FISHERY RESEARCH

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

ANNUAL PROGRESS REPORT
January 1, 2016 — December 31, 2016

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IDFG Report Number 17-01
January 2017

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Project Progress Report

2016 Annual Report

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Project Number 2010-031-00
Contract Number 69574

IDFG Report Number 17-01
January 2017

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ABSTRACT

This report summarizes the progress in the continuing 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, 2016 to December 31, 2016 that focused on the feasibility of developing and implementing PBT in the Snake River basin: Objective 1) annual sampling of hatchery broodstock, 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 broodstock (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 the PBT single nucleotide polymorphism (SNPs) 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) 2015 (Objective 2). We then use the data generated from the broodstock baselines to provide parentage analysis for a variety of management 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 over 40 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 is 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 tens 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. 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 sample size. By genotyping all parental broodstock, every juvenile is genetically "tagged."

While theoretically appealing (Anderson and Garza 2005; 2006), PBT technology still needs to be empirically tested and validated. Over the last several years, several committees and science review groups have recommended that two or more large-scale evaluations of the technology be performed (PFMC 2008; PSC 2008; ISRP/ISAB 2009).

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 monitoring 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, the PBT baselines were used to determine: 1) Origin of samples from sport fisheries in Columbia River Zones 1–6 during migration year 2015 (SY2016), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2015 (SY2016). 3) Origin of samples from sport fisheries in the lower Snake River in migration year 2015 (SY2016), 4) Origin of samples from various sport fisheries in Idaho in migration year 2014 (SY2015), 5) Parentage of SY2016 Upper Salmon B-run broodstock for real-time management of spawning, 6) Age composition and origin of the SY2015 broodstocks, and 7) stock composition of returning adults during SY2015 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2015), 2) Age composition and origin of SY2015 broodstocks, 3) Comparison of PIT- and PBT-derived estimates of abundance for particular release groups at Lower Granite Dam, 4) Evaluation of adult returns originating from experimental rearing conditions at Dworshak National Fish Hatchery.

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.

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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 broodstock 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 broodstock 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, but not included in summary tables.

Once the samples are 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 2016, we have collected and inventoried approximately 5,250 genetic samples from the steelhead broodstock (Table 1) spawned in the Snake River basin during spawn year (SY) 2015, and approximately 10,000 samples (Table 2) from spring/summer Chinook Salmon broodstock spawned in the Snake River basin during SY2015. We also report on Fall Chinook collected from the Lyons Ferry and Nez Perce Tribal Fish hatcheries for SY2015 (N = ~2,500; 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 but is also not recorded at some Snake River basin hatcheries simply because it is impractical and not part of their standard operating procedure.

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.

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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 SY2015.

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 2015. 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 sixth 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 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 (http://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 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 $\alpha = 0.05$.

Concordance Between Genotyping Platforms

Concordance of SNP genotypes between the Fluidigm platform and the new GT-seq protocols used on the Illumina NextSeq were compared. Concordance between the GT-seq and Fuidigm platforms was evaluated using 380 samples of Chinook. Each sample was genotyped at the PBT panel (96 loci) and at the GSI panel (96 loci) on each platform for a combined total of 72,960 genotypes generated for concordance.

Sex Markers

The accuracy of the sex-determining SNP assay for steelhead and Chinook Salmon was evaluated for hatchery stocks spawned in SY2014; 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 4, 5, and 6).

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%.

Testing Power of Sp/Su Chinook PBT Panel on Fall Chinook

Because the Chinook Salmon PBT marker set was developed on spring/summer Chinook Salmon stocks, we tested the power of these markers in identifying hatchery-origin Fall Chinook Salmon. For this test we simulated 1,000 offspring from each of the SY2011–SY2015 Lyon's Ferry and Nez Perce fish hatchery brood stocks. We also simulated 1,000 offspring from each of the SY2014–SY2016 collections of wild Fall Chinook Salmon sampled at the adult trapping facility at Lower Granite Dam. Data were simulated by performing 1x1 crosses within each brood. Females were simulated to spawn a single time, whereas males could be resampled from the population. To mimic variation in reproductive success we modeled whether or not a female produced offspring as a Bernoulli trial with the probability of success equal to 0.8. If a female was determined to have successfully reproduced her number of offspring was modeled using a Poisson distribution with a mean of 3. Alleles for simulated offspring were randomly selected from each parent at each locus. Offspring were modeled as having a missing genotype at any locus if one of the parents did not genotype at that same locus. A locus specific genotyping error rate of 0.02/locus was also introduced. If a locus was selected to have a genotyping error, the first allele at that locus was replaced with the second allele thereby creating an incorrect homozygous genotype for a locus that should have been heterozygous.

One of the main management goals for Fall Chinook sampled at Lower Granite Dam is to determine the proportion of hatchery-origin and wild-origin fish. We determined a potential false-negative error rate for Fall Chinook by summarizing the number of simulated hatchery-origin offspring that failed to assign when their parents were present in the baseline. We also determined a false positive rate by summarizing the number of simulated wild-origin offspring that incorrectly assigned to hatchery-origin parents as well as summarizing the number of simulated hatchery-origin offspring that incorrectly assigned to the wrong hatchery-origin parents. For assessing the false positive error rate we removed the parents in the dataset for all simulated wild-origin offspring from Lower Granite Dam broodstocks (SY2014–SY2016), as well as parents for all simulated hatchery-origin offspring from SY2014–SY2015 Lyon's Ferry and SY2011–SY2013 Nez Perce fish hatchery broodstocks. For analysis we also removed any individual that failed to genotype at 10 or more loci, and any duplicate samples that were similar at 90 of the 95 loci in

the PBT panel. The resulting simulated offspring were assigned parents using the program *SNPPIT* (Anderson and Garza 2006), with parent gender not specified *a priori*. Individual parentage assignments were discarded whose log odds of assignment (LOD score) were <14 and whose identified parents were of the same gender.

RESULTS

Concordance Between Genotyping Platforms

For Chinook, within the PBT panel of SNPs there were 36,480 genotype comparisons of which 308 comparisons received a 'No Call' designation either from Fluidigm or GT-seq. Of the remaining 36,172 comparisons, there were 226 discrepancies resulting in 99.38% concordance between Fluidigm and GT-seq. Of the 226 discrepancies, 172 were at the sex marker (76.1%). All were cases in which samples of phenotypic males were correctly called 'Male' by Fluidigm but incorrectly called 'Female' by GT-seq. It was determined that the SNP assay for the sex marker was inadvertently excluded from the pooled assay mix used in the preparation of the GT-seq library. This resulted in GT-seq erroneously identifying all samples as 'Female'. If the discrepancies of the sex marker are ignored the remaining 54 discrepancies resulted in 99.85% concordance of the PBT panel between Fluidigm and GT-seq.

Within the GSI panel of SNPs there were 36,480 genotype comparisons of which there were 241 comparisons that received a 'No Call' designation either from Fluidigm or GT-seq. Of the remaining 36,239 comparisons there were 131 discrepancies resulting in 99.64% concordance between Fluidigm and GT-seq. Of the 131 discrepancies, 109 were at Ots_111681-657 (83.2%). This marker was found to be incorrectly scored on the Fluidigm scatterplot. When this marker was scored correctly in Fluidigm and reanalyzed there were no discrepancies at this marker. When these discrepancies at Ots_111681-657 are corrected then the remaining 22 discrepancies result in 99.94% concordance of the GSI panel between Fluidigm and GT-seq.

Overall, when simultaneously considering concordance at both the PBT and GSI panels, there were 76 unresolved discrepancies among 72,411 comparisons resulting in 99.9% concordance between GT-seq and Fluidigm for Chinook SNPs.

Completion Rate and Missing Data

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

For steelhead SY2015, all 5,253 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 5,253 samples, 5,092 (96.9%) were genotyped with an acceptable level of missing data (Table 4). In this final SY2015 PBT baseline comprising the remaining 5,092 samples, there were just 3,246 missing genotypes due to SNP failure out of a possible 483,740 genotypes. This resulted in missing data for just 0.7% of the genotypes.

For spring/summer Chinook Salmon SY2014, all 10,056 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 10,056 samples, 9,824 (97.7%) were genotyped with an acceptable level of missing data (Table 5). In this final SY2015 PBT baseline comprising the remaining 9,824 samples, there were just 3,006 missing genotypes

due to SNP failure out of a possible 933,280 genotypes. This resulted in missing data for just 0.3% of the genotypes.

For Fall Chinook Salmon SY2015, all 2,492 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 2,492 samples, 2,421 (97.2%) were genotyped with an acceptable level of missing data (Table 6). In this final SY2015 PBT baseline comprising the remaining 2,492 samples, there were just 643 missing genotypes due to SNP failure out of a possible 236,740 genotypes. This resulted in missing data for just 0.3% of the genotypes.

Poor Performing Loci

Of the samples that genotyped with <10 missing SNPs, most SNPs had very high genotyping success. For SY2015 steelhead, only three loci failed to genotype at >3% of samples (Omy_105385-406 = 26.3%; Omy_aldB-165 = 11.2%; Omy_114315-438 = 4.9%). For SY2015 spring/summer Chinook Salmon, there were two loci that failed at >3% of the samples (Ots_MHC1 = 3.31%, and Ots_pigh-105 = 6.44%). For SY2015 Fall Chinook, there was one locus that failed at >3% of the samples (Ots_MHC1 = 11.9%).

Error Rate (Quality Control)

For steelhead SY2015, a subset of 367 samples were rerun at the 96 PBT loci and the resulting 35,232 genotypes were checked for discrepancies. Of these genotypes, there were 1,021 discrepancies, excluding any SNP failures in either the original or the rerun genotype, which resulted in an error rate of 2.9%. When the location of these discrepancies was plotted on a well map it showed that they were concentrated in a series of eight contiguous positions. Upon investigation, it was determined that cross-contamination of the i5 barcode occurred during library preparation resulting in inconsistent genotypes in the subset of samples. The issue was resolved by regenotyping affected samples in the baseline. When the discrepancies in these well positions are accounted for then resulting genotyping error rate is <0.01%.

For Chinook Salmon SY2015, a subset of 146 samples representing all extraction plates were rerun at 299 loci and checked for discrepancies. This resulted in 43,654 rerun genotypes being compared to the original genotypes. Of these genotypes, 386 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 43,268 genotypes with 93 discrepancies between the original and samples and a genotyping error rate of 0.21%.

Null Alleles

For steelhead SY2015, 93 of the 192 loci were found to exhibit a deficiency of heterozygotes in at least one population, but only eight of these loci had null frequencies estimated >5% (Table 7).

For spring/summer Chinook Salmon SY2014, 139 of the 299 loci were found to exhibit a deficiency of heterozygotes in at least one population, but only ten locus had a null frequency estimated >5% (Table 8).

For Fall Chinook Salmon SY2015, 64 of the 299 PBT loci were found to exhibit a deficiency of heterozygotes in at least one population. Sixteen had a null frequency estimated >5% (Table 9).

Average Heterozygosity

Levels of observed heterozygosity within steelhead broodstocks was ~0.29 for all hatcheries broodstocks (Table 13). For Chinook Salmon, levels of observed heterozygosity was ~0.21 in spring/summer stocks (Table 14) and ~0.25 in Fall stocks (Table 15).

Population Structure

Pairwise F_{ST} was calculated among the steelhead SY2015 hatchery broodstock (Table 16). Values ranged from a low of 0.001 between the Dworshak and SF Clearwater stocks, and a high of 0.036 between the Upper Salmon B-run and Little Sheep Creek stocks. All tests of genetic differentiation among stocks were significant except for the pairwise comparison of 0.003 between the Touchet and Tucannon stocks.

For spring/summer Chinook Salmon SY2015 pairwise F_{ST} values ranged from a low of 0.003 between the Dworshak stock and the S.F. Clearwater and Kooskia stocks a high of 0.054 between the Pahsimeroi and Tucannon stocks (Table 17). All tests of genetic differentiation among stocks were significant. Differentiation among the two Fall Chinook salmon stocks was very low (F_{ST} = 0.0001) albeit significant ($P < 0.01$).

Effective Population Size

Effective population size (N_e) for steelhead hatchery broodstock in SY2015 ranged from a low of 42.7 for the Cottonwood Cr. broodstock to a high of 234.2 for Dworshak National Fish Hatchery (DNFH) production broodstock (Table 18). The point estimates for EF Salmon, Touchet, and Tucannon broodstocks were not considered reliable given their wide or infinite CIs. Infinite estimates are an artifact of a sample size that is too small, such that the genetic signal in the data is driven by sample error rather than genetic drift (Waples and Do 2010).

Effective population size for spring/summer Chinook Salmon hatchery broodstock in SY2015 ranged from a low of 60.3 for Grande Ronde to a high of 474.0 for Rapid River (Table 19). The point estimates for Tucannon and Johnson creek broodstocks were not considered reliable given their wide or infinite CIs. Effective population size for the two Fall Chinook Salmon hatchery broodstocks were large with the Nez Perce stock estimated at 1,009.7 and the Lyons Ferry stock estimated at 1,143.0 (Table 20).

Sex Markers

The sex-specific assay for steelhead matched phenotypic sex in 99.0% of the samples (Table 10). Instances in which genetically-determined sex did not correspond to the phenotypic sex were nearly split between cases in which phenotypic females were misidentified as males and cases in which phenotypic males were misidentified as female. The assay either failed to genotype or provided ambiguous results for 2.7% of the samples.

The sex-specific assay for spring/summer Chinook Salmon matched phenotypic sex in 99.9% of the samples (Table 11). The four discrepancies were split equally between phenotypic males genetically identified as female and phenotypic females genetically identified as males. The assay inadvertently was not genotyped on approximately 2000 samples, which when combined with ambiguous results, or samples that failed to genotype, resulted in missing data for 24.7% of samples.

The sex-specific assay for Fall Chinook Salmon matched phenotypic sex in 99.7% of the samples (Table 12). The eight discrepancies were split equally between phenotypic males genetically identified as female and phenotypic females genetically identified as males. The assay either failed to genotype or provided ambiguous results for 2.0% of the samples.

Tagging Rate

Overall tagging rates were very high for steelhead (Table 4), spring/summer Chinook Salmon (Table 5), and Fall Chinook Salmon stocks (Table 6). All stock-level tag rates were greater than or approached 90% in steelhead broodstocks. Stock-level tag rates were greater than 90% in 13 of the 16 spring/summer Chinook salmon broodstocks. The three stocks that exhibited less than a 90% tag rate were: Imnaha = 83.4%, Lostine = 58.5%, and Tucannon = 57.8%. These three stocks represent just 5% of the total samples of spring/summer Chinook salmon within the Snake River basin. For Nez Perce and Lyons Ferry Fall Chinook hatchery stocks, all were tagged at 90% or greater for SY2015.

Testing Power of Sp/Su Chinook PBT Panel on Fall Chinook

A total of 12,359 simulated offspring from the 13 parental broodstocks had genotypes at greater than 85 loci. Of these 12,359 individuals, 4,524 had parents included in the analysis (Table 21). We successfully identified the parents of 4,468 (98.8%) simulated fish, and failed to identify parents for 44 (1.0%) individuals (Table 21). In addition, we incorrectly identified parents for 12 (0.3%) individuals whose parents were included in the analysis (Table 21). Of the 7,835 individuals without parents in the analysis, we falsely identified parentage for 82 (1.0%) individuals (Table 22).

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.

Concordance

The high concordance in genotypes (99.9%) reiterate initial assessments of GT-seq in that it produces equivalent genotypes as Fluidigm and that its automated scoring eliminates the subjectivity sometimes encountered during manual scoring of Fluidigm scatterplots.

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 eight 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 the first time 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. This year we expanded the panel of PBT SNPs to include loci found in the GSI panel. The genotyping of these 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 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 >3% 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. Pahsimeroi 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 (43.7), 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 for each species was ~95%.

Testing Power of Chinook PBT Panel on Fall Chinook

Despite the Chinook PBT marker panel being developed on spring/summer stocks we observed high power to correctly assign parentage in Fall Chinook Salmon. The observed false

negative rate (failing to assign an offspring when its parents are in the baseline) of 1.0% and the observed false positive rate (assigning an offspring to incorrect parents) of 0.76% in our data are within the ranges observed by Anderson (2010) during assessments of the accuracy of PBT in large-scale datasets. Regardless of these encouraging results, we have decided to regenotype the Fall Chinook baseline at the current complement of 298 SNPs are which will increase the power and accuracy of parentage assignments in Fall Chinook.

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SECTION 3: UTILIZATION OF PBT TO PROVIDE PARENTAL ASSIGNMENTS

INTRODUCTION

Several years' worth of broodstock genotypes have now been collected for both steelhead and spring/summer Chinook Salmon. 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, the PBT baselines were used to determine: 1) Origin of samples from sport fisheries in Columbia River Zones 1–6 during migration year 2015 (SY2016), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2015 (SY2016). 3) Origin of samples from sport fisheries in the lower Snake River in migration year 2015 (SY2016), 4) Origin of samples from various sport fisheries in Idaho in migration year 2014 (SY2015), 5) Parentage of SY2016 Upper Salmon B-run broodstock for real-time management of spawning, 6) Age composition and origin of the SY2015 broodstocks, and 7) stock composition of returning adults during SY2015 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2015), 2) Age composition and origin of SY2015 broodstocks, 3) Evaluation of adult returns originating from experimental rearing conditions at Dworshak National Fish Hatchery.

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 (river Zones 1–6) in 2015 (SY2016). A total of 1,086 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. (2016).

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) 2015 (e.g. spawn year 2016). A total of 2,253 samples from steelhead were analyzed. An example of the methods used for this annual sampling can be found in Byrne et al. (2016).

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 452 samples were processed for PBT assignment. An example of the methods used for this annual sampling can be found in Byrne et al. (2016).

Steelhead Sport Fisheries in Idaho

IDFG collected samples of steelhead harvested in the SY2015 sport fishery from various river systems including the Clearwater and Salmon. A total of 2,418 samples were processed for PBT assignment. A more detailed description of this project is in Warren et al. (*In prep*).

Broodstock Management of Upper Salmon B-run Steelhead

To minimize inbreeding during spawning of the upper Salmon B-run broodstock, all SY2016 broodstock were sampled at spawning. Genotyping of the samples was expedited to provide parentage results. Parentage results were used to identify inbred spawn crosses propagated by broodstock that shared one or both parents.

Age Composition of SY2015 Steelhead Broodstock

PBT was used to determine age composition of steelhead broodstocks in Idaho by assigning the SY2015 broodstocks back to all previously sampled broodstocks, thereby identifying the age of each fish. A total of 5,856 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 2015 and spring of 2016 (Warren et al. *In prep*) and 1,777 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 SY2015. A total of 1,773 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 SY2015 Chinook Salmon Broodstock

PBT was used to determine age composition of Chinook Salmon broodstocks in Idaho by assigning the SY2015 broodstocks back to previously sampled broodstocks, thereby identifying the age of each fish. A total of 10,142 hatchery-origin broodstock samples from ten different broodstocks were analyzed with PBT.

Evaluation of Increased Rearing Density to Increase Adult Returns of Spring Chinook

Dworshak National Fish Hatchery is interested in increasing smolt production to better meet mitigation goals. However, additional rearing space is currently unavailable. As an

alternative to increasing rearing space, the hatchery is interested in assessing whether increasing the density of smolts in the existing rearing space yields increased adult returns. To evaluate this, the hatchery created replicate normal and high density groups in BY2012. The first returning offspring reared under these conditions from BY2012 and tracked them as separate PBT release groups. The cohort originating from BY2012 has produced adult returns that have been detected in sport fisheries and during sampling at Lower Granite Dam during SY2015 and SY2016. These detections are summarized as a preliminary evaluation of the experiment. A more comprehensive evaluation will be conducted when genotyping of SY2016 broodstock is completed which will contain the bulk of adult returns as 4-year-olds.

RESULTS

Steelhead Sport Fisheries in Columbia River

Of the 1,086 samples analyzed, 690 assigned to the PBT baseline. After expanding by PBT rates, 65% 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 2,253 samples analyzed from clipped and unclipped steelhead, 1,224 assigned to the PBT baseline. After expanding by PBT rates, 56% 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 452 samples analyzed, 424 assigned to the PBT baseline. After expanding by PBT rates, the origin of 96% 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 2,418 samples analyzed, 2,180 assigned. After expanding by PBT rates, 94% 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.*).

Broodstock Management of Upper Salmon B-run Steelhead

Nine inbred spawn crosses were identified that resulted from crossing either full-siblings or half-siblings. The progeny from the inbred crosses were excluded from future production.

Stock Composition of Adult Steelhead at Lower Granite Dam

Of the samples collected, 1,764 were analyzed after removing duplicated samples or samples that failed to genotype. Of these, 1,689 assigned to the baseline. After expanding by the tag rates, the origin of 1,656 (93.4%) samples could be accounted for. A summary of stock composition and age will be provided in an upcoming IDFG technical report (Warren et al. *In prep*).

Age Composition of SY2015 Steelhead Broodstock

Of the samples collected 5,457 were analyzed with PBT after excluding duplicate and ungenotyped samples. Of these, 4,975 assigned (91.2%) to the baseline. After expanding by the tag rates, the origin of 5,205 (95.4%) samples could be accounted for. 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, 1,748 were analyzed after removal of duplicates and samples that failed to genotype. Of these, 1,710 received a PBT assignment. After expanding by PBT rates, the origin of 99.6% of the samples could be accounted for. 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 SY2015 Chinook Salmon Broodstock

Of the samples collected 9,989 were analyzed after removal of duplicated samples and samples that failed to genotype. Of these, 9,347 assigned (93.6%) to the PBT baseline. After expanding by the tag rates, the origin of 9,539 (95.5%) samples could be accounted for. Age composition for 3-, 4-, and 5-year olds in each hatchery stock will be provided in upcoming IDFG technical reports.

Evaluation of Increased Rearing Density to Increase Adult Returns of Spring Chinook

Adult Chinook originating from the experimental rearing conditions at Dworshak Hatchery during BY2012 were detected in several PBT projects. Adults were detected in the SY2015 Idaho sport fishery, of the SY2015 hatchery broodstock, and samples collected at Lower Granite Dam during SY2015 and SY2016 (Table 23). Individuals from all experimental rearing conditions (High/Low density, Burrows ponds, A-bank ponds) were represented in the samples.

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. 2016), will aide 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.*).

Broodstock Management of Upper Salmon B-run Steelhead

The ability to determine parentage and construct pedigrees using PBT allows hatchery managers an opportunity to minimize inbreeding among broodstocks. The upper Salmon River B-run broodstock has historically suffered from a small number of spawners and PBT was used to identify and remove inbred crosses from production. This ancillary application is a demonstration of the additional benefits of implementing PBT.

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 SY2015 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 the SY2015 broodstock. Determining age composition of the broodstock through PBT is another benefit of implementing the technology.

Evaluation of Increased Rearing Density to Increase Adult Returns of Spring Chinook

Results are intended to provide information to managers on return rates from experimental rearing conditions including rearing in high-density, burrows ponds, and A-bank ponds. The evaluation of different rearing densities at Dworshak National Fish Hatchery will continue over the next several years as 4- and 5-year-old progeny return. Combining all returning age-classes will increase sample sizes among treatments and allow the cumulative effect of rearing density across an entire cohort to be assessed. Examining the effect of rearing density on number of returns will be important for not only determining the potential benefits of increasing smolt production at Dworshak National Hatchery but will also be important in assessing the role of rearing density on domestication selection. A recent study proposed elevated rearing density as a proximate cause

for increased domestication in salmon hatcheries (Thompson and Blouin 2015). PBT will serve as an ideal tool evaluating the effects of rearing density at Dworshak National Fish Hatchery and for investigating the role of density in domestication selection and relative reproductive success of salmonids.

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ACKNOWLEDGEMENTS

The scope and magnitude of this project could not have been completed without the help and cooperation of many individuals from many different agencies and organizations. Thanks to Brian Leth, Chuck Warren, Chris Sullivan, and Forrest Bohlen from IDFG; Jeff Stephenson, Amanda Matala, Vanessa Morman, Stephanie Harmon, Megan Moore, Travis Jacobsen, Nick Hoffman, and Lori Maxwell from the Columbia River Inter-Tribal Fish Commission; and Amanda Boone, Lynn Schrader, Dylan Kovis, Laura Redfield, Kelly Heindel, and Thea Wickersham from the Pacific States Marine Fisheries Commission (IDFG Eagle Fish Genetics Lab). Special thanks to all IDFG, ODFW, WDFW, USFWS, NPT staff, and volunteers for help taking broodstock samples at hatcheries. Cheryl Leben's help with report formatting and editing is also appreciated. Helpful comments were also provided by Chuck Warren and Chris Sullivan. We also appreciate Barbara Shields' help in managing contract and reporting requirements. Primary funding for this project comes from the Bonneville Power Administration (#2010-031-00). Additional financial support was provided by the Pacific Coast Salmon Recovery Fund and the Idaho Power Company (Project #01608SC).

TABLES

Table 1. Total steelhead broodstock genetically sampled in SY2015 in the Snake River basin. Broodstock were sampled at 100% but only samples from broodstock producing offspring were included (samples from broodstock whose eggs were culled were not included).

Stock	Num. Samples
Sawtooth	688
E.F. Salmon River	34
Pahsimeroi	1,214
Upper Salmon R. B-run	367
Oxbow	361
Dworshak	1,260
S.F. Clearwater	382
Little Sheep Cr.	132
Tucannon R.	41
Touchet R.	29
Cottonwood Cr.	230
Wallowa	515
Total	**5,253**

Table 2. Total spring/summer Chinook Salmon broodstock sampled in SY2015 in the Snake River basin. Broodstock were sampled at 100% but only samples from broodstock producing offspring were included (samples from broodstock whose eggs were culled were not included).

Stock	Num. Samples
S.F. Clearwater	764
Dworshak	1,785
Kooskia	759
Johnson Cr.	72
Imnaha	264
Catherine Cr.	104
Lostine	153
Grande Ronde	175
Lookingglass Cr.	157
Tucannon	125
S.F. Salmon	1,249
Nez Perce Tribal FH	345
Pahsimeroi	990
Powell	455
Rapid River	1,895
Sawtooth	764
Total	**10,056**

Table 3. Total Fall Chinook Salmon broodstock sampled in SY2015 in the Snake River basin. Broodstock were sampled at 100% but only samples from broodstock producing offspring were included (samples from broodstock whose eggs were culled were not included).

Stock	Num. Samples
SY2015 Nez Perce	688
SY2015 Lyons Ferry	1,804
Total	2,492

Table 4. Sample sizes and genotyping completion rate of SY2015 steelhead broodstock. Samples with ≥10 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.

	2015		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
Sawtooth	688	684 (99.4%)	98.8%
E.F. Salmon River	34	34 (100%)	100.0%
Pahsimeroi	1,214	1,195 (98.4%)	96.8%
Upper Salmon R. B-run	367	345 (94.0%)	88.4%
Oxbow	361	352 (97.5%)	95.1%
Dworshak	1,260	1,195 (94.8%)	89.9%
S.F. Clearwater	382	373 (97.6%)	95.3%
Little Sheep Cr.	132	124 (93.9%)	88.2%
Tucannon R.	41	39 (95.1%)	90.4%
Touchet R.	29	29 (100%)	100.0%
Cottonwood Cr.	230	222 (96.5%)	93.1%
Wallowa	515	500 (97.1%)	94.3%
Total	**5,253**	**5,092 (96.94%)**	**94.0%**

Table 5. Sample sizes and genotyping completion rate of SY2015 spring/summer Chinook Salmon broodstock. Samples with ≥10 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.

	2015		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
S.F. Clearwater	764	762 (99.7%)	99.4%
Dworshak	1,785	1,771 (99.2%)	98.4%
Kooskia	759	756 (99.6%)	99.2%
Johnson Cr.	72	70 (97.2%)	94.5%
Imnaha	264	241 (91.3%)	83.4%
Catherine Cr.	104	103 (99.0%)	98.0%
Lostine	153	117 (76.5%)	58.5%
Grande Ronde	175	171 (97.7%)	95.5%
Lookingglass Cr.	157	152 (96.8%)	93.7%
Tucannon	125	95 (76.0%)	57.8%
S.F. Salmon	1,249	1,234 (98.8%)	97.6%
Nez Perce Tribal FH	345	337 (97.7%)	95.5%
Pahsimeroi	990	969 (97.9%)	95.8%
Powell	455	447 (98.2%)	96.4%
Rapid River	1,895	1,838 (97.0%)	94.1%
Sawtooth	764	761 (99.6%)	99.2%
Total	**10,056**	**9,824 (97.7%)**	**95.5%**

Table 6. Sample sizes and genotyping completion rate of SY2015 Fall Chinook Salmon broodstock. Samples with ≥10 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.

	2015		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
SY2015 Nez Perce	688	677 (98.4%)	96.8%
SY2015 Lyons Ferry	1,804	1,744 (96.7%)	93.5%
Total	2,492	2,421 (97.2%)	94.4%

Table 7. Ranked estimates of null allele frequencies (NullFreq) for the 93 loci in which 1 or more hatchery steelhead populations exhibited a deficiency of heterozygotes (H_ED) in SY2015.

SNP Name	H_ED	NullFreq
OMS00087	8	5.31%
Omy_b9164	8	5.26%
Omy_Il1b198	8	4.08%
OMS00095	7	14.81%
Omy_UT16_2173	7	13.89%
Omy_aldB165	7	8.19%
Omy_128996481	7	6.61%
OMS00129	7	6.36%
Omy_LDHB1_i2	7	5.70%
Omy_97954618	7	3.64%
OMS00174	7	3.41%
Omy_OmyP9180	7	2.88%
Omy_114315438	7	2.39%
OMS00077	7	2.38%
Omy_105385406	6	4.20%
Omy_crb106	6	3.91%
Omy_aromat280	6	3.60%
Omy_IL1b163	6	3.39%
Omy_101993189	6	2.06%
OMS00008	6	2.05%
OMS00154	6	1.71%
OMS00092	6	1.35%
OMS00138	6	1.25%
OMS00074	6	1.23%
Omy_impa155	5	3.75%
OMS00003	5	3.50%
OMS00105	5	3.36%
Omy_104519624	5	3.36%
OMS00179	5	2.91%
Omy_u0954311	5	2.49%
OMS00173	5	1.95%
OMS00119	5	1.85%
Omy_u0956119	5	1.84%
OMS00118	5	1.83%
Omy_rapd167	5	1.79%
Omy_hsf2146	5	1.75%
Omy_99300202	5	1.73%
Omy_bcAKala380rd	5	1.60%

OMS00072	5	1.46%
Omy_cin172	5	1.44%
Omy_Ogo4212	5	1.40%
Omy_anp17	5	1.38%
Omy_108007193	5	1.35%
Omy_sast264	5	1.34%
Omy_nkef241	5	1.09%
OMS00024	5	0.84%
Omy_109894185	4	4.86%
OMS00017	4	2.75%
M09AAD076	4	2.59%
Omy_110064419	4	2.01%
Omy_130524160	4	1.90%
Omy_hsp70aPro329	4	1.55%
Omy_105075162	4	1.46%
OMGH1PROM1SNP1	4	1.40%
OMS00121	4	1.24%
Omy_metA161	4	1.14%
Omy_9707773	4	0.56%
Omy_g1103	4	0.53%
Omy_pad196	3	2.49%
Omy_vamp5303	3	2.10%
Omy_colla1525	3	1.93%
OMS00101	3	1.43%
OMS00030	3	1.41%
Omy_128923433	3	1.25%
M09AAJ163	3	1.03%
Omy_97865196	3	0.93%
OMS00013	3	0.71%
OMS00143	3	0.61%
Omy_b1266	3	0.61%
Omy_hus152	3	0.43%
Omy_BACF5284	3	0.40%
Omy_CRBF11	3	0.33%
OMS00015	3	0.26%
OMS00070	2	1.56%
OMS00149	2	1.43%
OMS00114	2	1.19%
Omy_ndk152	2	1.18%
Omy_cox1221	2	1.09%
Omy_e1147	2	0.78%
Omy_105105448	2	0.76%
Omy_nxt2273	2	0.68%

Omy_tlr5205	2	0.59%
Omy_carban1264	1	1.04%
OMS00061	1	0.54%
OMS00133	1	0.46%
Omy_LDHB2_i6	1	0.30%
OMS00014	1	0.24%
M09AAC055	1	0.23%
Omy_nips299	1	0.10%
Omy_GHSR121	1	0.05%
Omy_mapK3103	1	0.05%
Omy_mcsf268	1	0.03%
Omy_u0952284	1	0.01%

Table 8. Ranked estimates of null allele frequencies (NullFreq) for the 139 loci in which 1 or more hatchery Chinook salmon populations exhibited a deficiency of heterozygotes ($H_E D$) in SY2015.

SNP Name	$H_E D$	NullFreq
Ots_crRAD6152371	11	12.02%
Ots_U512134	11	8.74%
Ots_crRAD1044725	11	7.53%
Ots_U230563	11	7.03%
Ots_OTALDBINT1SNP1	11	4.05%
Ots_105401325	10	31.36%
Ots_IsoT	10	7.19%
Ots_NAML12SNP1	10	6.98%
Ots_Tnsf	10	1.77%
Ots_MHC1	9	14.55%
Ots_crRAD506127	9	5.45%
Ots_10304152	9	4.65%
Ots_crRAD3034148	9	3.39%
Ots_108735302	9	2.31%
Ots_11230143	9	2.08%
Ots_u0717373	9	1.68%
Ots_126619400	9	1.67%
Ots_crRAD4458867	9	1.53%
Ots_pigh105	8	3.35%
Ots_crRAD3305462	8	2.78%
Ots_crRAD1752758	8	2.73%
Ots_crRAD7651228	8	2.72%
Ots_u1008108	8	2.20%
Ots_crRAD3531366	8	2.15%
Ots_AldB1122	8	1.83%
Ots_GDH81x	8	1.71%
Ots_E2275	8	1.53%
Ots_brp1664	8	1.28%
Ots_hsc713488	8	1.10%
Ots_crRAD5753724	7	2.41%
Ots_104048194	7	2.37%
Ots_crRAD3809529	7	2.20%
Ots_hnRNPL533	7	2.09%
Ots_u675	7	2.05%
Ots_GTH2B550	7	1.89%
Ots_ZR575	7	1.82%
Ots_u0707161	7	1.70%

Ots_unk526	7	1.69%
Ots_crRAD5547526	7	1.59%
Ots_crRAD5768734	7	1.58%
Ots_aldb177M	7	1.57%
Ots_95442b204	7	1.53%
Ots_NFYB147	7	1.38%
Ots_Prl2	7	1.23%
Ots_crRAD2536750	7	1.02%
Ots_SClkF2R2135	7	0.98%
Ots_117242136	7	0.92%
Ots_MetA	6	3.26%
Ots_Est1363	6	1.76%
Ots_101704143	6	1.48%
Ots_AldoB4183	6	1.45%
Ots_crRAD4205848	6	1.41%
Ots_P450	6	1.31%
Ots_unk183239	6	1.26%
Ots_u492	6	1.15%
Ots_Chin30up211	6	1.07%
Ots_100884287	6	1.06%
Ots_112208722	6	0.95%
Ots_crRAD4729755	6	0.92%
Ots_112820284	6	0.76%
Ots_GCSH	6	0.51%
Ots_111312435	5	1.72%
Ots_Thio	5	1.68%
Ots_12875761R	5	1.61%
Ots_118175479	5	1.19%
Ots_pop596	5	1.13%
Ots_crRAD6062051	5	1.11%
Ots_110201363	5	1.08%
Ots_mybp85	5	0.95%
Ots_107806821	5	0.94%
Ots_9766056	5	0.85%
Ots_crRAD2654147	5	0.78%
Ots_crRAD2867765	5	0.75%
Ots_U2362227	5	0.58%
Ots_129170683	4	14.93%
Ots_111084b619	4	1.34%
Ots_u202161	4	1.22%
Ots_104063132	4	0.95%
Ots_MHC2	4	0.95%
Ots_TCTA58	4	0.88%

Ots_crRAD7558170	4	0.80%
Ots_Est740	4	0.70%
Ots_mapK3309	4	0.70%
Ots_U2446123	4	0.65%
Ots_13072099	4	0.57%
Ots_117259271	4	0.55%
Ots_crRAD2026246	4	0.51%
Ots_128693461	4	0.51%
Ots_109693392	4	0.42%
Ots_crRAD1372551	4	0.25%
Ots_105897124	3	2.98%
Ots_u0720332	3	2.55%
Ots_102213210	3	1.82%
Ots_10728593	3	0.87%
Ots_zn593346	3	0.82%
Ots_IL11	3	0.79%
Ots_124774477	3	0.75%
Ots_EndoRB1486	3	0.68%
Ots_107607315	3	0.67%
Ots_FARSLA220	3	0.67%
Ots_crRAD29221	3	0.62%
Ots_cox1241	3	0.59%
Ots_hsc715453	3	0.58%
Ots_crRAD1893760	3	0.51%
Ots_113242216	3	0.46%
Ots_SERPC1209	3	0.42%
Ots_GH2	3	0.32%
Ots_OTSMTASNP1	3	0.30%
Ots_12830257	3	0.25%
Ots_crRAD4845974	3	0.23%
Ots_u1006171	3	0.18%
Ots_crRAD1849265	2	1.31%
Ots_u0753133	2	1.15%
Ots_12987055	2	0.66%
Ots_crRAD7476628	2	0.33%
Ots_slc7a271	2	0.25%
Ots_nramp321	2	0.21%
Ots_zP3b215	2	0.17%
Ots_aspat196	2	0.16%
Ots_GPDH338	1	3.66%
Ots_arp436	1	0.45%
Ots_RFC2558	1	0.38%
Ots_12241456	1	0.33%

Ots_crRAD9242025	1	0.30%
Ots_crRAD3439733	1	0.29%
Ots_102867609	1	0.25%
Ots_BMP2SNP1	1	0.25%
Ots_99550204	1	0.24%
Ots_crRAD375851	1	0.15%
Ots_Cath_D141	1	0.12%
Ots_crRAD7896846	1	0.12%
Ots_SL	1	0.12%
Ots_myo1a384	1	0.11%
Ots_96222525	1	0.10%
Ots_crRAD2111524	1	0.09%
Ots_Ots311101x	1	0.09%
Ots_97077179R	1	0.05%
Ots_PGK54	1	0.05%
Ots_u0719260	1	0.03%

Table 9. Ranked estimates of null allele frequencies (NullFreq) for the 64 loci in which 1 or more hatchery populations of Fall Chinook salmon exhibited a deficiency of heterozygotes (H_ED) in SY2015.

SNP Name	H_ED	NullFreq
Ots_MetA	2	61.95%
Ots_crRAD506127	2	35.90%
Ots_MHC1	2	35.75%
Ots_GPDH338	2	20.55%
Ots_104048194	2	16.10%
Ots_U230563	2	13.65%
Ots_127760569	2	12.60%
Ots_101704143	2	9.90%
Ots_11230143	2	8.90%
Ots_crRAD3607229	2	8.25%
Ots_NOD1	2	7.45%
Ots_hnRNPL533	2	6.90%
Ots_111312435	2	6.80%
Ots_sept978	2	6.35%
Ots_crRAD7651228	2	6.25%
Ots_u0725325	2	4.55%
Ots_112208722	2	4.45%
Ots_131802393	2	4.30%
Ots_ppie245	2	4.20%
Ots_u202161	2	4.15%
Ots_OTALDBINT1SNP1	2	4.00%
Ots_105401325	2	3.95%
Ots_124774477	2	3.65%
Ots_crRAD7558170	2	3.60%
Ots_u1008108	2	3.45%
Ots_126619400	2	3.15%
Ots_afmid196	2	3.15%
Ots_123921111	2	2.80%
Ots_crRAD5547526	2	2.65%
Ots_crRAD29221	2	2.25%
Ots_110689218	2	2.10%
Ots_unk351349	2	2.10%
Ots_PGK54	2	2.10%
Ots_10441588	2	1.95%
Ots_Est1363	2	1.85%
Ots_aldb177M	2	1.75%
Ots_crRAD3615244	2	1.70%
Ots_NAML12SNP1	2	1.70%

Ots_GST375	2	1.60%
Ots_u100275	2	1.35%
Ots_pop596	2	1.35%
Ots_109693392	2	1.25%
Ots_nramp321	2	0.85%
Ots_129170683	2	0.55%
Ots_Chin30up211	1	8.20%
Ots_crRAD4205848	1	4.50%
Ots_96899357R	1	2.15%
Ots_108390329	1	1.80%
Ots_106313729	1	1.30%
Ots_11055164	1	1.30%
Ots_txnip321	1	1.20%
Ots_crRAD4845974	1	1.15%
Ots_u1007124	1	0.85%
Ots_RAD454352	1	0.80%
Ots_crRAD3034148	1	0.75%
Ots_ARNT	1	0.65%
Ots_FARSLA220	1	0.65%
Ots_crRAD2867765	1	0.55%
Ots_crRAD5540059	1	0.50%
Ots_zP3b215	1	0.30%
Ots_u0757120	1	0.25%
Ots_u1006171	1	0.25%
Ots_SERPC1209	1	0.20%
Ots_crRAD5753724	1	0.15%

Table 10. Results of comparisons between phenotypic sex and genetically determined sex using the sex-specific assay for SY2015 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	688	2 (0.3%)	686 (99.7%)	670 (97.7%)	16 (2.3%)	5	11	344 (50.0%)	344 (50.0%)
E.F.Salmon R.	34	0 (0.0%)	34 (100%)	33 (97.1%)	1 (2.9%)	0	1	19 (55.9%)	15 (44.1%)
Pahsimeroi	1,214	8 (0.7%)	1,206 (99.3%)	1,194 (99.0%)	12 (1.0%)	11	1	607 (50.0%)	607 (50.0%)
Up. Sal. R. B-run	367	9 (2.5%)	358 (97.6%)	347 (96.9%)	11 (3.1%)	3	8	161 (43.9%)	206 (56.1%)
Oxbow	361	5 (1.4%)	356 (98.6%)	351 (98.6%)	5 (1.4%)	3	2	180 (49.9%)	181 (50.1%)
Dworshak	1,260	65 (5.2%)	1,195 (94.8%)	1,195 (100%)	0 (0.0%)	0	0	563 (44.7%)	697 (55.3%)
S.F. Clearwater	382	9 (2.4%)	373 (97.6%)	373 (100%)	0 (0.0%)	0	0	171 (44.8%)	211 (55.2%)
Little Sheep Cr.	132	8 (6.1%)	124 (93.9%)	124 (100%)	0 (0.0%)	0	0	66 (50.0%)	66 (50.0%)
Tucannon R.	41	0 (0.0%)	41 (100%)	41 (100%)	0 (0.0%)	0	0	15 (36.6%)	26 (63.4%)
Touchet R.	29	0 (0.0%)	29 (100%)	29 (100%)	0 (0.0%)	0	0	14 (48.3%)	15 (51.7%)
Cottonwood Cr.	230	11 (4.8%)	219 (95.2%)	218 (99.5%)	1 (0.5%)	0	1	104 (45.2%)	126 (54.8%)
Wallowa	515	22 (4.3%)	493 (95.7%)	490 (99.4%)	3 (0.7%)	1	2	246 (47.8%)	269 (52.2%)
Total	**5,253**	**139 (2.7%)**	**5,114 (97.4%)**	**5,065 (99.0%)**	**49 (1.0%)**	**23**	**26**	**2,490 (47.4%)**	**2,763 (52.6%)**

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 SY2015 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	764	2 (0.3%)	762 (99.7%)	762 (100%)	0 (0.0%)	0	0	345 (45.2%)	419 (54.8%)
Dworshak	1,785	952 (53.3%)	833 (46.7%)	832 (99.9%)	1 (0.1%)	1	0	767 (43.0%)	1,017 (57%)
Kooskia	759	517 (68.1%)	242 (31.9%)	242 (100%)	0 (0.0%)	0	0	308 (40.6%)	450 (59.3%)
Johnson Cr.	72	2 (2.8%)	70 (97.2%)	68 (97.1%)	2 (2.9%)	1	1	36 (50.0%)	36 (50.0%)
Imnaha	264	23 (8.7%)	241 (91.3%)	241 (100%)	0 (0.0%)	0	0	128 (48.5%)	135 (51.1%)
Catherine Cr.	104	1 (1.0%)	103 (99.0%)	103 (100%)	0 (0.0%)	0	0	51 (49.0%)	53 (51.0%)
Lostine	153	27 (17.7%)	126 (82.4%)	126 (100%)	0 (0.0%)	0	0	81 (52.9%)	72 (47.1%)
Grande Ronde	175	4 (2.3%)	171 (97.7%)	171 (100%)	0 (0.0%)	0	0	90 (51.4%)	85 (48.6%)
Lookingglass Cr.	157	4 (2.6%)	153 (97.5%)	153 (100%)	0 (0.0%)	0	0	82 (52.2%)	75 (47.8%)
Tucannon	125	30 (24.0%)	95 (76.0%)	95 (100%)	0 (0.0%)	0	0	50 (40.0%)	75 (60.0%)
S.F. Salmon	1,249	13 (1.0%)	1,236 (99.0%)	1,236 (100%)	0 (0.0%)	0	0	594 (47.6%)	655 (52.4%)
Nez Perce F.H.	345	8 (2.3%)	337 (97.7%)	337 (100%)	0 (0.0%)	0	0	164 (47.5%)	181 (52.5%)
Pahsimeroi	990	855 (86.4%)	135 (13.6%)	135 (100%)	0 (0.0%)	0	0	481 (48.6%)	509 (51.4%)
Powell	455	8 (1.8%)	447 (98.2%)	447 (100%)	0 (0.0%)	0	0	230 (50.6%)	225 (49.5%)
Rapid River	1,895	38 (2.0%)	1,857 (98.0%)	1,856 (99.9%)	1 (0.1%)	0	1	893 (47.1%)	1,002 (52.9%)
Sawtooth	764	3 (0.4%)	761 (99.4%)	761 (100%)	0 (0.0%)	0	0	439 (57.5%)	325 (42.5%)
Total	**10,056**	**2,487 (24.7%)**	**7,569 (75.3%)**	**7,565 (99.9%)**	**4 (0.1%)**	**2**	**2**	**5,311 (44.8%)**	**6,519 (55.0%)**

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 SY2015 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
SY2015 Nez Perce	688	11 (1.6%)	677 (98.4%)	677 (100%)	0 (0.0%)	0	0	298 (43.3%)	390 (56.7%)
SY2015 Lyons Ferry	1,804	40 (2.2%)	1,764 (97.8%)	1,756 (99.6%)	8 (0.5%)	4	4	572 (31.7%)	1,205 (66.8%)
Total	**2,492**	**51 (2.0%)**	**2,441 (98.0%)**	**2,433 (99.7%)**	**8 (0.3%)**	**4**	**4**	**870 (34.9%)**	**1,595 (64.0%)**

Table 13. Average observed and expected heterozygosity with associated standard deviation of hatchery steelhead stocks for SY2015.

Stock	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
Sawtooth	0.291	0.001	0.293	0.012
EF Salmon	0.297	0.006	0.300	0.013
Upper Salmon B	0.275	0.002	0.276	0.012
Oxbow	0.298	0.002	0.300	0.011
Pahsimeroi	0.296	0.001	0.298	0.011
Dworshak	0.267	0.001	0.271	0.012
SF Clearwater	0.265	0.002	0.269	0.012
Little Sheep Ck	0.285	0.003	0.288	0.012
Tucannon	0.306	0.005	0.304	0.012
Touchet	0.287	0.006	0.295	0.012
Cottonwood Ck	0.297	0.002	0.300	0.012
Wallowa	0.294	0.002	0.299	0.012

Table 14. Average observed and expected heterozygosity with associated standard deviation of hatchery spring/summer Chinook Salmon stocks in SY2015.

Stock	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
S.F. Clearwater	0.219	0.010	0.220	0.010
Dworshak	0.216	0.010	0.218	0.010
Kooskia	0.216	0.010	0.217	0.010
Johnson Cr.	0.198	0.011	0.197	0.011
Imnaha	0.210	0.010	0.214	0.010
Catherine Cr.	0.219	0.010	0.221	0.010
Lostine	0.209	0.010	0.207	0.010
Grande Ronde	0.217	0.011	0.217	0.011
Lookingglass Cr.	0.218	0.010	0.220	0.010
Tucannon	0.225	0.010	0.226	0.010
S.F. Salmon	0.202	0.010	0.203	0.010
Nez Perce Tribal FH	0.219	0.010	0.221	0.011
Pahsimeroi	0.204	0.011	0.206	0.011
Powell	0.216	0.011	0.218	0.011
Rapid River	0.206	0.011	0.208	0.011
Sawtooth	0.199	0.011	0.201	0.011

Table 15. Average observed and expected heterozygosity with associated standard deviation of hatchery Fall Chinook Salmon stocks in SY2015.

Stock	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
SY2015 Nez Perce	0.254	0.01	0.261	0.01
SY2015 Lyons Ferry	0.258	0.01	0.263	0.01

Table 16. Population structure (F_{ST}) (lower left) among steelhead hatchery stocks sampled in SY2015. Asterisks (*) in the upper right indicate that the genic differentiation (exact G test) were highly significant.

Population	Cottonwood	Dworshak	EF Salmon	Oxbow	Little Sheep	Pahsimeroi	Sawtooth	SF Clearwater	Touchet	Tucannon	Up Sal B
Dworshak	0.058	---	*	*	*	*	*	*	*	*	*
EF Salmon	0.029	0.041	---	*	*	*	*	*	*	*	*
Oxbow	0.025	0.051	0.014	---	*	*	*	*	*	*	*
Little Sheep	0.023	0.064	0.028	0.021	---	*	*	*	*	*	*
Pahsimeroi	0.024	0.053	0.013	0.006	0.022	---	*	*	*	*	*
Sawtooth	0.024	0.050	0.016	0.006	0.025	0.006	---	*	*	*	*
SF Clearwater	0.056	0.001	0.040	0.049	0.063	0.051	0.049	---	*	*	*
Touchet	0.021	0.052	0.021	0.023	0.023	0.023	0.028	0.052	---	NS	*
Tucannon	0.012	0.047	0.016	0.015	0.017	0.015	0.017	0.046	0.003	---	*
Up Sal B	0.053	0.016	0.043	0.051	0.068	0.052	0.047	0.016	0.054	0.051	---
Wallowa	0.007	0.050	0.023	0.021	0.022	0.020	0.020	0.049	0.019	0.008	0.048

Table 17. Population structure (F_{ST}) (lower left) among spring/summer Chinook Salmon hatchery stocks sampled in SY2015. Asterisks (*) in the upper right indicate that the genic differentiation (exact G test) were highly significant.

	Catherine Cr.	Dworshak	Grande Ronde	Imnaha	Johnson Cr.	Kooskia	Lookingglass	Lostine	Nez Perce TFH	Pahsimeroi	Powell	Rapid River	Sawtooth	S.F. Clearwater	S.F. Salmon
Dworshak	0.011	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.013	0.015	---	*	*	*	*	*	*	*	*	*	*	*	*
Imnaha	0.014	0.014	0.015	---	*	*	*	*	*	*	*	*	*	*	*
Johnson Cr.	0.027	0.025	0.028	0.024	---	*	*	*	*	*	*	*	*	*	*
Kooskia	0.011	0.003	0.014	0.016	0.024	---	*	*	*	*	*	*	*	*	*
Lookingglass	0.005	0.009	0.010	0.013	0.026	0.010	---	*	*	*	*	*	*	*	*
Lostine	0.028	0.025	0.032	0.023	0.040	0.029	0.026	---	*	*	*	*	*	*	*
Nez Perce TFH	0.012	0.005	0.018	0.017	0.024	0.005	0.011	0.028	---	*	*	*	*	*	*
Pahsimeroi	0.040	0.037	0.044	0.037	0.047	0.040	0.041	0.046	0.040	---	*	*	*	*	*
Powell	0.012	0.005	0.016	0.016	0.029	0.006	0.011	0.028	0.004	0.044	---	*	*	*	*
Rapid River	0.021	0.022	0.019	0.014	0.037	0.024	0.023	0.040	0.027	0.039	0.025	---	*	*	*
Sawtooth	0.027	0.026	0.033	0.026	0.029	0.028	0.028	0.038	0.029	0.036	0.033	0.035	---	*	*
S.F. Clearwater	0.009	0.003	0.013	0.011	0.022	0.004	0.009	0.025	0.005	0.036	0.005	0.017	0.025	---	*
S.F. Salmon	0.023	0.019	0.028	0.018	0.012	0.020	0.023	0.027	0.021	0.036	0.025	0.033	0.022	0.017	---
Tucannon	0.023	0.021	0.028	0.031	0.039	0.022	0.025	0.036	0.021	0.054	0.024	0.047	0.047	0.024	0.033

Table 18. Estimates of effective population size (N_E) and 95% confidence intervals for steelhead hatchery stocks in SY2015.

Stock	N_E	95% CI
Sawtooth	190.4	182.2 – 198.9
E.F. Salmon River	166.3	111.7 – 311.3
Pahsimeroi	202.0	194.7 – 209.6
Upper Salmon R. B-run	43.7	42.1 – 45.4
Oxbow	128.4	123.1 – 134.0
Dworshak	234.2	224.4 – 244.4
S.F. Clearwater	135.8	127.2 – 145.1
Little Sheep Cr.	173.5	151.8 – 201.2
Tucannon R.	245.9	164.7 – 465.2
Touchet R.	619.6	208.2 – Infinite
Cottonwood Cr.	42.7	41.0 – 44.5
Wallowa	180.5	171.5 – 190.1

Table 19. Estimates of effective population size and 95% confidence intervals for SY2015 spring/summer Chinook Salmon hatchery stocks.

Stock	Ne	95% CI
S.F. Clearwater	202.1	194.6 – 210.0
Dworshak	298.5	289.1 – 308.1
Kooskia	168.2	162.5 – 174.1
Johnson Cr.	415.4	285.8 – 737.3
Imnaha	186.4	173.3 – 201.1
Catherine Cr.	155.7	138.9 – 176.4
Lostine	168.5	150.9 – 190.0
Grande Ronde	60.3	57.5 – 63.2
Lookingglass Cr.	161.1	147.5 – 176.8
Tucannon	521.1	388.1 – 779.9
S.F. Salmon	236.1	227.9 – 244.7
Nez Perce Tribal FH	73.3	71.0 – 75.7
Pahsimeroi	152.6	147.6 – 157.7
Powell	91.8	88.6 – 95.1
Rapid River	474.0	455.1 – 493.9
Sawtooth	124.6	120.5 – 128.9

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

Stock	Ne	95% CI
SY2015 Nez Perce	1,099.7	1,006.1 – 1,209.2
SY2015 Lyons Ferry	1,143.0	1,084.6 – 1,206.1

Table 21. The number of simulated hatchery offspring that received a correct, incorrect, or no parentage assignment from an analysis in which potential parents were included in the analysis.

Parental Stock	Simulated offspring	Correct Assignments	Incorrect Assignments	Non-assigned individuals
Lyon's Ferry SY2011	974	964 (99.0%)	2 (0.2%)	8 (0.8%)
Lyon's Ferry SY2012	950	934 (98.3%)	3 (0.3%)	13 (1.4%)
Lyon's Ferry SY2013	968	958 (99.0%)	5 (0.5%)	5 (0.5%)
Nez Perce Fish Hatchery SY2014	782	773 (98.8%)	2 (0.3%)	7 (0.9%)
Nez Perce Fish Hatchery SY2015	850	839 (98.7%)	0 (0.0%)	11 (1.3%)
Total	4,524	4,468 (98.8%)	12 (0.3%)	44 (1.0%)

Table 22. The number of simulated wild-origin offspring that received an incorrect or no parentage assignment from an analysis in which potential parents were purposefully excluded from the analysis.

Parental Stock	Simulated offspring	Incorrect Assignments	Non-assigned individuals
Lower Granite Dam wild SY2014	1000	11 (1.1%)	989 (98.9%)
Lower Granite Dam wild SY2015	1000	7 (0.7%)	993 (99.3%)
Lower Granite Dam wild SY2016	1000	5 (0.5%)	995 (99.5%)
Lyon's Ferry SY2014	968	14 (1.4%)	954 (98.6%)
Lyon's Ferry SY2015	963	5 (0.5%)	958 (99.5%)
Nez Perce Fish Hatchery SY2011	904	12 (1.3%)	892 (98.7%)
Nez Perce Fish Hatchery SY2012	1000	19 (1.9%)	981 (98.1%)
Nez Perce Fish Hatchery SY2013	1000	9 (0.9%)	991 (99.1%)
Total	7,835	82 (1.0%)	7753 (99.0%)

Table 23. Summary of PBT detections of Dworshak-origin Chinook Salmon originating from the experimental rearing conditions in BY2012.

Project Name	Num. Detections
SY2015 Idaho Fishery	**70**
A-Bank Replicate 1	9
A-Bank Replicate 2	1
A-Bank Replicate 3	3
Burrows Ponds	16
Control	24
High Density Replicate 2	3
High Density Replicate 3	1
Low Density Replicate 1	6
Low Density Replicate 2	4
Low Density Replicate 3	3
SY2015 Evaluation of non-spawned Dworshak jacks	**84**
A-Bank Replicate 1	8
A-Bank Replicate 2	3
A-Bank Replicate 3	7
Burrows Ponds	34
Control	8
High Density Replicate 1	1
High Density Replicate 2	2
High Density Replicate 3	7
Low Density Replicate 1	7
Low Density Replicate 2	4
Low Density Replicate 3	3
SY2015 Hatchery broodstock	**20**
A-Bank Replicate 1	1
A-Bank Replicate 2	1
Burrows Ponds	6
Control	10
Low Density Replicate 3	2
SY2015 Lower Granite Dam (AD-clipped)	**30**
A-Bank Replicate 1	1
Burrows Ponds	9
Control	18
High Density Replicate 2	1
Low Density Replicate 3	1
SY2015 Lower Granite Dam (AD-intact)	**9**
A-Bank Replicate 1	1
Control	8

SY2016 Lower Granite Dam (AD-clipped)	**536**
A-Bank Replicate 1	26
A-Bank Replicate 2	27
A-Bank Replicate 3	20
Burrows Ponds	100
Control	257
Flow Replicate 1	2
Flow Replicate 2	2
Flow Replicate 3	1
High Density Replicate 1	17
High Density Replicate 2	25
High Density Replicate 3	23
Low Density Replicate 1	17
Low Density Replicate 2	9
Low Density Replicate 3	10
Grand Total	**749**

FIGURES

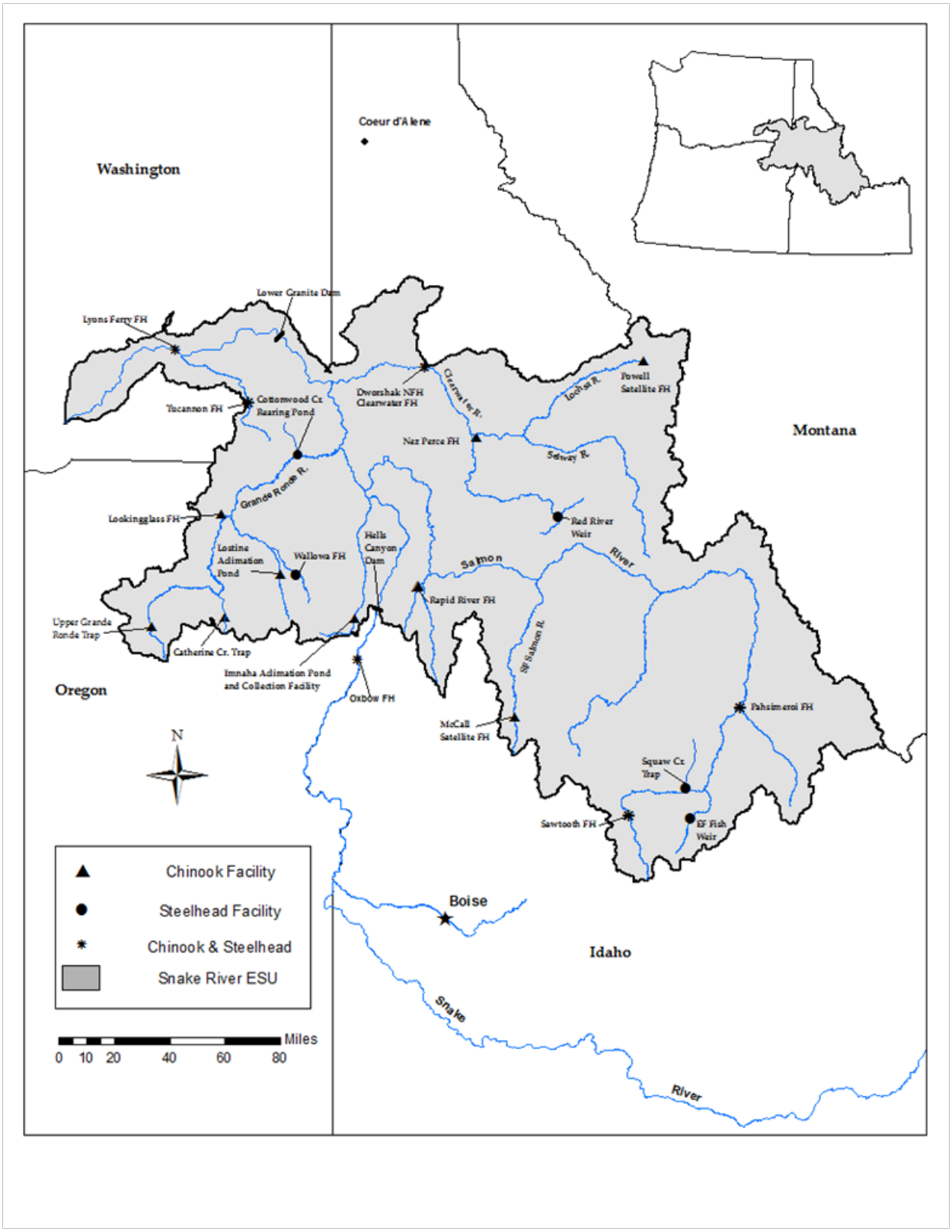

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

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