FISHERY RESEARCH

PARENTAGE-BASED TAGGING OF SNAKE RIVER HATCHERY STEELHEAD AND CHINOOK SALMON

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Project Progress Report

2017 Annual Report

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ABSTRACT

This report summarizes the continued development and evaluation of a genetic technology called Parentage-Based Tagging (PBT), a versatile tool for genetically tagging steelhead *Oncorhynchus mykiss* and Chinook Salmon *O*. *tshawytscha* in the Snake River basin. While PBT is potentially a more economical and efficient technique for tagging fish than coded wire tags (CWT), it also has the capability to address aspects of hatchery practices, salmonid life history, harvest patterns, and trait heritability. This report summarizes three objectives for this performance period of January 1, 2017 to December 31, 2017 that focused on the continued development and implementation of PBT in the Snake River basin: Objective 1) annual sampling of hatchery broodstocks, Objective 2) creation of genetic parental databases, and Objective 3) utilization of PBT to provide parentage assignments for hatchery fish of unknown origin. This project continues to sample and inventory nearly 100% of hatchery broodstocks (Objective 1) for steelhead (~5,000 individuals annually), spring/summer Chinook Salmon (~10,000 individuals annually), and Fall Chinook Salmon (~2,500) in the Snake River basin. In close collaboration with the Columbia River Inter-Tribal Fisheries Commission (CRITFC), we have used a powerful panel of single nucleotide polymorphism (SNPs) markers, identified for each species, to genotype nearly 100% of the steelhead and Chinook Salmon broodstocks sampled in the Snake River basin from spawn year (SY) 2016 (Objective 2). We then use the data generated from the broodstock baselines to provide parentage analysis to meet a variety of management and conservation objectives (Objective 3). Results continue to indicate that annual sampling, inventorying, and genotyping of all steelhead and Chinook Salmon broodstock in the Snake River basin is feasible and that the SNP sets identified for PBT are sufficient for accurate assignment of offspring to brood year and hatchery stock, thereby allowing an unprecedented ability to mark millions of hatchery-origin fish from the Snake River and an opportunity to address future objectives of parentage-based management.

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INTRODUCTION

For nearly 50 years, researchers and managers have used coded wire tags (CWTs) to monitor and assess harvest patterns and survival rates of salmon and steelhead in the Columbia River basin (Johnson 2004). Recovery of CWTs was one of the primary tools used by managers in Oregon, Washington, and Idaho to estimate the number of hatchery Chinook Salmon *Oncorhynchus tshawytscha* and steelhead *O. mykiss* contributing to in-state and out-of-state fisheries and to estimate harvest of individual hatchery stocks.

Despite the predominance of CWT technology in addressing management concerns, it has several limitations. The process of physically tagging hundreds of thousands of juveniles from different hatchery stocks is logistically difficult, labor intensive, and costly. These restrictions ultimately limit the total number of juveniles that are tagged each year, which in turn limits the number of CWT recoveries. The resulting small sample sizes greatly reduce statistical power to estimate stock contributions because the precision of these estimates are directly related to the number of CWTs recovered in fisheries or escapements (Hankin et al. 2009).

Parentage-based genetic tagging (described in Anderson and Garza [2005]), a technological alternative to CWT, would eliminate the problem of small sample sizes by increasing tag rates to near 100%. Parentage-based tagging (PBT) involves annual sampling and genotyping of hatchery broodstock and creating a database of parental genotypes. Progeny from any of these parents (collected either as juveniles or adults), can be non-lethally sampled and, if genotyped, be assigned back to their parents, thus identifying their hatchery of origin and their exact brood year. The exceptional advantage that PBT has over CWT technology is increased tagging rates. By genotyping all parental broodstock, every juvenile is genetically "tagged."

The prospect of implementing PBT was theoretically appealing (Anderson and Garza 2005; 2006) and several committees and science review groups recommended that large-scale evaluations of the technology be performed (PFMC 2008; PSC 2008; ISRP/ISAB 2009). Over the past several years PBT technology was empirically tested and validated in the Snake River basin (Steele et al. 2013).

Given these recent advancements, this project constructs the first PBT genetic baselines for steelhead and Chinook Salmon hatcheries in the Snake River basin. It also addresses both current and future objectives in creating PBT baselines within the Snake River basin that can be used for evaluating harvest of hatchery stocks but also for addressing additional issues, such as the origin of hatchery strays and steelhead kelts, effectiveness of hatchery mitigation programs, broodstock integration, and relative reproductive success of hatchery fish.

OBJECTIVES

For this performance period, the Snake River PBT project includes the following objectives:

Objective 1: Genetic Sampling of all Hatchery Chinook Salmon and Steelhead Broodstock in the Snake River Basin

Completion of this objective demonstrates the feasibility of sampling and inventorying all hatchery broodstock each year for steelhead and Chinook Salmon and recording accurate biological information (e.g. sex, length, spawn day) for every fish.

Objective 2: Creation of Parental Databases for Snake River Hatcheries

Completion of this objective demonstrates the ability to genotype all sampled broodstock and to create a database of parental genotypes for each spawn year (SY) of steelhead, spring/summer Chinook Salmon, and Fall Chinook Salmon.

Objective 3: Utilization of PBT Methods to Provide Accurate Parental Assignments

We demonstrate the application of this technology through “back end” projects that use the PBT baselines to assign parentage to samples of unknown origin. We demonstrate the versatility of PBT by summarizing several projects.

For steelhead returning in migration year 2015 (SY2016), the PBT baselines were used to determine: 1) Origin of samples from sport fisheries in the Columbia River, 2) Origin of samples from tribal fisheries in Columbia River Zone 6, 3) Origin of samples from sport fisheries in the lower Snake River, 4) Origin of samples from various sport fisheries in Idaho, 5) Age composition and origin of the broodstocks, and 6) stock composition of returning adults at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2016), 2) Age composition and origin of SY2016 broodstocks, 3.) stock composition of returning adults during SY2016 at Lower Granite Dam.

REPORT STRUCTURE

This report is divided into three sections, one for each of the objectives for this fiscal year. The first section reports on sampling efforts. The second section summarizes genetic data from the most recently genotyped broodstocks. The third section provides an overview of current implementation and results of PBT projects.

LITERATURE CITED

Anderson, E. C., and J. C. Garza. 2005. A description of full genotyping. Report submitted to the Pacific Salmon Commission, Vancouver, British Columbia. 11p. http://swfsc.noaa.gov/publications/FED/00675.pdf.

Anderson, E. C., and J. C. Garza. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics 172:2567–2582.

Hankin, D. G., J. Fitzgibbons, and T. Chen. 2009. Unnatural random mating policies select for younger age at maturity in hatchery Chinook Salmon (*Oncorhynchus tshawytscha*) populations. Canadian Journal of Fisheries and Aquatic Sciences 66:1505–1521.

ISRP/ISAB (Independent Scientific Review Panel and Independent Scientific Advisory Board). 2009. Tagging Report. A comprehensive review of Columbia River Basin fish tagging technologies and programs. ISRP/ISAB 2009-1. Northwest Power and Conservation Council. Portland, Oregon.

Johnson, K. J. 2004. Regional overview of coded wire tagging of anadromous salmon and steelhead in Northwest America. Regional Mark Processing Center, Pacific Sates Marine Fisheries Commission. Portland, Oregon.

PFMC (Pacific Fishery Management Council). 2008. Research and data needs. http://www.pcouncil.org/wp-content/uploads/Res_Data_Needs_2008_Final_OCT08.pdf.

PSC (Pacific Salmon Commission). 2008. Recommendations for Application of Genetic Stock Identification (GSI) Methods to Management of Ocean Salmon Fisheries. Special Report of the GSI Steering Committee and the Pacific Salmon Commission's Committee on Scientific Cooperation. January 2008. Pacific Salmon Commission. Technical Report No. 23. http://www.psc.org/GSIWorkshop?GSI_Final_Report.pdf.

Steele, C. A., E. C. Anderson, M. A. Ackerman, M. A. Hess, N. R. Campbell, S. R. Narum, and M. R. Campbell. 2013. A validation of parentage-based tagging using hatchery steelhead in the Snake River basin. Canadian Journal of Fisheries and Aquatic Sciences 70: 1046–1054.

SECTION 1: ANNUAL SAMPLING OF HATCHERY STEELHEAD AND SPRING/SUMMER CHINOOK SALMON BROODSTOCKS IN THE SNAKE RIVER BASIN

INTRODUCTION

The implementation of PBT methods requires a complete sampling of broodstocks from all hatcheries contributing to the production of steelhead and Chinook Salmon (Figure 1). This objective addresses the feasibility of annually sampling tissue from 100% of the hatchery broodstocks for spring/summer Chinook Salmon and steelhead in the Snake River basin.

METHODS

The overall goal is to obtain high quality tissue samples and accurate biological data from every adult that contributes to spawning. This includes species, sex, hatchery/stock, date sampled/spawned, tag information, and markings. Hatcheries also record length and cross information whenever possible. Tissue samples are collected in the form of fin tissue stored on absorbent sheets of Whatman 3mm chromatography paper (LaHood et al. 2008; and see https://www.monitoringmethods.org/; *Genetic sampling and storage using chromatography filter paper v1.0, Method ID# 4087*). The samples are shipped to the IDFG genetics lab in Eagle, Idaho. Care is taken to avoid contamination during sampling by rinsing scissors or hole-punch tools in water or ethanol and wiping with a paper towel between each tissue sample.

Each sample is labeled with a field identification number, which is used to track the samples until they arrive at the lab, at which time they are given a standardized lab database code. The associated data is reviewed at the lab to ensure accurate information was recorded for every fish sampled. Any discrepancies that are discovered are solved via correspondence with the hatchery employee in charge of recording data. Samples from spawned adults whose eggs were culled due to disease or surplus are now genotyped and included in summary tables.

Once the DNA from the samples is extracted and genotyped, genetic data are recorded into a Progeny SQL database (Progeny Software, South Bend, Indiana, USA) and stored with collection information and individual fish data. Due to the scope of this project, this database was created to manage, organize, and track physical tissue samples along with their associated DNA extractions and genotypes. Progeny allows genetic data to be exported along with individual fish data in a variety of formats, which has proven to be essential for the transfer of data between the collaborating IDFG and CRITFC laboratories.

Complete sampling methods can be found at https://www.monitoringmethods.org/; *Tissue sampling for Parentage Based Tagging v1.0*, *Method ID# 1432.*

RESULTS

For fiscal year 2017, we have collected and inventoried approximately 5,253 genetic samples from the steelhead broodstock (Table 1) spawned in the Snake River basin during spawn year (SY) 2016, and approximately 10,056 samples (Table 2) from spring/summer Chinook Salmon broodstock spawned in the Snake River basin during SY2016. We also report on Fall Chinook Salmon collected from the Lyons Ferry and Nez Perce Tribal Fish hatcheries for SY2016 (N = 2,492; Table 3). Most hatcheries provided biological information on all fish sampled (sex, length, etc.) as well as individual cross information. Missing biological information

is usually due to inadvertently overlooking the recording of the data; missing cross-information can be due to the same reason.

DISCUSSION

We continue to demonstrate the feasibility of large-scale sampling and inventorying of thousands of broodstock fish each year. The annual completion of this objective lays the foundation for the use of PBT baselines in the Snake River basin.

LITERATURE CITED

LaHood, E. S., J. J. Miller, C. Apland, and M. J. Ford. 2008. A Rapid, Ethanol-Free Fish Tissue Collection Method for Molecular Genetic Analyses. Transactions of the American Fisheries Society 137:1104-1107.

SECTION 2: CREATION OF GENETIC DATABASES FOR BROODSTOCKS OF STEELHEAD AND SPRING/SUMMER CHINOOK SALMON IN THE SNAKE RIVER BASIN

This section presents summary information for the genetic data collected from steelhead and Chinook Salmon broodstocks in SY2016.

INTRODUCTION

Previously, sets of 96 single nucleotide polymorphism (SNP) markers were identified for steelhead and Chinook Salmon, and it was demonstrated that the selected SNPs provide sufficient resolving power for dual-parentage assignments (Steele et al. 2011). These sets of markers were again used to genotype broodstock samples collected in 2016. Primer and probe sequence information for these markers are available on http://www.FishGen.net: CRITFC/IDFG Chinook Salmon 96 PBT v5.1 and CRITFC/IDFG Steelhead 96 PBT v5.1.

During the seventh year of this project, IDFG and CRITFC labs extracted and genotyped all samples for steelhead and Chinook Salmon broodstocks (~7,500 IDFG, ~7,500 CRITFC = ~15,000 total samples).

Beginning in SY2015, our lab adopted Genotyping-in-Thousands (GT-seq) protocols developed by the CRITFC genetics lab (Campbell et al. 2014) to genotype PBT baselines (also see https://www.monitoringmethods.org; *SNP genotyping using Genotyping in Thousands (GT-seq) on Illumina Sequencer platform v1.0*, *Method ID# 5446*). This technology utilizes a next-generation DNA sequencing instrument (Illumina brand Nextseq). This instrument was purchased in September 2015 via a grant from the Pacific Coast Salmon Recovery Fund. It sequences multiplexed PCR (polymerase chain reaction) products to genotype samples with a minimum of 192 SNP loci at reduced consumable costs. The screening of additional numbers of SNPs for this project continues to allow the two labs (IDFG and CRITFC) to remain standardized and may allow the assignment of single parents in situations where one parent was either inadvertently not sampled or not successfully genotyped.

The continued creation of these parental genetic databases establishes an unprecedented ability to mark millions of hatchery-origin fish from the Snake River basin and an opportunity to address a variety of parentage-based research and management objectives.

METHODS

Laboratory Protocol

Genomic DNA extraction followed the methods described in Matala et al. (2011) and was extracted using the Nexttec Genomic DNA Isolation Kit from XpressBio (Thurmont, Maryland) or Qiagen DNeasy (Valencia, California). Protocols of library preparation for GT-Seq followed Campbell et al. (2014). Library preparation begins with an initial multiplex PCR reaction that is used to ligate a pair of sequencing primers to the target sequences which contain a known single-nucleotide polymorphism (SNP). In a subsequent PCR reaction the sample is "barcoded" by ligating an additional sequence to the target that identifies the sample's tray of origin (i7 barcode) and its position on the tray (i5 barcode). After barcoding the quantity of DNA must be normalized for each sample. A SequalPrep™ Normalization Plate Kit (Applied Biosystems) is used to bind a standard amount of amplicon product and normalize

concentrations. All 96 samples are then pooled into a single 'plate library'. All plate libraries are quantified by qPCR, and concentrations are normalized again before being pooled. Loci are genotyped by sequencing the target location on the Illumina NextSeq. A bioinformatics pipeline is used to assign resulting sequences and the genotypes back to individual samples using the unique combination of i5 and i7 barcodes.

Standardized parental genotypes were stored on a Progeny database server housed at the Eagle Fish Genetics Laboratory (EFGL). Progeny software (www.progenygenetics.com) is already used by the majority of Genetic Analysis of Pacific Salmon (GAPS) labs throughout the Pacific Northwest: Idaho Department of Fish and Game, University of Washington, NOAA-Northwest Fisheries Science Center, Washington Department of Fish and Wildlife, Columbia River Intertribal Fish Commission, and U.S. Fish and Wildlife Service. Parentage analysis of broodstock spawned in the Snake River basin is conducted annually. Results are stored at EFGL in the Progeny database and available to GAPS labs upon request.

Data quality was inferred from estimates of completion rate, missing data, poor performing loci, and error rates. The program ML-NULLFREQ (Kalinowski and Taper 2006) was used to run a HWE (Hardy-Weinberg equilibrium) test for heterozygote deficiency on each locus. For this test, a significant P-value suggests a deficiency of heterozygotes which may be due to null alleles at the locus. Significance thresholds were adjusted using the modified B-Y Method proposed by Narum (2006) to account for the multiple tests across the different hatchery populations. For loci that were identified as containing null alleles the same program was then used to estimate the proportion of null alleles at the locus. Basic diversity indices were calculated for the brood years. This included average observed (H_O) and expected (H_E) heterozygosity using the program GenAlEx ver 6.5 (Peakall and Smouse 2006, 2012), estimates of differentiation among stocks through estimates of pairwise F_{ST} and tests of allelic differentiation using Genepop (Rousset 2008), and effective population size (N_E) using NeEstimator v.2 (Do et al. 2014). Estimates of N_E using NeEstimator v.2 were employed assuming a random-mating model and $\alpha = 0.05$.

Sex Markers

The accuracy of the sex-determining SNP assay for steelhead and Chinook Salmon was evaluated for hatchery stocks spawned in SY2016; comparisons were made between the phenotypic sex of samples, which was determined at time of spawning, and the genetically determined sex of samples.

Tagging Rate

A small portion of hatchery-origin offspring were genetically "untagged" because genotypes from 100% of the broodstock were not always obtained for all hatchery stocks. This "untagged" portion of hatchery-origin fish cannot be assigned back to their parental pair or hatchery of origin because genotypes were missing from one or both of their parents and genotypes from both parents are needed for accurate PBT assignment. However, we can easily estimate the proportion of "untagged" progeny of each hatchery stock for each brood year based on the proportion of successfully genotyped broodstock. Assuming that males and females were successfully genotyped at equal rates, the proportion of PBT-tagged offspring can also be estimated by squaring the total proportion of successfully genotyped broodstock. We used this method to estimate the proportion of PBT-tagged offspring from each stock (Tables 1, 2, and 3).

Whether PBT can serve as an efficient and accurate tag at scales finer than the stock level depends on the ability of the hatchery to track families through the culture phase of their life cycle. If managers want to use PBT to evaluate different release sites within a fishery then an effort must be made during the rearing stage not to split families into groups destined for different release sites. Splitting families in this manner means that when the progeny are sampled at a later date their parents can be identified with PBT, but because offspring were released at two different sites it is impossible to determine at which release site the sampled offspring was released. Hatchery steelhead management in Idaho is complicated and approximately 7.8 million steelhead are released annually from 7 stocks (5 hatcheries) at ~30 different release sites. Hatcheries have had to devise a PBT tracking system that allowed family groups to be tracked from PBT-sampled parents to egg tray incubators to vats, raceways, and then to unique release sites. While this report uses PBT rates at the stock level, PBT rates for Idaho hatchery steelhead can be calculated at the release group level. Average realized PBT tagging rates at the level of release site are generally over 90%.

RESULTS

Completion Rate and Missing Data

If a sample failed to genotype at 10% or more of the SNPs it was re-extracted and re-genotyped. If that sample failed a second time at 10% or more of the SNPs, it was automatically excluded from future PBT analyses because the excess missing data can prevent accurate parentage assignment.

For steelhead SY2016, all 5,368 samples were extracted and genotyped with the expanded panel of 268 SNPs and the sex-identification assay. Of the 5,368 samples, 5,250 (97.8%) were genotyped with an acceptable level of missing data (Table 4). In this final SY2016 PBT baseline comprising the remaining 5,250 samples, there were 27,265 missing genotypes due to SNP failure out of a possible 1,564,500 genotypes. This resulted in missing data for just 1.7 % of the genotypes.

For spring/summer Chinook Salmon SY2016, all 9,711 samples were extracted and genotyped with the expanded panel of 298 PBT SNPs and the sex-identification assay. Of the 9,711 samples, 9,469 (97.5%) were genotyped with an acceptable level of missing data (Table 5). In this final SY2016 PBT baseline comprising the remaining 9,469 samples, there were just 39,267 missing genotypes due to SNP failure out of a possible 2,821,762 genotypes. This resulted in missing data for just 1.4% of the genotypes.

For Fall Chinook Salmon SY2016, all 2,628 samples were extracted and genotyped with the expanded panel of 298 PBT SNPs and the sex-identification assay. Of the 2,628 samples, 2,530 (96.3%) were genotyped with an acceptable level of missing data (Table 6). In this final SY2016 PBT baseline comprising the remaining 2,530 samples, there were just 2,530 missing genotypes due to SNP failure out of a possible 753,940 genotypes. This resulted in missing data for just 0.3% of the genotypes.

Poor Performing Loci

Most SNPs in the samples that passed the genotyping threshold had high genotyping success. For SY2016 steelhead, 22 loci failed to genotype at >5% of samples (Table 4). For SY2016 spring/summer Chinook Salmon, there were 17 loci that failed at >5% of the samples

(Table 6). For SY2016 Fall Chinook, there were 27 loci that failed at >5% of the samples (Table 7).

Error Rate (Quality Control)

For steelhead SY2016, a subset of 137 samples were rerun at the complete panel of 269 loci and the resulting 36,853 genotypes were checked for discrepancies. Of these genotypes, there were 62 discrepancies, excluding any SNP failures in either the original or the rerun genotype, which resulted in a genotype error rate of 0.17%.

For Chinook Salmon SY2016, a subset of 441 samples representing all extraction plates were rerun at 299 loci and checked for discrepancies. This resulted in 131,859 rerun genotypes being compared to the original genotypes. Of these genotypes, 9,635 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 122,224 genotypes with 10 discrepancies between the original and samples and a genotyping error rate of 0.008%.

Null Alleles

For steelhead SY2016, 175 of the 268 loci were found to exhibit a deficiency of heterozygotes in at least one population, but only 27 of these loci had null frequencies estimated >5% (Table 8).

For spring/summer Chinook Salmon SY2016, 214 of the 298 loci were found to exhibit a deficiency of heterozygotes in at least one population, but only 15 loci had a null frequency estimated >5% (Table 9).

For Fall Chinook Salmon SY2016, 87 of the 298 PBT loci were found to exhibit a deficiency of heterozygotes in at least one population. Twenty-four had a null frequency estimated >5% (Table 10).

Average Heterozygosity

Levels of observed heterozygosity within steelhead broodstocks was ~0.25 for all hatchery broodstocks (Table 14). For Chinook Salmon, levels of observed heterozygosity was ~0.21 in spring/summer stocks (Table 15) and ~0.25 in fall stocks (Table 16).

Population Structure

Pairwise F_{ST} was calculated among the steelhead SY2016 hatchery broodstock (Table 17). Values ranged from a low of 0.001 between the Dworshak and SF Clearwater stocks, and a high of 0.062 between the Upper Salmon B-run and Little Sheep Creek stocks. All tests of genetic differentiation among stocks were significant.

For spring/summer Chinook Salmon SY2016 pairwise F_{ST} values ranged from a low of 0.001 between the Dworshak and the Kooskia stocks and a high of 0.049 between the Sawtooth and Tucannon stocks (Table 18). All tests of genetic differentiation among stocks were significant. Differentiation among the two Fall Chinook Salmon stocks was very low (F_{ST} = 0.0002) albeit significant ($P < 0.01$).

Effective Population Size

Effective population size (N_e) for steelhead hatchery broodstock in SY2016 ranged from a low of 36.2 for the Upper Salmon River B-run broodstock to a high of 507.7 for the Tucannon broodstock (Table 19).

Effective population size for spring/summer Chinook Salmon hatchery broodstock in SY2016 ranged from a low of 85.7 for Grande Ronde to a high of 515.8 for Dworshak (Table 20). Effective population size for the two Fall Chinook Salmon hatchery broodstocks were large with the Nez Perce stock estimated at 1,571.5 and the Lyons Ferry stock estimated at 1,432.8 (Table 21).

Sex Markers

The sex-specific assay for steelhead matched phenotypic sex in 99.1% of the samples (Table 10). In the instances (n = 46) in which genetically-determined sex did not correspond to the phenotypic sex, it was twice as likely that phenotypic males were genetically misidentified as female than phenotypic females being genetically misidentified as males. The assay either failed to genotype or provided ambiguous results for 4.8% of the samples.

The sex-specific assay for spring/summer Chinook Salmon matched phenotypic sex in 99.7% of the samples (Table 11). In the instances (n = 21) in which genetically-determined sex did not correspond to the phenotypic sex, more phenotypic females were misidentified as males rather than phenotypic males genetically identified as female. The assay either failed to genotype or provided ambiguous results for 2.0% of the samples.

The sex-specific assay for Fall Chinook Salmon matched phenotypic sex in 99.7% of the samples (Table 12). Of the nine discrepancies, most were cases of phenotypic females genetically identified as males. The assay either failed to genotype or provided ambiguous results for 1.5% of the samples.

Tagging Rate

Overall tagging rates were very high for steelhead (Table 1), spring/summer Chinook Salmon (Table 2), and Fall Chinook Salmon stocks (Table 3). All stock-level tag rates were greater than or approached 90% in steelhead broodstocks. Stock-level tag rates were greater than 90% in 12 of the 16 spring/summer Chinook salmon broodstocks. For Nez Perce and Lyons Ferry Fall Chinook hatchery stocks, all were tagged at 90% or greater for SY2016.

DISCUSSION

We continue to demonstrate the ability to routinely genotype thousands of broodstock samples collected each year. Genotypes are stored and organized in an on-site database where they can be exported for PBT analysis. The creation of these PBT baselines also provides the ability to assess several measures of genetic diversity and relatedness among the broodstocks, which provide the added benefit of genetic monitoring of hatchery populations. The completion of this objective allows parental genotypes to be queried in parentage analyses resulting in the identification of hatchery fish originating from the Snake River basin.

Completion Rate and Missing Data

The high rate of genotyping success for samples and the low rate of missing data demonstrate the feasibility of collecting high quality data from nearly all Snake River basin broodstock samples.

Poor Performing Loci

Our panels of SNP loci for steelhead and spring/summer Chinook Salmon have been genotyped on nine years' worth of hatchery broodstock in the Snake River basin (>100,000 samples). We observe very few loci that do not exhibit high genotyping success. Transitioning to GT-Seq has helped reduce the number of poor performing loci. The GT-Seq protocol uses an automated procedure to score loci, thereby removing inconsistency in the scoring. This is perhaps why we observed fewer poor performing loci in Fall Chinook when using GT-Seq, despite a larger panel of SNPs being screened, than we did last year when Fall Chinook broodstock were genotyped for using the Fluidigm platform. While it is interesting to identify loci that have differential genotyping success rates, we have decided that it is not necessary that these loci be replaced in any of the SNP panels, especially since the PBT panel has been expanded using GT-Seq. The number of SNP loci in the PBT panel is approaching 300 markers for steelhead and Chinook salmon and the presence of several poorly genotyping loci is not critical for accurate parentage analysis given the remaining number of successfully genotyping loci in the panel.

Error Rate (Quality Control)

To minimize false negatives in parentage assignments, genetic markers need to exhibit low genotyping error rates and researchers should accommodate estimated error rates during data analysis (Kalinowski et al. 2007). Genotyping error rates for microsatellite markers are variable but have often been reported between 1-2% (Pearse et al. 2009; Hauser et al. 2011). For the parentage software programs CERVUS and SNPPIT, the default error rate used is 1%. We consistently observed error rates ≤1% for both the steelhead and Chinook Salmon PBT panels of SNPs across several years.

Null Alleles

Since the initiation of this project we have evaluated our panels of SNP loci for null alleles in order to identify loci in which we do not recover both alleles. This helps to identify loci that could potentially be removed from the panel as they do not provide accurate genotypes. We have generally found that while null alleles are probably present within certain hatchery stocks, the frequency of these alleles is low and they have minimal impact on the accuracy of parentage assignment. The genotyping of additional loci across the entire broodstock was made possible through the adoption of the GT-Seq genotyping platform. The additional information ultimately increases the power of the parental analysis. However, identifying loci with null alleles in this expanded panel is somewhat irrelevant because the SNPs used for GSI (Genetic Stock Identification) are purposefully selected to provide maximum discrimination between populations. This means that these SNPs display a high frequency of one allele within one population while displaying a high frequency of the other allele in different populations. This pattern can appear as if the locus is displaying null alleles when in fact it is not. We report the estimated frequency of null alleles but most of the loci with values >5% originated from the GSI panel and their values should be viewed as an artifact of the function of these new loci.

Population Structure

Within steelhead, the highest pairwise F_{ST} values are seen between the Dworshak Hatchery stock (and its derivatives such as the Upper Salmon B-run stock and SF Clearwater stock) and other locations. The larger degree of divergence between Dworshak and the other stocks reflects the distinctness of Clearwater origin fish to those in the Salmon and Snake rivers. The lowest F_{ST} values are also consistently seen between populations that are geographically proximate, such as the Touchet and Tucannon stocks in Washington State, or among stocks with shared founding ancestries. For example, Oxbow, Sawtooth, and Pahsimeroi stocks were recently derived from stocks whose brood source came from wild adult steelhead trapped at Hells Canyon Dam on the Snake River in the late 1960s (Nielsen et al. 2009). This shared ancestry is reflected in their low differentiation from one another.

Within Chinook Salmon, the highest pairwise F_{ST} values are consistently seen among the most geographically distant stocks (e.g. Sawtooth and Tucannon). This is a common pattern of isolation-by-distance indicating genetic differentiation increases with geographic distance. The lowest pairwise F_{ST} values tended to be among stocks within the Clearwater drainage (Dworshak, Powell, Nez Perce, and Clearwater). Chinook Salmon stocks in the Clearwater drainage were extirpated following the construction of Lewiston Dam in 1927. Present-day stocks were derived predominantly from Rapid River origin broodstock. Current management practices treat broodstock from different hatcheries within the Clearwater basin as a single stock and transportation of eggs among facilities is allowed, thereby generating low degrees of genetic differentiation among these hatcheries.

Effective Population Size

Effective population size (N_E) is an important parameter for hatchery managers to measure and monitor because it summarizes the magnitude of genetic drift and increase in inbreeding occurring in their populations (Wright 1931). For this report, we calculated the effective population size of all hatchery broodstocks using the commonly employed linkage disequilibrium estimator. Results indicate that while we observe variation in N_E between larger hatchery programs (e.g. Dworshak steelhead, Lyons Ferry Fall Chinook Salmon) and smaller programs (e.g., Cottonwood Creek steelhead and Powell spring/summer Chinook Salmon), N_E is generally fairly large (>150) for hatchery broodstock populations spawned annually in the Snake River basin. Only the Upper Salmon River B-run stock was estimated to have an N_E <50 (36.2), which based on genetic theory would put the population at risk of inbreeding (Franklin 1980). During the last couple of years, the Upper Salmon River B-run stock has been supplemented with additional B-run broodstock from the Dworshak Fish Hatchery. This additional adult diversity, along with increased smolt production, should increase N_E in this population over time, although this will have to be monitored.

Sex Markers

The steelhead and Chinook salmon sex markers continue to provide an accurate (~99%) method of sex determination for both species.

Tagging Rates

This project continues to demonstrate that it is possible to achieve high PBT tagging rates even when tens of thousands of fish require tissue sampling and genotyping. The overall tag rate was ≥95% for steelhead and spring/summer Chinook, and ~93% for fall Chinook.

LITERATURE CITED

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2014. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 15(4):855–867.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14(1):209–214.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135 – 140 in: M.E. Soule and B.A. Wilcox (eds.), Conservation Biology: An Evolutionary Ecological Perspective. Sunderland, Mass. Sinauer Associates.

Hauser, L., M. Baird, R. Hilborn, L. W. Seeb, and J. E. Seeb. 2011. An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon *(Oncorhynchus nerka*) population. Molecular Ecology Resources. 11 (Suppl 1): 150–161.

Kalinowski, S. T., and M. L. Taper. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conservation Genetics 7:991–995.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1106.

Matala, A. P., J. E. Hess, and S. R. Narum. 2011. Resolving adaptive and demographic divergence among Chinook Salmon populations in the Columbia River Basin. Transactions of the American Fisheries Society 140:783–807.

Narum, S. R. 2006. Beyond Bonferroni: Less conservative analyses for conservation genetics. Conservation Genetics 7(5):783–787.

Nielsen, J. L., A. Byrne, S. L. Graziano, and C. C. Kozfkay. 2009. Steelhead genetic diversity at multiple spatial scales in a managed basin: Snake River, Idaho. North American Journal of Fisheries Management 29:680–701.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288–295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537–2539.

Pearse, D. E., S. A. Hayes, M. H. Bond, C. V. Hanson, E. C. Anderson, and J. C. Garza. 2009. Over the Falls? Rapid Evolution of Ecotypic Differentiation in Steelhead/Rainbow Trout (Oncorhynchus mykiss). Journal of Heredity 100:515–525.

Rousset, F. 2008. GENEPOP'007: a complete reimplementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103–106.

Steele, C. A., M. Ackerman, J. McCane, M. Hess, N. Campbell, S. R. Narum, M. R. Campbell. 2011. Parentage Based Tagging Snake River hatchery steelhead and Chinook Salmon. Annual Report. BPA (Project No. 2010-031-00 Contract Number 48348). United States Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, P.O. Box 3621, Portland, Oregon 97208.

Wright, S. 1931. Evolution in Mendelian Populations. Genetics. 16(2):97-159.

SECTION 3: UTILIZATION OF PBT TO PROVIDE PARENTAL ASSIGNMENTS

INTRODUCTION

Broodstock genotypes have now been collected for both steelhead and spring/summer Chinook Salmon since 2008. Projects can now be implemented to use PBT in addressing a multitude of research and management questions involving hatchery stocks. We report the results from various projects that collected samples from particular spawn years (SY) or collection years (CY) and have utilized these PBT baselines for questions pertaining to Chinook Salmon and steelhead. All PBT projects presented here were instigated by fisheries managers and biologists to answer their specific research or monitoring questions. Brief descriptions of their projects are presented here, but complete descriptions of the specific study objectives, design, results, and interpretation are presented in their respective reports.

For steelhead returning in migration year 2015 (SY2016), the PBT baselines were used to determine: 1) Origin of samples from sport fisheries in the Columbia River, 2) Origin of samples from tribal fisheries in Columbia River Zone 6, 3) Origin of samples from sport fisheries in the lower Snake River, 4) Origin of samples from various sport fisheries in Idaho, 5) Age composition and origin of the broodstocks, and 6) stock composition of returning adults during at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2016), 2) Age composition and origin of SY2016 broodstocks, 3.) stock composition of returning adults during SY2016 at Lower Granite Dam.

METHODS

Samples collected for these various “back end” projects were inventoried and genotyped using the same procedures as the broodstock. The program SNPPIT was used to conduct parentage analysis. Unless indicated otherwise, the criteria for accepting a PBT assignment was an LOD score (log of odds) >14.

Steelhead Sport Fisheries in Columbia River

IDFG coordinated the sampling of steelhead harvested in the lower Columbia River sport fishery in 2016 (SY2017). A total of 1,521 samples were processed for PBT assignment. An example of the methods used for this annual sampling and PBT assignment results can be found in Byrne et al. (In Prep).

Steelhead Tribal Fisheries in Zone 6 of Columbia River

IDFG coordinated sampling of steelhead harvested in the tribal fishery between Bonneville Dam and McNary Dam (Zone 6) during collection year (CY) 2016 (e.g. spawn year 2017). A total of 1,889 samples from steelhead were analyzed. Description of the methods used for this annual sampling can be found in Byrne et al. (2015) and Byrne et al. (*In Prep*).

Steelhead Sport Fisheries in Lower Snake River

Washington Department of Fish and Wildlife (WDFW) collected samples of steelhead harvested in the SY2016 lower Snake River sport fishery from the mouth of the Snake River to

the Idaho/Washington border. A total of 525 samples were processed for PBT assignment. Description of the methods used for this annual sampling can be found in Byrne et al. (2015) and Byrne et al. (*In prep*).

Steelhead Sport Fisheries in Idaho

IDFG collected samples of steelhead harvested in the SY2016 sport fishery from various river systems including the Clearwater and Salmon. A total of 2,298 samples were processed for PBT assignment. A more detailed description of this project is in Warren et al. (*In prep*).

Age Composition of SY2016 Steelhead Broodstock

PBT was used to determine age composition of steelhead broodstocks in Idaho by assigning the SY2016 broodstocks back to all previously sampled broodstocks, thereby identifying the age of each fish. A total of 5,022 samples from eight different broodstocks were analyzed with PBT.

Stock Composition of Adult Steelhead at Lower Granite Dam

Representative samples of the adult steelhead run across Lower Granite Dam were collected in the fall of 2016 and spring of 2017 (Warren et al. *In prep*) and 2,000 samples were analyzed with PBT.

Chinook Salmon Sport Fishery in Idaho

Fisheries managers within IDFG continued PBT sampling of Chinook Salmon harvested in the sport fisheries in SY2016. A total of 1,286 samples representative of the various time strata and river sections were analyzed with PBT. Complete methodology and results are presented in Sullivan et al. (*In prep.*).

Age Composition of SY2016 Chinook Salmon Broodstock

PBT was used to determine age composition of Chinook Salmon broodstocks in Idaho by assigning the SY2016 broodstocks back to previously sampled broodstocks, thereby identifying the age of each fish. A total of 10,132 hatchery-origin broodstock samples from ten different broodstocks were analyzed with PBT.

Stock Composition of Adult Chinook at Lower Granite Dam

Representative samples of the adult Chinook run across Lower Granite dam were collected in 2017 (Sullivan et al. *In prep*) and 2,003 samples were analyzed with PBT.

RESULTS

Steelhead Sport Fisheries in Columbia River

Of the 1,521 samples analyzed, 1,075 assigned to the PBT baseline. After expanding by PBT rates, ~72% of the sport samples assigned to hatcheries in the Snake River basin. A detailed breakdown of stock composition in these fisheries is presented in Byrne et al. (*In Prep*.).

Steelhead Tribal Fisheries in Zone 6 of Columbia River

Of the 1,889 samples analyzed from clipped and unclipped steelhead, 1,307 assigned to the PBT baseline. After expanding by PBT rates, ~71% of the Zone 6 samples assigned to hatcheries in the Snake River basin. A detailed breakdown of stock composition in this fishery is presented in Byrne et al. (*In Prep*.).

Steelhead Sport Fisheries in Lower Snake River

Of the 525 samples analyzed, 489 assigned to the PBT baseline. After expanding by PBT rates, the origin of ~95% of samples could be accounted for. A breakdown of stock and cohort proportions will be presented in Byrne et al. (*In Prep.*).

Steelhead Sport Fisheries in Idaho

Of the 2,298 samples analyzed, 1,971 assigned. After expanding by PBT rates, ~88% of the samples assigned to hatcheries in the Snake River basin. A detailed breakdown of stock composition in this fishery is presented in Warren et al. (*In Prep.*).

Stock Composition of Adult Steelhead at Lower Granite Dam

Of the samples collected, 1,926 were analyzed after removing duplicated samples or samples that failed to genotype. Of these, 1,831 assigned to the baseline. A summary of stock composition and age will be provided in an upcoming IDFG technical report (Warren et al. *In prep*).

Age Composition of SY2016 Steelhead Broodstock

Of the samples collected, 4,650 were analyzed with PBT after excluding duplicate and ungenotyped samples. Of these, 4,428 assigned (95.2%) to the baseline. Age composition for 3-, 4-, and 5-year olds in each hatchery stock will be provided in upcoming IDFG technical reports.

Chinook Salmon Sport Fishery in Idaho

Of the samples collected, 1,217 were analyzed after removal of duplicates and samples that failed to genotype. Of these, 1,200 received a PBT assignment. A detailed breakdown of stock and age composition of the harvest in this fishery is presented in Sullivan et al. (*In Prep.*).

Age Composition of SY2016 Chinook Salmon Broodstock

Of the samples collected, 9,732 were analyzed after removal of duplicated samples and samples that failed to genotype. Of these, 9,159 assigned (94.1%) to the PBT baseline. Age composition for 3-, 4-, and 5-year olds in each hatchery stock will be provided in upcoming IDFG technical reports.

Stock Composition of Adult Chinook at Lower Granite Dam

Of the samples collected, 1,829 were analyzed after removing duplicated samples or samples that failed to genotype. Of these, 1,783 assigned to the baseline. A summary of stock

composition and age will be provided in an upcoming IDFG technical report (Sullivan et al. *In prep*).

DISCUSSION

The PBT baselines being developed and maintained are made available to fisheries managers to help address a variety of management questions for steelhead and Chinook Salmon. While specific implications and interpretations are presented in separate reports, the number and diversity of projects that made use of the PBT baselines is noteworthy, especially since many of these projects would not have been possible without access to this technology.

Steelhead Sport Fisheries in Columbia River

This project represents some of the first comprehensive attempts to categorize the stock composition of the steelhead harvest in the Lower Columbia sport fishery. Results from this year's sampling (Byrne et al. *In Prep*), as well as results from previous years (Byrne et al. 2015), will aid in monitoring needs for the *U.S. v Oregon* Management Agreement and in the management of ESA-listed B-run steelhead that return to the Dworshak Fish Hatchery.

Steelhead Tribal Fisheries in Zone 6 of Columbia River

This project also represents some of the first comprehensive attempts to categorize the stock composition of the steelhead harvest in the Zone 6 fishery. Implications of the results are more thoroughly explored in Byrne et al. (*In Prep.*).

Steelhead Sport Fisheries in Lower Snake River

This project represents some of WDFW's first evaluations of stock composition from in-state fisheries using PBT. A breakdown of stock and cohort proportions will be presented in Byrne et al. (*In Prep*).

Steelhead Sport Fisheries in Idaho

This project represents some of IDFG's first evaluations of stock composition of in-state fisheries using PBT. A complete evaluation can be found in Warren et al. (*In Prep.*).

Chinook Salmon Sport Fishery in Idaho

This effort represents the continuation of IDFG's first implementations of PBT for estimating the stock and age composition of a Chinook Salmon fishery in Idaho. A complete discussion is presented in Sullivan et al. (*In Prep*.).

Age Composition of Broodstocks

One broodstock metric of interest to managers is age composition. Traditionally, coded wire tags are read from a sample of the broodstock and the age composition of the sample is expanded to the entire broodstock. In this case, PBT was used to assign the entire broodstock back to their brood years of origin. PBT samples are already being collected and genotyped to genetically mark the progeny of subsequent broodstock. Determining age composition of the broodstock through PBT is another benefit of implementing the technology.

LITERATURE CITED

Byrne, A., J. Hymer, S. Ellis, R. Dick II, K. Keller, C. A. Steele, J. E. Hess, M. Begay, and T. Miller (*In Prep*). A Genetic Analysis of the Summer Steelhead Stock Composition in the Columbia River and Snake River Tribal and Sport Fisheries from June 2016 to March 31, 2017.

Byrne, A., J. Hymer, S. Ellis, R. Dick, K. Keller, C. A. Steele, M. E. Hess, M. Begay, and T. Miller. 2015. A genetic analysis of the summer steelhead stock composition in the Columbia River and Snake River tribal and sport fisheries. Idaho Department of Fish and Game, Report 15-06, Boise.

Sullivan, C., S. Rosenberger, F. Bohlen. (*In Prep*). 2017 Calendar Year Hatchery Chinook Salmon Report: IPC and LSRCP Monitoring and Evaluation Programs in the State of Idaho.

Warren, Charles D., S. Rosenberger, F. Bohlen. (*In Prep*). 2017 Calendar Year Hatchery Steelhead Report: IPC and LSRCP Monitoring and Evaluation Programs in the State of Idaho.

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TABLES

Table 1. Sample sizes and genotyping completion rate of SY2016 steelhead broodstock. Samples with ≥27 failed PBT SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

	2016		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
Sawtooth	676	663 (98.1%)	96.2%
E.F. Salmon River	30	30 (100.0%)	100.0%
Pahsimeroi	1,096	1,079 (98.4%)	96.9%
Upper Salmon R. B-run	518	515 (99.4%)	98.8%
Oxbow	493	462 (93.7%)	87.7%
Dworshak	1,050	1,025 (97.6%)	95.3%
S.F. Clearwater	522	514 (98.5%)	97.0%
Little Sheep Cr.	133	128 (96.2%)	92.6%
Tucannon R.	55	55 (100.0%)	100.0%
Touchet R.	29	27 (93.1%)	86.7%
Cottonwood Cr.	288	284 (98.6%)	97.2%
Wallowa	478	468 (97.9%)	95.9%
Total	**5,368**	**5,250 (97.8%)**	**95.7%**

Table 2. Sample sizes and genotyping completion rate of SY2016 spring/summer Chinook Salmon broodstock. Samples with ≥30 failed PBT SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

	2016		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
S.F. Clearwater	675	672 (99.6%)	99.1%
Dworshak	1,855	1,747 (94.2%)	88.7%
Kooskia	855	792 (92.6%)	85.8%
Johnson Cr.	67	65 (97.0%)	94.1%
Imnaha	245	244 (99.6%)	99.2%
Catherine Cr.	76	71 (93.4%)	87.3%
Lostine	140	132 (94.3%)	88.9%
Grande Ronde	149	148 (99.3%)	98.7%
Lookingglass Cr.	161	156 (96.9%)	93.9%
Tucannon	125	122 (97.6%)	95.3%
S.F. Salmon	769	765 (99.5%)	99.0%
Nez Perce Tribal FH	573	556 (97.0%)	94.2%
Pahsimeroi	667	666 (99.9%)	99.7%
Powell	345	340 (98.2%)	97.1%
Rapid River	2,004	1,983 (99.0%)	97.9%
Sawtooth	1,053	1,051 (99.8%)	99.6%
Total	**9,759**	**9,510 (97.4%)**	**95.0%**

Table 3. Sample sizes and genotyping completion rate of SY2016 Fall Chinook Salmon broodstock. Samples with ≥30 failed PBT SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

	2016		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
SY2015 Nez Perce	763	742 (97.2%)	94.6%
SY2015 Lyons Ferry	1,865	1,788 (95.9%)	91.9%
Total	**2,628**	**2,530 (96.3%)**	**92.7%**

Table 4. List of loci from the 268 PBT markers that failed to genotype >5% of the steelhead SY2016 samples.

Locus	Fail Rate
OMS00129	6%
OMS00169	6%
Omy_cyp17153	6%
OMGH1PROM1SNP1	7%
Omy_cd59b112	7%
OMS00058	8%
OMS00180	8%
OMS00095	8%
Omy_UT16_2173	8%
Omy_ndk152	9%
Omy_RAD7469149	9%
Omy_colla1525	10%
Omy_RAD6958333	10%
Omy_114315438	11%
OMY1011SNP	12%
Omy_111084526	12%
Omy_118175396	12%
OMS00003	14%
Omy_aldB165	34%
Omy_gsdf291	40%
Omy_118205116	57%
Omy_104569114	70%

Table 5. List of loci from the 298 PBT markers that failed to genotype >5% of the Chinook SY2016 samples.

Locus	Fail Rate
Ots_pigh105	6%
Ots_110495380	6%
Ots_crRAD1044725	6%
Ots_crRAD2654147	7%
Ots_MHC2	8%
Ots_crRAD5753724	8%
Ots_crRAD3615244	10%
Ots_NAML12SNP1	11%
Ots_crRAD5547526	12%
Ots_crRAD4845974	12%
Ots_crRAD3034148	15%
Ots_crRAD5768734	17%
Ots_105401325	18%
Ots_crRAD1752758	25%
Ots_U230563	29%
Ots_crRAD4205848	32%
Ots_129170683	38%

Table 6. List of loci from the 298 PBT markers that failed to genotype >5% of the Fall Chinook SY2016 samples.

Locus	Fail Rate
Ots_110689218	6%
Ots_pigh105	6%
Ots_u0757120	6%
Ots_104048194	6%
Ots_12830257	7%
Ots_112208722	9%
Ots_crRAD1044725	9%
Ots_crRAD3615244	9%
Ots_Thio	10%
Ots_MHC2	10%
Ots_Est1363	10%
Ots_crRAD5753724	10%
Ots_108735302	11%
Ots_MHC1	13%
Ots_sept978	13%
Ots_crRAD506127	15%
Ots_GPDH338	16%
Ots_u202161	17%
Ots_crRAD1752758	18%
Ots_crRAD3034148	18%
Ots_crRAD3531366	18%
Ots_crRAD3607229	18%
Ots_U230563	20%
Ots_crRAD5547526	20%
Ots_crRAD4205848	23%
Ots_129170683	28%
Ots_MetA	52%

Table 7. Ranked estimates of null allele (NullFreq) frequencies > 5% from the 268 PBT loci and the number of hatchery steelhead populations that exhibit a deficiency of heterozygotes (H_ED) in SY2016.

SNP Name	H_ED	NullFreq
Omy_RAD4795551	3	5.6%
Omy_impa155	3	6.1%
Omy_b9164	3	6.7%
Omy_97954618	3	7.1%
OMS00087	3	9.6%
OMGH1PROM1SNP1	3	16.6%
OMS00180	4	5.3%
Omy_aromat280	4	5.7%
Omy_cd59b112	4	6.5%
Omy_RAD6958333	4	7.7%
OMS00058	5	5.3%
Omy_crb106	5	6.0%
Omy_IL1b163	5	6.2%
Omy_128996481	5	11.4%
OMS00169	6	5.2%
Omy_GH1P1_2	6	5.2%
OMS00129	6	11.5%
Omy_96222125	6	16.6%
OMS00095	6	30.5%
Omy_RAD7469149	7	5.1%
Omy_114315438	7	6.2%
Omy_114976223	7	10.9%
OMY1011SNP	7	11.3%
Omy_cyp17153	7	16.5%
Omy_RAD6621858	8	11.2%
Omy_gsdf291	8	52.8%
Omy_aldB165	8	59.1%

Table 8. Ranked estimates of null allele (NullFreq) frequencies >5% from the 298 PBT loci and the number of hatchery Chinook populations that exhibit a deficiency of heterozygotes (H_ED) in SY2016.

SNP Name	H_ED	NullFreq
Ots_crRAD7651228	5	7.7%
Ots_crRAD2654147	5	5.8%
Ots_OTALDBINT1SNP1	5	5.8%
Ots_crRAD1044725	5	5.4%
Ots_MHC2	7	10.8%
Ots_U512134	7	10.0%
Ots_NAML12SNP1	9	7.8%
Ots_crRAD3034148	10	11.7%
Ots_crRAD6152371	10	9.7%
Ots_105401325	11	34.4%
Ots_MHC1	11	20.7%
Ots_GPDH338	11	19.3%
Ots_U230563	11	14.2%
Ots_IsoT	11	12.0%
Ots_crRAD29221	11	5.1%

Table 9. Ranked estimates of null allele (NullFreq) frequencies >5% from the 298 PBT loci and the number of hatchery Fall Chinook populations that exhibit a deficiency of heterozygotes (H_ED) in SY2016.

SNP Name	H_ED	NullFreq
Ots_MetA	2	72.5%
Ots_crRAD506127	2	38.3%
Ots_MHC1	2	37.3%
Ots_GPDH338	2	35.2%
Ots_crRAD3034148	2	15.7%
Ots_sept978	2	15.5%
Ots_u202161	2	14.9%
Ots_crRAD3607229	2	14.2%
Ots_104048194	2	11.3%
Ots_11230143	2	10.9%
Ots_101704143	2	8.7%
Ots_NOD1	2	8.6%
Ots_u0725325	2	8.6%
Ots_crRAD4205848	2	8.5%
Ots_127760569	2	8.3%
Ots_crRAD29221	2	7.7%
Ots_111312435	1	7.1%
Ots_U230563	2	6.9%
Ots_MHC2	1	6.1%
Ots_crRAD5547526	2	6.0%
Ots_hnRNPL533	1	5.7%
Ots_crRAD7651228	1	5.4%
Ots_129170683	2	5.3%
Ots_afmid196	1	5.2%

Table 10. Results of comparisons between phenotypic sex and genetically determined sex using the sex-specific assay for SY2016 steelhead (Omy1_2SEXY).

	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotypes	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Sawtooth	676	39 (5.8%)	637 (94.2%)	629 (98.7%)	8 (1.3%)	8	0	338 (50.0%)	338 (50.0%)
E.F. Salmon R.	30	1 (3.3%)	29 (96.7%)	29 (100%)	0 (0.0%)	0	0	15 (50.0%)	15 (50.0%)
Pahsimeroi	1,096	71 (6.5%)	1,025 (93.5%)	1,023 (99.8%)	2 (0.2%)	1	1	548 (50.0%)	548 (50.5%)
Up. Sal. R. B-run	518	27 (5.2%)	491 (94.8%)	490 (99.8%)	1 (0.2%)	0	1	217 (41.9%)	301 (58.1%)
Oxbow	493	50 (10.1%)	443 (89.9%)	432 (97.5%)	11 (2.5%)	8	3	265 (53.8%)	228 (46.2%)
Dworshak	1,050	26 (2.5%)	1,024 (97.5%)	1,024 (100%)	0 (0.0%)	0	0	461 (43.9%)	589 (56.1%)
S.F. Clearwater	522	8 (1.5%)	514 (98.5%)	514 (100%)	0 (0.0%)	0	0	236 (45.2%)	286 (54.8%)
Little Sheep Cr.*	133	4 (3.0%)	129 (97.0%)	129 (100%)	0 (0.0%)	0	0	65 (48.9%)	66 (49.6%)
Tucannon R.	55	2 (3.6%)	53 (96.4%)	53 (100%)	0 (0.0%)	0	0	26 (47.3%)	29 (52.7%)
Touchet R.	29	4 (13.8%)	25 (86.2%)	25 (100%)	0 (0.0%)	0	0	13 (44.8%)	16 (55.2%)
Cottonwood Cr.	288	15 (5.2%)	273 (94.8%)	250 (91.6%)	23 (8.4%)	13	10	144 (50.0%)	144 (50.0%)
Wallowa *	478	11 (2.3%)	467 (97.7%)	466 (99.8%)	1 (0.2%)	0	1	238 (49.8%)	238 (49.8%)
Total	**5,368**	**258 (4.8%)**	**5,110 (95.2%)**	**5,064 (99.1%)**	**46 (0.9%)**	**30**	**16**	**2,566 (47.8%)**	**2,798 (52.1%)**

* Phenotypic sex not known for some samples

Table 11. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for spring/summer Chinook Salmon (Ots_SEXY3-1) from the SY2016 broodstocks.

	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotypes	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
S.F. Clearwater	675	0 (0.0%)	675 (100.0%)	675 (100%)	0 (0.0%)	0	0	292 (43.3%)	383 (56.7%)
Dworshak	1,855	59 (3.2%)	1,796 (96.8%)	1,796 (100%)	0 (0.0%)	0	0	810 (43.7%)	1,045 (56.3%)
Kooskia	855	59 (6.9%)	796 (93.1%)	794 (99.7%)	2 (0.3%)	0	2	367 (42.9%)	488 (57.1%)
Johnson Cr.	67	2 (3.0%)	65 (97.0%)	64 (98.5%)	1 (1.5%)	0	1	35 (52.2%)	32 (47.8%)
Imnaha	245	1 (0.4%)	244 (99.6%)	243 (99.6%)	1 (0.4%)	1	0	110 (44.9%)	135 (55.1%)
Catherine Cr.	76	3 (3.9%)	73 (96.1%)	73 (100%)	0 (0.0%)	0	0	38 (50.0%)	38 (50.0%)
Lostine	140	3 (2.1%)	137 (97.9%)	135 (98.5%)	2 (1.5%)	0	2	66 (47.1%)	74 (52.9%)
Grande Ronde	149	0 (0.0%)	149 (100.0%)	149 (100%)	0 (0.0%)	0	0	79 (53.0%)	70 (47.0%)
Lookingglass Cr. *	161	4 (2.5%)	157 (97.5%)	153 (97.5%)	1 (0.6%)	0	1	81 (50.3%)	77 (47.8%)
Tucannon *	125	1 (0.8%)	124 (99.2%)	122 (100%)	0 (0.0%)	0	0	48 (38.4%)	75 (60.0%)
S.F. Salmon	769	7 (0.9%)	762 (99.1%)	761 (99.9%)	1 (0.1%)	1	0	373 (48.5%)	396 (51.5%)
Nez Perce F.H.	573	8 (1.4%)	565 (98.6%)	555 (98.2%)	10 (1.8%)	1	9	235 (41.0%)	338 (59.0%)
Pahsimeroi	667	2 (0.3%)	665 (99.7%)	665 (100%)	0 (0.0%)	0	0	292 (43.8%)	375 (56.2%)
Powell	345	2 (0.6%)	343 (99.4%)	343 (100%)	0 (0.0%)	0	0	174 (50.4%)	171 (49.6%)
Rapid River	2,004	40 (2.0%)	1,964 (98.0%)	1,999 (99.9%)	3 (0.1%)	0	3	992 (49.5%)	1,012 (50.5%)
Sawtooth	1,053	3 (0.4%)	1,050 (99.6%)	1,050 (100%)	0 (0.0%)	0	0	538 (51.1%)	515 (48.9%)
Total	**9,759**	**194 (2.0%)**	**9,565 (98.0%)**	**9,577 (99.7%)**	**21 (0.2%)**	**3**	**18**	**4,530 (46.4%)**	**5,224 (53.5%)**

* Phenotypic sex not known for some samples

Table 12. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for Fall Chinook Salmon (Ots_SEXY3-1) from the SY2016 broodstocks.

Stock	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotypes	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
SY2016 Nez Perce	763	21 (2.8%)	742 (97.2%)	763 (100%)	0 (0.0%)	0	0	299 (40.3%)	443 (59.7%)
SY2016 Lyons Ferry *	1,865	18 (1.0%)	1,847 (99.5%)	1,836 (99.5%)	9 (0.5%)	2	7	646 (35.0%)	1,199 (65.0%)
Total	**2,628**	**39 (1.5%)**	**2,589 (98.5%)**	**2,599 (99.7%)**	**9 (0.3%)**	**2**	**7**	**945 (36.5%)**	**1,642 (63.5%)**

* Phenotypic sex not known for some samples

Table 13. Average observed and expected heterozygosity with associated standard deviation of hatchery steelhead stocks for SY2016.

Stock	Avg. het. (Obs)	SE	Avg. het. (Exp)	SE
Sawtooth	0.25	0.01	0.26	0.01
EF Salmon	0.26	0.01	0.26	0.01
Upper Salmon B	0.25	0.01	0.24	0.01
Oxbow	0.27	0.01	0.27	0.01
Pahsimeroi	0.26	0.01	0.27	0.01
Dworshak	0.24	0.01	0.25	0.01
SF Clearwater	0.25	0.01	0.25	0.01
Little Sheep Ck	0.25	0.01	0.25	0.01
Tucannon	0.26	0.01	0.27	0.01
Touchet	0.25	0.01	0.26	0.01
Cottonwood Ck	0.26	0.01	0.26	0.01
Wallowa	0.26	0.01	0.27	0.01

Table 14. Average observed and expected heterozygosity with associated standard deviation of hatchery spring/summer Chinook Salmon stocks in SY2016.

Stock	Avg. het. (Obs)	SE	Avg. het. (Exp)	SE
S.F. Clearwater	0.21	0.01	0.21	0.01
Dworshak	0.21	0.01	0.22	0.01
Kooskia	0.21	0.01	0.21	0.01
Johnson Cr.	0.20	0.01	0.20	0.01
Imnaha	0.21	0.01	0.21	0.01
Catherine Cr.	0.22	0.01	0.22	0.01
Lostine	0.21	0.01	0.21	0.01
Grande Ronde	0.21	0.01	0.22	0.01
Lookingglass Cr.	0.22	0.01	0.22	0.01
Tucannon	0.22	0.01	0.22	0.01
S.F. Salmon	0.20	0.01	0.20	0.01
Nez Perce Tribal FH	0.21	0.01	0.22	0.01
Pahsimeroi	0.20	0.01	0.20	0.01
Powell	0.20	0.01	0.20	0.01
Rapid River	0.20	0.01	0.21	0.01
Sawtooth	0.19	0.01	0.20	0.01

Table 15. Average observed and expected heterozygosity with associated standard deviation of hatchery Fall Chinook Salmon stocks in SY2016.

Stock	Avg. het. (Obs)	SE	Avg. het. (Exp)	SE
SY2016 Nez Perce	0.25	0.01	0.26	0.01
SY2016 Lyons Ferry	0.25	0.01	0.26	0.01

Table 16. Population structure (F_{ST}) (below diagonal) among steelhead hatchery stocks sampled in SY2016. Asterisks (*) indicate that the genotypic differentiation (exact G test) were highly significant (above diagonal).

Population	Cottonwood	Dworshak	EF Salmon	Little Sheep	Oxbow	Pahsimeroi	Sawtooth	SF Clearwater	Touchet	Tucannon	Up Sal B
Dworshak	0.051	---	*	*	*	*	*	*	*	*	*
EF Salmon	0.026	0.037	---	*	*	*	*	*	*	*	*
Oxbow	0.024	0.059	0.032	---	*	*	*	*	*	*	*
Little Sheep	0.021	0.053	0.018	0.021	---	*	*	*	*	*	*
Pahsimeroi	0.022	0.051	0.016	0.022	0.008	---	*	*	*	*	*
Sawtooth	0.022	0.051	0.018	0.025	0.010	0.007	---	*	*	*	*
SF Clearwater	0.050	0.001	0.035	0.057	0.051	0.049	0.050	---	*	*	*
Touchet	0.023	0.052	0.032	0.025	0.025	0.025	0.027	0.050	---	*	*
Tucannon	0.014	0.041	0.014	0.018	0.017	0.015	0.016	0.038	0.003	---	*
Up Sal B	0.056	0.020	0.045	0.062	0.053	0.053	0.051	0.019	0.055	0.046	---
Wallowa	0.008	0.045	0.025	0.024	0.019	0.020	0.020	0.044	0.019	0.012	0.049

Table 17. Population structure (F_{ST}) (below diagonal) among spring/summer Chinook Salmon hatchery stocks sampled in SY2016. Asterisks (*) indicate that the genotypic differentiation (exact G test) were highly significant (above diagonal).

	Cath.Cr.	Dwor.	Gr. Ronde	Imn.	Johns. Cr.	Koos.	Looking.	Lost.	NPTFH	Pahs.	Pow.	Rap. R.	Sawt.	S.F. Clear.	S.F. Sal.
Dworshak	0.010	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.013	0.011	---	*	*	*	*	*	*	*	*	*	*	*	*
Imnaha	0.012	0.010	0.013	---	*	*	*	*	*	*	*	*	*	*	*
Johnson Cr.	0.027	0.024	0.025	0.026	---	*	*	*	*	*	*	*	*	*	*
Kooskia	0.013	0.001	0.015	0.013	0.026	---	*	*	*	*	*	*	*	*	*
Lookingglass	0.006	0.009	0.011	0.011	0.026	0.013	---	*	*	*	*	*	*	*	*
Lostine	0.022	0.024	0.028	0.022	0.042	0.028	0.028	---	*	*	*	*	*	*	*
Nez Perce TFH	0.011	0.001	0.010	0.010	0.024	0.003	0.009	0.025	---	*	*	*	*	*	*
Pahsimeroi	0.033	0.030	0.037	0.033	0.037	0.033	0.030	0.037	0.031	---	*	*	*	*	*
Powell	0.022	0.017	0.022	0.019	0.014	0.019	0.022	0.026	0.018	0.029	---	*	*	*	*
Rapid River	0.014	0.012	0.014	0.012	0.029	0.017	0.012	0.028	0.010	0.035	0.024	---	*	*	*
Sawtooth	0.030	0.026	0.032	0.032	0.031	0.029	0.027	0.040	0.027	0.023	0.024	0.030	---	*	*
S.F. Clearwater	0.013	0.004	0.013	0.013	0.030	0.006	0.011	0.026	0.004	0.030	0.021	0.011	0.027	---	*
S.F. Salmon	0.023	0.017	0.022	0.020	0.014	0.019	0.022	0.028	0.018	0.028	0.002	0.024	0.024	0.021	---
Tucannon	0.022	0.022	0.026	0.028	0.038	0.022	0.028	0.033	0.023	0.047	0.030	0.038	0.049	0.025	0.033

Table 18. Estimates of effective population size (N_E) and 95% confidence intervals for steelhead hatchery stocks in SY2016.

Stock	N_E	95% CI
Sawtooth	235.1	227.7 – 242.9
E.F. Salmon River	342.5	196.7 – 1213.3
Pahsimeroi	180.4	176.0 – 184.8
Upper Salmon R. B-run	36.2	35.4 – 37.1
Oxbow	122.6	119.0 – 126.3
Dworshak	268.7	259.8 – 277.9
S.F. Clearwater	132.1	127.8 – 136.5
Little Sheep Cr.	163.4	149.9 – 179.1
Tucannon R.	507.7	332.9 – 1035.7
Touchet R.	150.5	108.8 – 239.3
Cottonwood Cr.	70.5	68.4 – 72.7
Wallowa	202.3	194.6– 210.4

Table 19. Estimates of effective population size and 95% confidence intervals for SY2016 spring/summer Chinook Salmon hatchery stocks.

Stock	Ne	95% CI
S.F. Clearwater	208.1	201.6 – 214.9
Dworshak	515.8	500.1 – 532.1
Kooskia	321.7	310.4 – 333.5
Johnson Cr.	376.1	275.6 – 581.6
Imnaha	374.0	340.7 – 413.2
Catherine Cr.	181.1	156.6 – 213.5
Lostine	153.5	141.9 – 166.9
Grande Ronde	85.7	81.8 – 90.0
Lookingglass Cr.	125.7	118.6 – 133.5
Tucannon	265.6	235.0 – 304.1
S.F. Salmon	202.4	196.2 – 208.8
Nez Perce Tribal FH	264.4	254.1 – 275.3
Pahsimeroi	201.3	194.7 – 208.2
Powell	106.6	103 – 110.4
Rapid River	429.2	417.4 – 441.4
Sawtooth	299.0	288.8 – 309.6

Table 20. Estimates of effective population size and 95% confidence intervals for Fall Chinook Salmon hatchery stocks.

Stock	Ne	95% CI
SY2016 Nez Perce	1,571.5	1,412.4 – 1,765.8
SY2016 Lyons Ferry	1,432.8	1,363.8 – 1,507.3

FIGURES

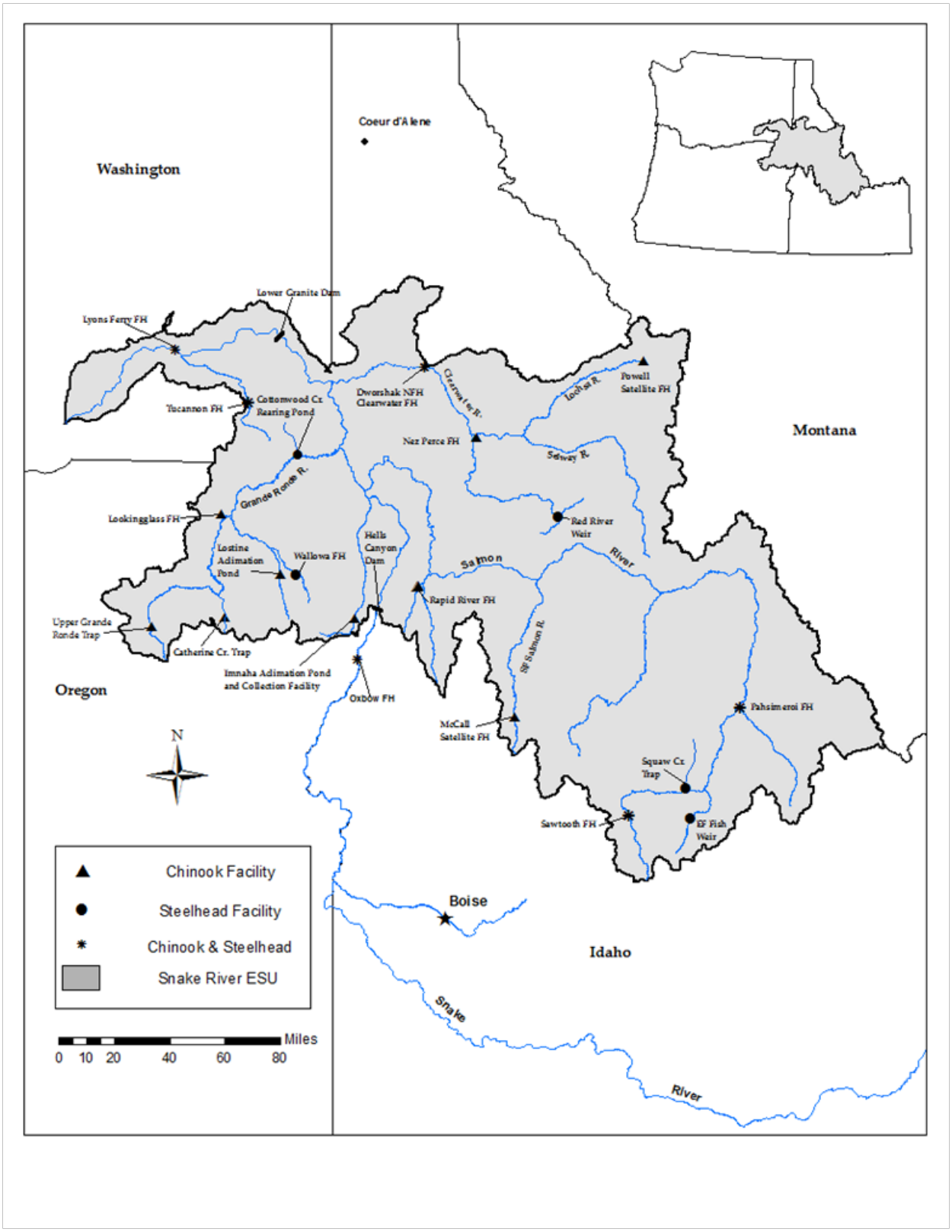

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

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