64,998) (Table 9, Fig. 3). These estimates include relative abundance estimated from PBT-assigned fish that were mostly adipose clipped, however a large portion of the PBT-assigned fish were found to have their adipose intact. Therefore PBT assignments improved our ability to accurately identify hatchery-origin steelhead and estimate total stock relative abundance (Fig. 3), particularly for three reporting groups MGILCS, South Fork Clearwater R., and Upper Salmon R. Further, using PBT assignments we can now provide relative abundance, run-timing, and size and age information at a spatial scale of a particular hatchery (Table 10). At the level of hatchery, we estimated less than 1,000 fish for Squaw SQUW and Tucannon TUCW. The following eight hatchery stocks produced greater than 1,000 fish: E.F. Salmon EFSW (1,313), Little Sheep Cr. LSCR (3,264), Catherine CGRW (6,304), Wallowa WALL (10,343), Oxbow OXBO (10,697), Lyons Ferry LYON (14,974), Dworshak DWOR (18,021), Sawtooth SAWT (21,400), and Pahsimeroi PAHH (29,090). These hatchery stocks could also be classified into the larger stock groups utilized by GSI analyses, such that the MGILCS reporting group would include Little Sheep Cr., Catherine, Wallowa, and Lyons Ferry hatchery stocks. The SFCLWR reporting group would include the Dworshak hatchery stock; and the UPSALM reporting group would include E.F. Salmon, Oxbow, Sawtooth, and Pahsimeroi hatchery stocks.

**Table 9. Stock-specific relative abundance and run-timing distributions of hatchery-origin steelhead stocks passing Bonneville Dam in 2013.**

Reporting group	Estimated abundance				Run-timing distribution						
	Total		Management period								
	mean	95% C.I.	Skamania Summer Apr. 1 - Jun. 30	Summer A/B Index Jul. 1 - Oct. 31	Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Interquartile range (d)
WCOAST	0	0 – 47	0	0	-	-	-	-	-	-	-
LOWCOL	67	0 – 418	67	0	170	157	176	135	180	19-Jun	19
SKAMAN	2666	2054 – 3412	1951	714	175	163	190	138	221	24-Jun	27
WILLAM	0	0 – 227	0	0	-	-	-	-	-	-	-
BWSALM	0	0 – 44	0	0	-	-	-	-	-	-	-
KLICKR	55	0 – 536	55	0	170	157	176	135	180	19-Jun	19
MGILCS	44858†	41467 – 45806	1167	43691	221	209	240	190	263	9-Aug	31
YAKIMA	0	0 – 240	0	0	-	-	-	-	-	-	-
UPPCOL	6568	5536 – 9546	112	6456	220	212	226	194	235	8-Aug	14
SFCLWR	18579†	18387 – 18851	0	18579	263	248	272	238	284	20-Sep	24
UPCLWR	0	0 – 334	0	0	-	-	-	-	-	-	-
SFSALM	0	0 – 247	0	0	-	-	-	-	-	-	-
MFSALM	478	40 – 1152	0	478	252	244	260	239	275	9-Sep	16
UPSALM	64998†	63418 – 67047	0	64998	223	218	239	202	264	11-Aug	21
Total	138269		3352	134917							

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 during the sampling period. † These estimates were combined with PBT-estimated stock abundance.





**Figure 3. Estimated relative abundance of reporting groups for steelhead passing Bonneville Dam in 2013.** Reporting group proportions were estimated using GSI and PBT on monthly pooled mixtures of steelhead passing Bonneville Dam and then these proportions were multiplied with monthly tallies of steelhead at the Bonneville Dam fish counting window. “PBT” refers to the relative abundance of all fish (marked and unmarked) that could be assigned using PBT to Snake River hatchery stocks, “HOR” refers to relative abundance of all marked fish (excluding those assigned with PBT), and “NOR” refers to the relative abundance of unmarked fish (excluding unmarked Snake River hatchery fish that were assigned with PBT). Exclusion of these unmarked hatchery fish helps to more accurately estimate relative abundance of fish that are truly natural origin.

**Table 10. Hatchery broodstock-specific relative abundance and run-timing distributions of unclipped and clipped PBT-assigned steelhead passing Bonneville Dam.**

GSI reporting group	Broodstock collection	Tagging rate	Estimated abundance		Run-timing distribution								Sex and size					
			Total	Skamania	Summer	1st		3rd	5th	95th	Median	Interquartile	Female		Male		Total	
				Apr. 1 - Jun. 30	Jul. 1 - Oct. 31	Median	quartile	quartile	percentile	percentile	date	range (d)	N	>78cm length	N	>78cm length	N	>78cm length
MGILCS	CGRW10S	100.0%	2333	0	2333	229	214	249	196	264	17-Aug	35	7	0.0%	5	0.0%	12	0.0%
	CGRW11S	96.0%	3971	0	3971	224	216	242	198	262	12-Aug	26	7	0.0%	11	0.0%	18	0.0%
	LSCR10S	89.1%	947	0	947	223	208	249	194	273	11-Aug	41	3	0.0%	2	0.0%	5	0.0%
	LSCR11S	100.0%	2317	0	2317	209	202	222	189	250	28-Jul	20	9	0.0%	5	0.0%	14	0.0%
	LYON10S	100.0%	2520	758	1762	205	178	225	151	254	24-Jul	47	21	0.0%	4	0.0%	25	0.0%
	LYON11S	100.0%	12454	408	12045	215	203	227	185	252	3-Aug	24	36	0.0%	38	0.0%	74	0.0%
	TUCW10S	77.9%	160	0	160	202	197	206	186	209	21-Jul	9	1	0.0%	-	-	1	0.0%
	TUCW11S	68.6%	181	0	181	202	197	206	186	209	21-Jul	9	1	0.0%	-	-	1	0.0%
	WALL10S	97.6%	2725	0	2725	235	221	253	213	275	23-Aug	32	7	0.0%	6	0.0%	13	0.0%
	WALL11S	98.3%	7618	0	7618	228	219	245	210	265	16-Aug	26	9	0.0%	24	0.0%	33	0.0%
SFCLWT	DWOR10S	97.1%	14178	0	14178	260	247	272	238	284	17-Sep	25	54	44.4%	49	89.8%	103	66.0%
	DWOR11S	98.9%	3843	0	3843	266	251	273	240	285	23-Sep	22	-	-	31	0.0%	31	0.0%
	SQUW10S	95.6%	91	0	91	273	269	279	266	288	30-Sep	10	1	0.0%	-	-	1	0.0%
UPSALM	EFSW10	100.0%	282	0	282	221	218	228	211	236	9-Aug	10	1	0.0%	-	-	1	0.0%
	EFSW11S	100.0%	1031	0	1031	243	221	257	200	277	31-Aug	36	2	0.0%	4	0.0%	6	0.0%
	OXBO09S	88.0%	577	0	577	234	220	248	213	261	22-Aug	28	2	0.0%	-	-	2	0.0%
	OXBO10S	95.5%	2408	0	2408	227	219	243	209	264	15-Aug	24	6	0.0%	4	0.0%	10	0.0%
	OXBO11S	91.6%	7712	0	7712	222	217	233	204	257	10-Aug	16	15	0.0%	15	0.0%	30	0.0%
	PAHH10S	97.1%	5103	0	5103	224	214	245	197	269	12-Aug	31	20	0.0%	7	0.0%	27	0.0%
	PAHH11S	86.3%	23987	0	23987	222	217	235	200	263	10-Aug	18	41	0.0%	53	0.0%	94	0.0%
	SAWT10S	99.8%	3135	0	3135	240	221	252	205	267	28-Aug	31	11	0.0%	3	0.0%	15	0.0%
	SAWT10S_yankee	100.0%	1139	0	1139	229	218	246	201	260	17-Aug	28	3	0.0%	2	0.0%	5	0.0%
Total			116733	1167	115566								291		308		600	

Note: GSI reporting groups indicate which group the broodstock sources are most genetically similar. Collection abbreviations can be found in Table 12. The date ranges listed under “Skamania” and “Summer” were chosen by steelhead fishery managers, and for each hatchery source stock we provide the abundance that has passed within these time periods. Under “sex and size” we provide number of each sex as determined by a genetic marker and length shows the percent of fish larger than 78 cm for each category.

There were five major stocks (abundance > 1000) represented in the total estimated relative abundance (n=91,381) of wild steelhead (Table 11, Fig. 3). These stocks in order of magnitude were upper Clearwater R. (1,335), Middle Fork Salmon R. (2,104), Upper Salmon R. (7,584), Upper Columbia R. (9,667), and MGILCS-middle Columbia R. (67,040). There were six minor stocks with abundance estimates below 1000 fish and include lower Columbia R. (334), South Fork Salmon R. (512), Klickitat R. (588), Skamania (631), South Fork Clearwater R. (754), and Yakima R. (833).

#### *Run-timing of steelhead stocks in 2013*

We were able to obtain sufficient sample sizes to characterize the run-timing distributions of 8 hatchery steelhead stocks and 11 wild steelhead stocks in 2013 (Tables 9, 11). Results for the hatchery stocks indicate three main run-timing categories of stocks. An early run-timing category has been observed previously to primarily include Skamania summer-run (Median date Jun. 24), however this year the lower Columbia R. and Klickitat R. stocks also appeared early (Median date Jun. 19). These latter two stocks had very low sample sizes and could be explained by assignment errors. An intermediate run-timing category includes the following four hatchery steelhead stocks (ordered by median dates): upper Columbia R. UPPCOL (Aug. 8), MGILCS (Aug. 9), upper Salmon R. UPSALM (Aug. 11<sup>th</sup>), and Middle Fork Salmon R. MFSALM (Sep 9). Finally, a late run-timing category includes South Fork Clearwater R. SFCLWR (Sep. 20). The late run-timing category is typically thought to be characteristic of B-run steelhead that return after August 25<sup>th</sup> at Bonneville Dam.

**Table 11. Stock-specific relative abundance and run-timing distributions of natural-origin (adipose unclipped) steelhead passing Bonneville Dam in 2013.**

Reporting group	Estimated abundance				Run-timing distribution						
	Total		Management period								
	mean	95% C.I.	Skamania Apr. 1 - Jun. 30	Summer Jul. 1 - Oct. 31	Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Interquartile range (d)
WCOAST	0	0 – 43	0	0	-	-	-	-	-	-	-
LOWCOL	334	63 – 1231	240	94	178	170	192	152	207	27-Jun	22
SKAMAN	631	120 – 1274	241	391	205	177	218	159	231	24-Jul	41
WILLAM	0	0 – 366	0	0	-	-	-	-	-	-	-
BWSALM	0	0 – 66	0	0	-	-	-	-	-	-	-
KLICKR	588	83 – 1260	120	468	215	197	227	166	276	3-Aug	30
MGILCS	67040	65277 – 76115	471	66569	216	205	223	193	251	4-Aug	18
YAKIMA	833	0 – 749	0	833	214	205	221	193	233	2-Aug	16
UPPCOL	9667	4374 – 12319	250	9417	219	211	231	193	254	7-Aug	20
SFCLWR	754	305 – 1523	0	754	269	223	274	213	286	26-Sep	51
UPCLWR	1335	505 – 2629	0	1335	251	243	258	239	273	8-Sep	15
SFSALM	512	107 – 1429	0	512	255	245	269	239	283	12-Sep	24
MFSALM	2104	527 – 3225	0	2104	239	218	251	202	264	27-Aug	33
UPSALM	7584	2996 – 10164	0	7584	225	215	245	200	262	13-Aug	30
Total	91381		1321	90060							

Note: These summary statistics of run-timing distributions were calculated using a method to estimate relative 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 wild steelhead stocks generally fit the same run-timing categories as characterized for hatchery steelhead (Table 11). The wilds stocks ordered by median date were lower Columbia R. (Jun. 27), Skamania (Jul. 24), Yakima R. (Aug. 2), Klickitat R. (Aug. 3), MGILCS (Aug. 4), upper Columbia R. (Aug. 7), Upper Salmon R. (Aug. 13), M.F. Salmon R. (Aug. 27), upper Clearwater R. (Sep. 8), S.F. Salmon R. (Sep. 12), and S.F. Clearwater R. (Sep. 26).

Using the PBT-assigned steelhead, we also characterized the run-distributions at a very fine scale according to hatchery and spawn year (Table 10). This analysis of broodstock sources allowed us to group them into categories according to their median date. The following broodstock sources all had median dates before August: Tucannon 2010-2011, Lyons Ferry 2010, and Little Sheep Cr. 2011. Broodstock sources with median dates between August 1<sup>st</sup> – 24<sup>th</sup> were: Lyons Ferry 2011, Sawtooth EFSR 2010, Oxbow 2009-2011, Pahsimeroi 2010-2011, Little Sheep Cr. 2010, Grande Ronde Cottonwood 2010-2011, Sawtooth 2010-2011 (Yankee), and Wallowa 2010-2011. Finally, broodstock sources with median dates occurring after August 25<sup>th</sup> were the following: Sawtooth 2010, Sawtooth EFSR 2011, Dworshak 2010-2011, and Squaw 2010.

#### *Parentage based tagging analyses of steelhead in 2013*

We were able to assign 600 steelhead sampled at Bonneville Dam in 2013 to the 2008-2011 steelhead broodstock from eleven different Snake River hatcheries (Table 12). The largest portion of the PBT-assigned fish originated from the Dworshak Hatchery (n=134, 22%), and the next greatest portions originated from the Pahsimeroi (20%), Lyons Ferry (17%), and Sawtooth (17%) hatcheries. Using these known hatchery-of-origin steelhead, we compared the individual assignments based on GSI analysis and used these assignments to help classify them into the most genetically similar reporting group (Table 12). Those groupings were used to combine results of PBT-hatchery abundance estimates with the GSI estimated abundance of hatchery stocks (Fig. 3). Adipose marking rates did not vary much across source hatcheries. There were eight hatcheries with sample sizes above 15 fish assignments, and all but one of them had adipose marking rates below 10% unmarked. The exception was Dworshak hatchery which had a sample size of 134 fish but 20% were unmarked (Table 12).

We examined which of the hatchery sources was contributing the size range of fish typically classified as B-run steelhead (Table 10). Fish with a fork length greater than 78 cm were found to only originate from the Dworshak broodstock source, and specifically only from the 2010 spawn year (2-ocean age). The percent of fish greater than 78 cm from this broodyear were found to be 44% and 90% for females and males respectively. 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 Fork Salmon R. are generally thought to be the largest sources of B-run steelhead. Dworshak and Squaw broodstock fit within the South Fork Clearwater R. genetic stock and so are expected to produce large, older steelhead.

Table 12. Summary information for Snake River steelhead broodstock sources and numbers of assignments of Bonneville Dam steelhead in 2013.

GSI		PBT	Broodstock			Adipose clip		
reporting	source	source	Tagging	Age of PBT				
group	hatchery	abbreviation	rate	assigned adults	Y	N	Total	
MGILCS	LSRCP/WDFW-L.F. (G.R. cottonwood)	CGRW10S	100.0%	2-ocean	12		12	
		CGRW11S	96.0%	1-ocean	18		18	
	LSRCP/ODFT - Little Sheep Cr. FH	LSCR10S	89.1%	2-ocean	5		5	
		LSCR11S	100.0%	1-ocean	14		14	
	LSRCP/WDFW-Lyons Ferry	LYON10S	100.0%	2-ocean	25		25	
		LYON11S	100.0%	1-ocean	72	2	74	
	LSRCP/WDFW-L.F. (Tucannon)	TUCW10S	77.9%	1-ocean		1	1	
		TUCW11S	68.6%	1-ocean	1		1	
	LSRCP/ODFW-Wallowa F.H.	WALL10S	97.6%	2-ocean	13		13	
		WALL11S	98.3%	1-ocean	33		33	
SFCLWR	LSRCP/IDFG/USFWS Dworshak/C.W.	DWOR10S	97.1%	2-ocean	84	19	103	
		DWOR11S	98.9%	1-ocean	23	8	31	
	LSRCP/IDFG Sawtooth (USB/Squaw)	SQUW10S	95.6%	2-ocean		1	1	
UPSALM	LSRCP/IDFG Sawtooth (EFSR)	EFSW10S	100.0%	2-ocean		1	1	
		EFSW11S	100.0%	1-ocean		6	6	
	Idaho Power/IDFG,Oxbow F.H.	OXBO09S	88.0%	3-ocean	2		2	
		OXBO10S	95.5%	2-ocean	10		10	
		OXBO11S	91.6%	1-ocean	30		30	
	Idaho Power/IDFG, Pahsimeroi F.H.	PAHH10S	97.1%	2-ocean	27		27	
		PAHH11S	86.3%	1-ocean	93	1	94	
	LSRCP/IDFG Sawtooth (IDFG & SBT)	SAWT10S	99.8%	2-ocean	14	1	15	
		SAWT10S	100.0%	2-ocean	2	3	5	
		SAWT11S	99.7%	1-ocean	77	2	79	
Total			94.7%		555	45	600	

Note: The GSI reporting group is the group that is most genetically similar to the listed hatchery broodstock sources. The hatchery abbreviations include digits that indicate the spawn year, e.g. 10S = 2010 spawn year. The tagging rate is the percent of broodstock parents that were genotyped. Adipose clipped fish (Y) and non-clipped fish (N) refer to the number of adult steelhead passing Bonneville Dam in 2013 that were assigned to parents from a particular broodstock source.

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

An ongoing study employing PIT-tags for fish tagged as adults to estimate sockeye salmon escapement relies on detection of tagged fish at upstream dams in order to identify stocks (Fryer et al. 2014). For example, detections at Tumwater, Wells, and Lower Granite dams provide identification of Wenatchee, Okanagan, and Snake River sockeye stocks, respectively. However, in many years there is a portion of fish that cannot be assigned due to failure to tag, tag loss, or for reasons related to detection failure (either mortality of the fish, lock passage or equipment sensitivity at the dam detection arrays). In 2013, there were 223 unassigned sockeye salmon (28%) out of the total 799 that were PIT-tagged (Table 2). Therefore, GSI can provide the stock information missing for over a quarter of the fish sampled at the Adult Fish Facility at Bonneville Dam. The concordance between methods has been previously determined to be high, e.g. 97.9% to 99.5% for Wenatchee and Okanagan stocks, respectively (Hess et al. 2013). These concordance results allow us to combine the stock identification results from both PIT-tag and genetic methods. For example, we primarily relied on PIT-tag stock ID, and in the absence of this PIT-tag ID, we utilized the genetic stock ID. The results from combining PIT-tag and genetic stock ID provided stock information for the entire set of 799 fish that were sampled at the Adult Fish Facility in 2013.

**Table 13. Stock-specific relative abundance and run-timing distributions of sockeye salmon passing Bonneville Dam in 2013.**

Reporting group	Sample size	Estimated relative abundance	Run-timing distribution						
			Median	1st quartile	3rd quartile	5th percentile	95th percentile	Median date	Interquartile range (d)
Deschutes	2	642	-	-	-	-	-	-	-
Yakima	3	600	-	-	-	-	-	-	-
Wenatchee	223	53245	177	171	182	165	190	26-Jun	11
Entiat	3	1075	-	-	-	-	-	-	-
Okanagan	560	127256	178	171	187	163	197	27-Jun	16
Snake	8	1677	187	179	192	164	198	6-Jul	13
Whatcom	0	0	-	-	-	-	-	-	-
Total	799	184494	-	-	-	-	-	-	-

Note: The estimated relative abundance was calculated based on weekly proportions of each stock using a combined PIT-tag and Genetic stock ID, and these proportions were multiplied by the total number of sockeye counted at Bonneville Dam in a particular week (sampled time period from statistical week 23-30). The Entiat and Deschutes River “stocks” are still in question, because they may represent “dip-ins” rather than a spawning aggregate, however the “Entiat” fish were last detected in the mid and upper sections of that tributary suggesting they may be more permanent (J. Fryer personal communication). The run-timing distributions are indicated by ordinal day of passage in which a specific proportion of the total abundance was estimated to have passed Bonneville Dam. The interquartile range is marked in days.



### *Estimated relative abundance and run-timing of sockeye salmon stocks in 2013*

Abundance was estimated during a span of eight statistical weeks in which sample numbers were 14 or greater per week (Table 2). The PIT-tagged sockeye salmon stocks passing Bonneville Dam were estimated with the following abundance in decreasing magnitude: Okanagan (127,256), Wenatchee (53,245), Snake River (1677), Entiat (1075), Deschutes (642), and Yakima (600) (Table 13). Genetic assignments of fish from three stocks (Okanagan, Wenatchee, and Snake River) were utilized in cases in which it was not possible to assign them with PIT-tag data due to lack of detections of fish at their terminal dam. These genetic assignments had the most significant impact on the proportion of Snake River sockeye (i.e. 0% versus 1% estimated proportion of the total run for PIT-tag versus GSI methods, respectively), however, sample sizes of this rare stock are very low and difficult to accurately measure.

We characterized the run-timing distributions of the three main genetic stocks of sockeye salmon (Table 13). These stocks could be ordered by median date as follows: Wenatchee (Jun. 26), Okanagan (Jun. 27), and Snake River (Jul. 6).

### *Parentage based tagging analyses of Pacific lamprey*

None of the 181 Lower Granite Dam (LGR) juveniles assigned to the 2007-translocation-year adults. Estimation of the effective number of spawners represented by these LGR juveniles yielded 501 (95%CI 413–634), which is relatively low given that it would represent the effective spawner abundance of nearly the entire Snake River Basin.

## 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 securing a higher level of funding to genotype a greater number of samples both from hatchery broodstock, Bonneville Dam, and from harvest; further, most 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 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 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 ten hatchery stocks and twelve wild stocks of Chinook salmon estimated to have relative abundance greater than 1,000 fish pass Bonneville Dam in 2013. 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, spring and summer. In fact, we observed more than 5,000 natural-origin and 10,000 hatchery-origin fish from spring-run adult and jack Chinook salmon reporting groups (mostly Snake R. origin) that are estimated to return during the summer management (June 16 – July 31). Data from fish PIT tagged as juveniles suggest the majority of these late timed fish are jacks. There were some consistent run-timing results with those from previous analyses, e.g. Salmon R. stocks have relatively late runs compared to other spring stocks. We

showed our GSI accuracy has improved from previous analyses and we have begun distinguishing the following five stocks of fall-run Chinook: West Cascade, Spring Creek tule, and Deschutes R., upper Columbia R. summer/fall-run, and Snake R. stocks; however, this year's method of sampling only the even-numbered weeks precluded us from estimating accurate total abundance of these stocks. The results also demonstrate that substantial assignment errors continue to occur (e.g. hatchery origin fish assigning to JDR and MF Salmon). PBT analysis has allowed us to demonstrate consistent run-timing difference between jacks and 4-year olds from the same hatchery. In most cases in which the two age classes could be compared from a single hatchery the jacks were found to come later than the 4-year olds which is the same pattern as is typically observed for fish PIT tagged as juveniles.

For steelhead, we identified five wild- and five hatchery- steelhead stocks with an estimated relative abundance greater than 1000 fish passing Bonneville Dam in 2013. 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 that arrives after August 25<sup>th</sup> and includes South Fork Clearwater R., and upper Clearwater R. Characteristics of the steelhead that assigned to Snake River steelhead hatchery broodstock sources generally support the typical A-run and B-run steelhead life history categories. The relatively large (greater than 78 cm) steelhead were found only 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. This analysis did not specifically address run sizes of A or B Index steelhead (steelhead smaller or larger than 78cm) which is currently used to manage fall season fisheries, although the U.S. v. Oregon Technical Advisory Committee utilizes the same steelhead data collected at the Bonneville AFF trap to estimate these run sizes. It is also 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 second year we were able to analyze sockeye salmon using GSI, and our results provided some useful insight. Sockeye salmon relative abundance can be estimated with a mark-recapture (recapture via detection arrays) approach using PIT-tagging of adults at Bonneville Dam. However, each of the PIT-tagged fish must survive and be detected at its terminal dam for the method to successfully assign all individuals to a particular stock. Therefore, there is often an "unknown" group of fish that cannot be assigned to stock using this PIT-tag method alone, which provides a potentially useful role that GSI can fill. In this study, the additional information that GSI provided for the "unknown" category of fish helped to improve abundance estimates on the three sockeye populations from the Okanagan River, Wenatchee River, and Snake River. We were able to use stock composition estimates of sockeye salmon at Bonneville Dam to compare to the stock proportions estimated in the harvest in fisheries (Section 3). However, Snake River sockeye represent such a small proportion of the run and sample rates at Bonneville and in the fisheries were so low that it is difficult to draw conclusions about the number of Snake River sockeye at Bonneville or in the harvest. The fact that the Snake River sockeye estimates at Bonneville are inconsistent with PIT tag data from a larger number of fish PIT tagged as juveniles, places into question the validity of estimates for this group at Bonneville Dam. While no independent comparison can be made of the estimates of Snake River Sockeye in the fishery, these estimates may or may not have similar issues.

This was the first year we were able to analyze Pacific lamprey with parentage analysis and utilize this tool to track offspring from a translocation event. Although we did not identify any of the offspring from translocated adults from Newsome Creek when we analyzed the juvenile lamprey from Lower Granite Dam, we will continue to perform this analysis for subsequent years. We know that in 2012, nearly 100% of juvenile lamprey that were collected in a screw trap at the mouth of Newsome Creek (Clearwater subbasin of the Snake River) assigned to parents that had been translocated in 2007 (Hess et al. in review). Therefore, we were able to age these juveniles as 5-year-olds. We expect that this same 2007 cohort of juveniles will continue to work their way downstream and will eventually be possible to identify via parentage at Lower Granite Dam. However, based on results from this study, it is unlikely that they were present at Lower Granite Dam in 2011 (i.e. 4-year-old juveniles may not be expected to migrate this far downstream). If we do find a positive detection of this 2007 cohort of juveniles at Lower Granite Dam, it will likely be the first direct observation of a genetically-aged lamprey at this large distance from its natal site (i.e. ~250 km downstream). At a minimum, this genetic analysis of juvenile lamprey at Lower Granite Dam is providing a means to estimate effective number of spawners for the entire Snake River basin. As such, this method can provide a means for monitoring adult abundance of lamprey for this large and important region of its native range.

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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 trout, sockeye salmon and kokanee, and coho salmon; 3) implement Genetic Stock Identification (GSI) programs for mainstem Chinook salmon, sockeye salmon, and steelhead trout fisheries and 4) GSI of fish passing Bonneville Dam (steelhead, sockeye, and Chinook salmon) and juvenile Pacific lamprey passing Lower Granite Dam.

SNP discovery and evaluation goals (Objective 1) were achieved with a total of three projects that included one project each on Chinook salmon, steelhead trout, and Pacific lamprey which identified SNP markers using restriction associated DNA sequence (RAD-seq) technology. For genetic baseline expansion (Objective 2), the 96-SNP baseline for sockeye salmon and kokanee was expanded with the addition of kokanee collections. In addition, a RAD-seq project on Chinook salmon identified 20,000+ quality-filtered SNPs across 50 collections distributed throughout the Northeastern Pacific range. These SNPs were useful in characterizing genetic diversity of hatchery and wild Chinook salmon stocks, and may help to genetically differentiate among these stocks. This study included two broad applications of GSI; namely, stock composition of Chinook salmon, sockeye salmon, and steelhead trout fisheries (Objective 3), and stock composition of Chinook salmon, sockeye salmon, and steelhead trout passing Bonneville Dam and juvenile Pacific lamprey passing Lower Granite Dam (Objective 4). Chinook salmon and steelhead trout 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 addressed 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. We also estimated stock composition for the mark-selective fall-run Chinook salmon sport fishery and observed an increase in estimated proportion of upriver stocks relative to the W\_Cascade\_fa stock from the lower Columbia River, as compared to the previous year.

Sockeye salmon and steelhead trout 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 steelhead trout, we quantified unmarked hatchery stocks that were present among unclipped steelhead trout caught in Zone 6. These data will be combined into a

data analysis that includes clipped steelhead trout that were harvested in Zone 6 and the lower Columbia and will be reported by IDFG (in collaboration with CRITFC and WDFW).

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 trout stocks in 2013. We also combined PIT-tag and genetic analyses to estimate run-timing distributions and abundance of sockeye salmon stocks. The stock-specific data on abundance and run-timing of these species were used as a context for interpreting harvest stock composition.

Lastly, Pacific lamprey juveniles were collected as mortalities on the screens of turbine units at Lower Granite Dam (LGR) in 2011. Objective 4 included an analysis to test whether any of the juveniles from the dam could assign to adult lamprey that were translocated in the Snake River basin in the year 2007. Although none of the juveniles assigned as progeny of the translocated adults, we estimated these juveniles represented a total of 501 effective spawners that contribute to total lamprey production from the entire Snake River Basin.