FISHERY RESEARCH

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

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Parentage-Based Tagging of Snake River Hatchery Steelhead and Chinook Salmon

Project Progress Report

2020 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, 2020 to December 31, 2020 that focused on the continued development and implementation of 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 all 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 used a highly variable panel of single nucleotide polymorphism (SNPs) markers, identified for each species, to genotype steelhead and Chinook Salmon broodstocks sampled in the Snake River basin from spawn year (SY) 2019 (Objective 2). We then used the data generated from the broodstock baselines to provide parentage results to inform a variety of management and conservation programs (Objective 3). Results continued 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 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, 2006), 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."

Snake River Chinook Salmon and Steelhead Parentage Based Tagging was initiated in 2010, partly in response to Independent Scientific Review Panel (ISRP) and Independent Scientific Advisory Board (ISAB) recommendations that proof-of-concept trials be completed on Parentage Based Tagging (PBT) technology (ISRP/ISAB 2009). The technology involves the annual sampling and genotyping of all hatchery broodstock and creating a genetic database of parental genotypes. The project's initial accomplishments are detailed in Steele et al (2013): empirically confirming the number of genetic markers needed for PBT, comparing the power of microsatellite markers and single nucleotide polymorphisms (SNPs), and demonstrating that assignments made with PBT match those using coded-wire tags. Since the publication of Steele et al (2013), the primary objectives of this project have been to oversee the sampling and genotyping of parental broodstock at all Snake River hatcheries, and to genotype these samples with powerful genetic marker panels that are standardized between our main collaborating lab (Columbia River Inter-Tribal Fish Commission) and other NW salmon genetic labs. In addition, we help organize and summarize projects that use the Snake River PBT baseline, along with offspring recoveries, to address conservation and management issues of importance to the Council and state and tribal fisheries managers. A review of our accomplishments over the last 10 years in areas related to tracking family groups, estimating PBT tag rates, storing PBT baseline data and making it publicly available, is published in Steele et al (2019).

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 the Columbia River during migration year 2019 (SY2020), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2019 (SY2020), 3) Origin of samples from various sport fisheries in Idaho in migration year 2019 (SY2020), 4) Age composition and origin of the SY2019 broodstocks, and 5) stock composition of returning adults during SY2020 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2019), 2) Age composition and origin of SY2019 broodstocks, 3.) Stock composition of returning adults during SY2019 at Lower Granite Dam.

REPORT STRUCTURE

This report is divided into three sections, one for each of the objectives for this reporting period. 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 and included in summary tables.

Once the samples are extracted and genotyped, genetic data are recorded into a Progeny SQL database (Ambry Genetics, Aliso Viejo, California, 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

We collected and inventoried approximately 4,865 genetic samples from steelhead broodstock (Table 1) spawned in the Snake River basin during SY2019, and approximately 9,427 samples (Table 2) from spring/summer Chinook Salmon broodstock spawned in the Snake River basin during SY2019. We also report on fall Chinook collected from the Lyons Ferry and Nez Perce Tribal Fish hatcheries for SY2019 (N = 2,360; 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 SY2019.

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 2018. 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 ninth 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 laboratory adopted Genotyping-in-Thousands (GT-seq) protocols developed by the CRITFC genetics lab (Campbell et al. 2015) 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 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. Including the vast majority of the original 96 markers for each species, Chinook Salmon were genotyped at a total of 299 or 343 markers and steelhead were genotyped at a total of either 379 or 368 markers, respectively. The only Chinook Salmon collection genotyped with 299 markers was the Lyon's Ferry fall Chinook, and so statistics calculated for the fall Chinook populations are restricted to the markers overlapping between the 299 and 343 marker panels. The steelhead collections were approximately evenly split between being genotyped with the 379 and 368 panels due to panel improvement and optimization occurring partway through the year. All statistics calculated for the steelhead collections are restricted to the markers overlapping between the 379 and 368 marker panels.

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. (2015). 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. Basic diversity indices were calculated for the brood years. This included average observed (H_O) and expected (H_E) heterozygosity custom R scripts, 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 minimum allele frequency of 0.05.

Sex Markers

The accuracy of the sex-determining SNP assay for steelhead and Chinook Salmon was evaluated for hatchery stocks spawned in SY2019; comparisons were made between the phenotypic sex of samples, which was determined at time of spawning, and the genetically determined sex of samples. Note that the steelhead stock Cottonwood Cr., which has been included in previous reports, was not spawned in 2019 and so is not included in this report.

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 rearing 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%.

Microhaplotype Discovery

Microhaplotypes are multiple, tightly linked SNPs within an amplicon that exhibit contrasting allele frequencies across populations. To exploit the additional information gained from considering microhaplotypes, we performed SNP discovery using reads from the existing GTseq panels. We then genotyped the samples for any resulting microhaplotypes and validated the microhaplotypes.

Reference amplicon sequences were created by pooling reads for nine samples for a given species and, for each locus, extracting the unique sequence with highest depth that began with the forward primer and contained one of the in *silico probes*. Reads for all samples were aligned to these reference sequences with bowtie2 (Langmead and Salzberg 2012) using the following parameters: --end-to-end -N 1 --rdg 0,5 --rfg 0,5 --score-min L,0,-.76. The main function of these parameters compared to the defaults is to allow more differences between the read and the reference for an alignment to still be made. This is justified as the amplicons are expected to contain one or more polymorphisms and the relatively small number of amplicon sequences implies that reads originating from different regions are not likely to be highly similar by chance. Only reads matching the forward strand were retained.

In addition to the known SNPs in these loci, candidate substitution SNPs were identified by utilizing the “mpileup” and “call” routines in samtools/bcftools 1.9 (Li 2011). Loci with more than 8 candidate SNPs were removed as it was determined these were more likely primer sets that were amplifying multiple paralogous sequences. Samples were then genotyped for the candidate SNPs with a microhaplotype aware genotyper, microTyper (https://github.com/delomast/microTyper), that we developed. Genotypes were called based on a posterior probability greater than 0.99, a uniform prior, and a minimum depth of 10 reads. Posterior probabilities were calculated using a multinomial likelihood with errors considered equally likely to be any other allele (--count option in microTyper’s genoCaller routine) and assuming an error rate of 0.01 (1%).

Loci were then filtered in multiple ways to remove primer pairs that may be amplifying multiple paralogous sequences or have high rates of allelic bias. First, loci with observed heterozygosity greater than 0.7 across all samples were removed. Then read count plots were visually evaluated and loci were removed if they showed either a systematic deviation of allele balance in heterozygotes from 0.5 or distinct clusters of heterozygotes with different mean allele balances. Finally, candidate SNPs that were non-variable (as genotyped by microTyper) were

removed. In total, we identified 92 loci that exhibited three or more alleles in the steelhead panel and 53 loci in the Chinook panel.

Using these loci, we calculated expected and observed heterozygosity with the microhaplotypes and compared it to those calculated using just the SNPs in those loci targeted by GTseq. For the steelhead and spring/summer Chinook Salmon stocks, SY19 broodstock were used to calculate heterozygosity. For the fall Chinook stocks, sequence data for the SY19 broodstock was not available, and so SY18 returning adults collected at Lower Granite Dam were used. These samples were assigned to the two constituent populations through PBT analysis. Given the large size of the fall Chinook stocks, changes in heterozygosity between SY18 and SY19 are expected to be minimal.

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 SY2019, some broodstocks were extracted and genotyped with an older expanded panel of 378 SNPs and the sex-identification assay while other broodstocks were genotyped with a newer expanded panel of 368 SNPs and the sex-identification assay. Of the 4,865 total samples collected, 4,768 (98.0%) were genotyped with an acceptable level of missing data (Table 1).

For spring/summer Chinook Salmon SY2019, all samples were extracted and genotyped with the expanded panel of 343 PBT SNPs and the sex-identification assay. Of the 9,427 total samples collected, 9,360 (99.3%) were genotyped with an acceptable level of missing data (Table 2).

For fall Chinook Salmon SY2019, all Lyons Ferry FH samples were genotyped with an older expanded panel of 298 PBT SNPs and the sex-identification assay, while the Nez Perce Tribal FH samples were genotyped with a newer expanded panel of 342 SNPs and the sex-identification assay. Of the 2,360 total samples collected, 2,324 (98.5%) were genotyped with an acceptable level of missing data (Table 3).

Poor Performing Loci

Most SNPs in the samples that passed the genotyping threshold had high genotyping success. For SY2019 steelhead, 37 loci failed to genotype at >5% of samples (Table 4). For SY2019 spring/summer Chinook Salmon, 20 loci failed at >5% of the samples (Table 5). For SY2019 fall Chinook, 11 loci failed at >5% of the samples (Table 6).

Error Rate (Quality Control)

For steelhead SY2019, a subset of 84 samples were rerun and the resulting 29,467 genotypes (limited to non-missing genotypes in both runs) were checked for discrepancies. Of

these genotypes, there were 6 discrepancies, excluding any SNP failures in either the original or the rerun genotype, which resulted in a genotype error rate of 0.02%.

For Chinook Salmon SY2019, a subset of 123 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 41,119 rerun genotypes being compared to the original genotypes. This resulted in 4 discrepancies, excluding any SNP failures in either the original or the rerun genotype, between the original and samples and a genotyping error rate of 0.01%.

Average Heterozygosity

Levels of observed heterozygosity within steelhead broodstocks were 0.23-0.26 for all hatchery broodstocks (Table 10). For Chinook Salmon, levels of observed heterozygosity were 0.19-0.24 in spring/summer stocks (Table 11) and 0.27 in fall stocks (Table 12).

Population Structure

Pairwise F_{ST} was calculated among the steelhead SY2019 hatchery broodstock (Table 13). Values ranged from a low of 0.0036 between the Dworshak and SF Clearwater stocks, and a high of 0.0780 between the Little Sheep Creek and Upper Salmon River B-run stocks. All tests of genetic differentiation among stocks were significant ($p < .01$).

For spring/summer Chinook Salmon SY2019, pairwise F_{ST} values ranged from a low of 0 between the Dworshak/Kooskia and the Nez Perce FH stocks and a high of 0.0872 between the Lostine and Rapid River stocks (Table 14). Excluding six comparisons involving the Nez Perce FH stock, all tests of genetic differentiation were statistically significant ($p < .01$). Differentiation among the two fall Chinook Salmon stocks was very low ($F_{ST} = 0.0045$) and genotypic differentiation was not statistically significant.

Effective Population Size

Effective population size (N_e) for steelhead hatchery broodstock in SY2019 ranged from a low of 40.2 for the E.F. Salmon River broodstock to a high of 258.0 for the Touchet R. broodstock (Table 15).

Effective population size for spring/summer Chinook Salmon hatchery broodstock in SY2019 ranged from a low of 95.1 for Grande Ronde to a high of 1,350.4 for Nez Perce FH (Table 16). Effective population size for the two fall Chinook Salmon hatchery broodstocks were large with the Nez Perce stock estimated at 1,270.6 and the Lyons Ferry stock estimated at 1,642.6 (Table 17).

Sex Markers

The sex-specific assay for steelhead matched phenotypic sex in 99.5% of the samples (Table 7). In the instances ($n = 22$) in which genetically-determined sex did not correspond to the phenotypic sex, it was slightly more likely that phenotypic females were misidentified as males than the opposite. The assay either failed to genotype or provided ambiguous results for 0.6% of the samples.

The sex-specific assay for spring/summer Chinook Salmon matched phenotypic sex in 99.9% of the samples (Table 8). In the instances ($n = 10$) in which genetically-determined sex did

not correspond to the phenotypic sex, all were phenotypic females misidentified as males. The assay either failed to genotype or provided ambiguous results for 0.4% of the samples.

The sex-specific assay for fall Chinook Salmon matched phenotypic sex in 99.9% of the samples (Table 9). The two discrepancies were split with one of each type. The assay either failed to genotype or provided ambiguous results for 0% 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 met or exceeded 90% in steelhead broodstocks. Stock-level tag rates were greater than 90% in all but one of the spring/summer Chinook Salmon broodstocks. Both Nez Perce and Lyons Ferry fall Chinook hatchery broodstocks were tagged at 95% or greater for SY2019.

Microhaplotype Discovery

Heterozygosity of the microhaplotypes was higher than that of just the corresponding SNPs. In steelhead, expected heterozygosity of the microhaplotypes and SNPs had range of 0.29-0.34 and 0.23-0.26, respectively (Table 18). In spring/summer Chinook Salmon, expected heterozygosity of the microhaplotypes and SNPs had range of 0.25-0.34 and 0.23-0.26 respectively (Table 19). In fall Chinook Salmon, expected heterozygosity in both stocks of the microhaplotypes and SNPs was 0.33 and 0.26, respectively (Table 20).

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 twelve 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. 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 close to or

above 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 SNPs vary depending on the technique used to genotype them. For methods that rely on genotyping-by-sequencing, error rates are also dependent upon the sequencing depth. With genotyping-by-sequencing, SNP genotyping error rates have been estimated at less than 1% with depths greater than 30 reads per locus (Fountain et al. 2016). With our GT-seq panels, mean depth is typically ~150 reads per locus, suggesting that our error rates should be lower. 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.

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., Powell spring/summer Chinook Salmon), N_E is frequently fairly large (>150) for hatchery broodstock populations spawned annually in the Snake River basin. Only the E.F. Salmon River

and Upper Salmon River B-run steelhead stocks were estimated to have N_E <50, which based on genetic theory would put the population at risk of inbreeding (Franklin 1980).

Sex Markers

The steelhead and Chinook Salmon sex markers continue to provide an accurate (~99%) method of identifying phenotypic sex in 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 the Snake River basin was 95.6% for steelhead, 97.0% for spring/summer Chinook, and 96.7% for fall Chinook.

Microhaplotype Discovery

The added variation of the microhaplotypes we discovered will improve accuracy of relationship inference and increase the opportunity to utilize single parent assignments in future analyses.

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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, the PBT baselines were used to determine: 1) Origin of samples from sport fisheries in the Columbia River during migration year 2019 (SY2020), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2019 (SY2020), 3) Origin of samples from various sport fisheries in Idaho in migration year 2019 (SY2020), 4) Age composition and origin of the SY2019 broodstocks, and 5) stock composition of returning adults during SY2020 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2019), 2) Age composition and origin of SY2019 broodstocks, 3) stock composition of returning adults during SY2019 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 2019 (SY2020). A total of 250 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) 2019 (e.g., spawn year 2020). A total of 204 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 Idaho

IDFG collected samples of steelhead harvested in the SY2019 sport fishery from various river systems including the Clearwater and Salmon. A total of 747 samples were processed for

PBT assignment. A more detailed description of this project is in LeCheminant et al. (*In prep*). Results from a previous year are available in Warren et al. (2018).

Age Composition of SY2019 Steelhead Broodstock

PBT was used to determine age composition of steelhead broodstocks in Idaho by assigning the SY2019 broodstocks back to all previously sampled broodstocks, thereby identifying the age of each fish. A total of 4,768 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 2018 and spring of 2019 (LeCheminant et al. *In prep*) and 2,542 samples were analyzed with PBT. Results from a previous year are available in Warren et al. (2018).

Chinook Salmon Sport Fishery in Idaho

Fisheries managers within IDFG continued PBT sampling of Chinook Salmon harvested in the sport fisheries in SY2019. A total of 480 samples representative of the various time strata and river sections were analyzed with PBT. Complete methodology and results are presented in Belnap et al. (*In prep*). Results for a previous year are available in Sullivan et al. (2018).

Age Composition of SY2019 Chinook Salmon Broodstock

PBT was used to determine age composition of Chinook Salmon broodstocks in Idaho by assigning the SY2019 broodstocks back to previously sampled broodstocks, thereby identifying the age of each fish. A total of 9,360 hatchery-origin broodstock samples 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 2019 (Belnap et al. *In prep*) and 2,377 samples were analyzed with PBT. Results for a previous year are available in Sullivan et al. (2018).

RESULTS

Steelhead Sport Fisheries in Columbia River

Of the 249 samples analyzed, 96 assigned to the PBT baseline. 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 203 samples analyzed from clipped and unclipped steelhead, 109 assigned to the PBT baseline. A detailed breakdown of stock composition in this fishery is presented in Byrne et al. (*In prep*).

Steelhead Sport Fisheries in Idaho

Of the 967 samples analyzed, 913 assigned. A detailed breakdown of stock composition in this fishery is presented in LeCheminant et al. (*In prep*).

Stock Composition of Adult Steelhead at Lower Granite Dam

Of the samples collected, 2,690 were analyzed after removing duplicated samples or samples that failed to genotype. Of these, 2,494 assigned to the baseline. A summary of stock composition and age will be provided in an upcoming IDFG technical report (LeCheminant et al. *In prep*).

Age Composition of SY2019 Steelhead Broodstock

Of the samples collected, 4,768 were analyzed with PBT after excluding duplicate and ungenotyped samples. Of these, 4,412 assigned 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, 448 were analyzed after removal of samples that failed to genotype. Of these, 387 received a PBT assignment. A detailed breakdown of stock and age composition of the harvest in this fishery is presented in Belnap et al. (*In prep*).

Age Composition of SY2019 Chinook Salmon Broodstock

Of the samples collected, 9,395 were analyzed after removal of samples that failed to genotype. Of these, 8,683 assigned (92.4%) 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, 2,373 were analyzed after removing samples that failed to genotype. Of these, 2,271 assigned to the baseline. A summary of stock composition and age will be provided in an upcoming IDFG technical report (Belnap 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 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 LeCheminant 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 Belnap 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.

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TABLES

Table 1. Sample sizes and genotyping completion rate of SY2019 steelhead broodstock. Samples with ≥10% failed SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock. Note that Lyons Ferry did not spawn a Cottonwood Creek broodstock in SY2019.

Stock	Samples	Genotyped (%)	Tagging Rate (%)
Dworshak	1149	1120 (97.5)	95.0
E.F. Salmon R.	25	25 (100.0)	100.0
Little Sheep Cr.	126	124 (98.4)	96.9
Oxbow	470	451 (96.0)	92.1
Pahsimeroi	1004	993 (98.9)	97.8
S.F. Clearwater	211	208 (98.6)	97.2
Sawtooth	864	833 (96.4)	93.0
Touchet R.	29	29 (100.0)	100.0
Tucannon R.	58	57 (98.3)	96.6
Up. Sal. R. B-run	274	274 (100.0)	100.0
Wallowa	655	654 (99.8)	99.7
Total	4865	4768 (98.0)	96.0

Table 2. Sample sizes and genotyping completion rate of SY2019 spring/summer Chinook Salmon broodstock. Samples with ≥10% failed SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

Stock	Samples	Genotyped (%)	Tagging Rate (%)
Catherine Cr.	93	93 (100.0)	100.0
Dworshak	1155	1154 (99.9)	99.8
Grande Ronde	163	163 (100.0)	100.0
Imnaha	261	256 (98.1)	96.2
Johnson Cr.	62	62 (100.0)	100.0
Kooskia	870	850 (97.7)	95.5
Lookingglass Cr.	155	154 (99.4)	98.7
Lostine	145	138 (95.2)	90.6
Nez Perce FH	17	17 (100.0)	100.0
Pahsimeroi	473	472 (99.8)	99.6
Powell	81	81 (100.0)	100.0
Rapid River	2994	2991 (99.9)	99.8
S.F. Clearwater	981	981 (100.0)	100.0
S.F. Salmon	1106	1093 (98.8)	97.7
Sawtooth	786	776 (98.7)	97.5
Tucannon	85	79 (92.9)	86.4
Total	9427	9360 (99.3)	98.6

Table 3. Sample sizes and genotyping completion rate of SY2019 fall Chinook Salmon broodstock. Samples with ≥10% failed SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

Stock	Samples	Genotyped (%)	Tagging Rate (%)
Lyons Ferry	1673	1638 (97.9)	95.9
Nez Perce	687	686 (99.9)	99.7
Total	2360	2324 (98.5)	97.0

Table 4. List of loci that failed to genotype in >5% of the steelhead SY2019 samples.

Locus	Fail Rate (%)
OMS00169	6
Omy_113490.159	6
Omy_arp.630	7
Ocl_gshpx.357	7
Omy_RAD27740.55	7
OMS00095	7
Omy_hsp70aPro.329	7
Omy_GREB1_05	8
Omy_114315.438	8
Omy_101554.306	8
OMS00039	9
Omy_RAD366.7	9
OMS00074	9
OMS00129	9
OMS00003	10
Omy_RAD55997.10	10
Omy_RAD78147.27	10
OMS00058	10
Omy_Ogo4.212	11
Omy_RAD19578.59	11
Omy_99300.202	11
Omy_RAD78502.57	12
Omy_RAD36848.7	12
Omy_109894.185	13
Omy_RAD43694.41	13
Omy_RAD66402.36	14
Omy_RAD9004.13	15
OMY1011SNP	18
Omy_cd28.130	18
Omy_cd59b.112	19
Omy_impa1.55	21
Omy_RAD62596.38	24
Omy_RAD98715.53	27
OMS00180	32
Omy_ndk.152	41
Omy_118205.116	50
Omy_104569.114	52

Table 5. List of loci that failed to genotype in >5% of the Spring/Summer Chinook SY2019 samples.

Locus	Fail Rate (%)
Ots28_11205423	6
Ots_u07.25.325	7
Ots_TNF	7
Ots_NAML12.SNP1	7
Ots28_11071377	7
Ots_NOD1	9
Ots28_11207428	9
Ots4_41638710	10
Ots28_11210919	11
Ots29_18791740	11
Ots28_11077016	11
Ots_105385.421	12
Ots_131906.141	12
Ots_pigh.105	13
Ots_U5121.34	13
Ots19_46172427	16
Ots28_11143508	18
Ots_CHI06027687_143477	18
Ots_105401.325	19
Ots_MHC2	20

Table 6. List of loci that failed to genotype >5% of the fall Chinook SY2019 samples.

Locus	Fail Rate (%)
Ots_112208.722	6
Ots_crRAD17527.58	7
Ots_u202.161	11
Ots_GPDH.338	12
Ots_crRAD55475.26	13
Ots_u07.57.120	13
Ots_sept9.78	14
Ots_129170.683	14
Ots_crRAD36072.29	15
Ots_u07.07.161	26
Ots_MetA	46

Table 7. Results of comparisons between phenotypic sex and genetically determined sex using the sex-specific assay for SY2019 steelhead (Omy1_2SEXY).

Stock	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotype	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Dworshak	1120	1 (0.1%)	1119 (99.9%)	1116 (99.7%)	3 (0.3%)	2	1	451	669
E.F. Salmon R.	25	0 (0.0%)	25 (100.0%)	25 (100.0%)	0 (0.0%)	0	0	13	12
Little Sheep Cr.	124	1 (0.8%)	123 (99.2%)	123 (100.0%)	0 (0.0%)	0	0	61	63
Oxbow	451	3 (0.7%)	448 (99.3%)	442 (98.7%)	6 (1.3%)	2	4	225	226
Pahsimeroi	993	5 (0.5%)	988 (99.5%)	986 (99.8%)	2 (0.2%)	0	2	493	500
S.F. Clearwater	208	2 (1.0%)	206 (99.0%)	206 (100.0%)	0 (0.0%)	0	0	91	117
Sawtooth	833	16 (1.9%)	817 (98.1%)	807 (98.8%)	10 (1.2%)	0	10	417	416
Touchet R.	29	0 (0.0%)	29 (100.0%)	29 (100.0%)	0 (0.0%)	0	0	15	14
Tucannon R.	57	0 (0.0%)	57 (100.0%)	57 (100.0%)	0 (0.0%)	0	0	23	34
Up. Sal. R. B-run	274	1 (0.4%)	273 (99.6%)	273 (100.0%)	0 (0.0%)	0	0	105	169
Wallowa	654	0 (0.0%)	654 (100.0%)	653 (99.8%)	1 (0.2%)	1	0	321	333

Table 8. 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 SY2019 broodstocks.

Stock	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotype	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Catherine Cr.	93	0 (0.0%)	93 (100.0%)	93 (100.0%)	0 (0.0%)	0	0	49	44
Dworshak	1154	10 (0.9%)	1144 (99.1%)	1143 (99.9%)	1 (0.1%)	0	1	529	625
Grande Ronde	163	1 (0.6%)	162 (99.4%)	162 (100.0%)	0 (0.0%)	0	0	90	73
Imnaha	256	0 (0.0%)	256 (100.0%)	256 (100.0%)	0 (0.0%)	0	0	117	139
Johnson Cr.	62	0 (0.0%)	62 (100.0%)	62 (100.0%)	0 (0.0%)	0	0	29	33
Kooskia	850	15 (1.8%)	835 (98.2%)	830 (99.4%)	5 (0.6%)	0	5	394	456
Lookingglass Cr.	154	1 (0.6%)	153 (99.4%)	153 (100.0%)	0 (0.0%)	0	0	78	76
Lostine	138	0 (0.0%)	138 (100.0%)	138 (100.0%)	0 (0.0%)	0	0	67	71
Nez Perce FH	17	0 (0.0%)	17 (100.0%)	17 (100.0%)	0 (0.0%)	0	0	8	9
Pahsimeroi	472	0 (0.0%)	472 (100.0%)	472 (100.0%)	0 (0.0%)	0	0	210	262
Powell	81	2 (2.5%)	79 (97.5%)	78 (98.7%)	1 (1.3%)	0	1	41	40
Rapid River	2991	0 (0.0%)	2991 (100.0%)	2991 (100.0%)	0 (0.0%)	0	0	1495	1496
S.F. Clearwater	981	7 (0.7%)	974 (99.3%)	971 (99.7%)	3 (0.3%)	0	3	495	486
S.F. Salmon	1093	1 (0.1%)	1092 (99.9%)	1092 (100.0%)	0 (0.0%)	0	0	560	533
Sawtooth	776	0 (0.0%)	776 (100.0%)	776 (100.0%)	0 (0.0%)	0	0	391	385
Tucannon	79	0 (0.0%)	79 (100.0%)	79 (100.0%)	0 (0.0%)	0	0	34	45

Table 9. 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 SY2019 broodstocks.

Stock	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotype	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Lyons Ferry	1638	0 (0.0%)	1638 (100.0%)	1637 (99.9%)	1 (0.1%)	1	0	503	1135
Nez Perce	686	0 (0.0%)	686 (100.0%)	685 (99.9%)	1 (0.1%)	0	1	264	422

Table 10. Average observed and expected heterozygosity with associated standard error of hatchery steelhead stocks for SY2019.

Stock	Avg. Het. (Obs)	SE	Avg. Het. (Exp)	SE
Dworshak	0.23	0.01	0.24	0.01
E.F. Salmon R.	0.25	0.01	0.25	0.01
Little Sheep Cr.	0.24	0.01	0.25	0.01
Oxbow	0.25	0.01	0.26	0.01
Pahsimeroi	0.25	0.01	0.26	0.01
S.F. Clearwater	0.23	0.01	0.23	0.01
Sawtooth	0.24	0.01	0.25	0.01
Touchet R.	0.25	0.01	0.26	0.01
Tucannon R.	0.26	0.01	0.26	0.01
Up. Sal. R. B-run	0.23	0.01	0.23	0.01
Wallowa	0.25	0.01	0.26	0.01

Table 11. Average observed and expected heterozygosity with associated standard error of hatchery spring/summer Chinook Salmon stocks in SY2019.

Stock	Avg. Het. (Obs)	SE	Avg. Het. (Exp)	SE
Catherine Cr.	0.21	0.01	0.21	0.01
Dworshak	0.21	0.01	0.21	0.01
Grande Ronde	0.2	0.01	0.21	0.01
Imnaha	0.24	0.01	0.23	0.01
Johnson Cr.	0.21	0.01	0.22	0.01
Kooskia	0.21	0.01	0.21	0.01
Lookingglass Cr.	0.21	0.01	0.21	0.01
Lostine	0.23	0.01	0.23	0.01
Nez Perce FH	0.2	0.01	0.2	0.01
Pahsimeroi	0.22	0.01	0.22	0.01
Powell	0.22	0.01	0.22	0.01
Rapid River	0.19	0.01	0.2	0.01
S.F. Clearwater	0.21	0.01	0.21	0.01
S.F. Salmon	0.21	0.01	0.21	0.01
Sawtooth	0.21	0.01	0.21	0.01
Tucannon	0.22	0.01	0.22	0.01

Table 12. Average observed and expected heterozygosity with associated standard error of hatchery fall Chinook Salmon stocks in SY2019.

Stock	Avg. Het. (Obs)	SE	Avg. Het. (Exp)	SE
Lyons Ferry	0.27	0.01	0.27	0.01
Nez Perce	0.27	0.01	0.28	0.01

Table 13. Population structure (F_{ST}) (below diagonal) among steelhead hatchery stocks sampled in SY2019. P-values are shown above the diagonal. Asterisks (*) indicate that the genotypic differentiation (exact G test) was highly significant and the combined p-value (Fisher's method) could not be calculated.

Stock	Oxbow	Pahsimeroi	Up. Sal. R. B-run	Sawtooth	E.F. Salmon R.	Tucannon R.	Touchet R.	Wallowa	Little Sheep Cr.	Dworshak	S.F. Clearwater
Oxbow	---	*	*	*	*	*	*	*	*	*	*
Pahsimeroi	0.008	---	*	*	*	*	*	*	*	*	*
Up. Sal. R. B-run	0.066	0.067	---	*	*	*	*	*	*	*	*
Sawtooth	0.010	0.009	0.066	---	*	*	*	*	*	*	*
E.F. Salmon R.	0.022	0.021	0.060	0.023	---	*	*	*	*	*	*
Tucannon R.	0.022	0.024	0.069	0.025	0.029	---	0.001	*	*	*	*
Touchet R.	0.027	0.028	0.072	0.030	0.034	0.009	---	*	*	*	*
Wallowa	0.021	0.022	0.062	0.022	0.029	0.017	0.021	---	*	*	*
Little Sheep Cr.	0.026	0.027	0.078	0.030	0.036	0.028	0.027	0.028	---	*	*
Dworshak	0.067	0.069	0.019	0.068	0.055	0.061	0.060	0.059	0.071	---	*
S.F. Clearwater	0.063	0.064	0.020	0.063	0.053	0.059	0.058	0.056	0.069	0.004	---

Table 14. Population structure (F_{ST}) (below diagonal) among spring/summer Chinook Salmon hatchery stocks sampled in SY2019. P-values are shown above the diagonal. Asterisks (*) indicate that the genotypic differentiation (exact G test) was highly significant and the combined p-value (Fisher's method) could not be calculated.

Stock	Rapid River	Tucan-non	S.F. Salmon	Pahsimeroi	Sawtooth	S.F. Clear-water	Kooskia	Dworshak	Nez Perce FH	Powell	Imnaha	Lostine	Grande Ronde	Looking-glass Cr.	Catherine Cr.	Johnson Cr.
Rapid River	---	*	*	*	*	*	*	*	< .001	*	*	*	*	*	*	*
Tucannon	0.046	---	*	*	*	*	*	*	< .001	*	*	*	*	*	*	*
S.F. Salmon	0.040	0.043	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Pahsimeroi	0.067	0.069	0.030	---	*	*	*	*	*	*	*	*	*	*	*	*
Sawtooth	0.040	0.053	0.020	0.027	---	*	*	*	*	*	*	*	*	*	*	*
S.F. Clearwater	0.015	0.023	0.028	0.055	0.032	---	*	*	0.999	*	*	*	*	*	*	*
Kooskia	0.020	0.021	0.031	0.060	0.036	0.004	---	*	1	*	*	*	*	*	*	*
Dworshak	0.019	0.021	0.029	0.057	0.032	0.002	0.002	---	1	*	*	*	*	*	*	*
Nez Perce FH	0.018	0.018	0.021	0.049	0.025	0.000	0.000	0.000	---	*	*	*	0.050	0.667	0.725	*
Powell	0.045	0.046	0.002	0.028	0.018	0.032	0.035	0.033	0.025	---	*	*	*	*	*	*
Imnaha	0.066	0.069	0.031	0.031	0.038	0.060	0.063	0.061	0.053	0.025	---	*	*	*	*	*
Lostine	0.087	0.075	0.038	0.042	0.049	0.072	0.075	0.072	0.061	0.034	0.020	---	*	*	*	*
Grande Ronde	0.016	0.031	0.038	0.062	0.040	0.011	0.012	0.013	0.011	0.043	0.064	0.080	---	*	*	*
Lookingglass Cr.	0.020	0.025	0.027	0.050	0.032	0.009	0.010	0.009	0.006	0.030	0.049	0.060	0.010	---	*	*
Catherine Cr.	0.020	0.026	0.034	0.057	0.036	0.011	0.012	0.012	0.010	0.038	0.060	0.075	0.014	0.008	---	*
Johnson Cr.	0.070	0.068	0.020	0.041	0.036	0.056	0.062	0.059	0.050	0.016	0.026	0.034	0.066	0.051	0.063	---

Table 15. Estimates of effective population size (N_E) and 95% confidence intervals for steelhead hatchery stocks in SY2019.

Stock	Ne	CI (95%)
Oxbow	108.7	96.7 - 122.3
Pahsimeroi	181.7	171.1 - 193.1
Up. Sal. R. B-run	49.2	42.5 - 57.0
Sawtooth	248.2	230.5 - 267.6
E.F. Salmon R.	40.2	20.9 - 149.2
Tucannon R.	58.6	38.1 - 105.1
Touchet R.	258.0	92.3 - Infinite
Wallowa	222.4	205.0 - 241.6
Little Sheep Cr.	90.6	70.8 - 120.1
Dworshak	172.8	161.8 - 184.6
S.F. Clearwater	101.9	82.8 - 127.9

Table 16. Estimates of effective population size and 95% confidence intervals for SY2019 spring/summer Chinook Salmon hatchery stocks.

Stock	Ne	CI (95%)
Rapid River	532.8	509.0 - 557.6
Tucannon	143.0	92.2 - 278.0
S.F. Salmon	210.5	196.0 - 226.1
Pahsimeroi	200.9	175.0 - 232.2
Sawtooth	248.5	224.8 - 275.5
S.F. Clearwater	241.0	222.7 - 261.1
Kooskia	306.6	278.7 - 338.2
Dworshak	486.1	445.8 - 531.4
Nez Perce FH	1350.4	141.5 - Infinite
Powell	146.2	99.8 - 250.2
Imnaha	193.8	160.5 - 239.1
Lostine	157.0	119.4 - 219.8
Grande Ronde	95.1	78.4 - 117.5
Lookingglass Cr.	160.8	122.8 - 222.7
Catherine Cr.	118.6	84.6 - 184.1
Johnson Cr.	631.3	266.7 - Infinite

Table 17. Estimates of effective population size and 95% confidence intervals for fall Chinook Salmon SY2019 hatchery stocks.

Stock	Ne	CI (95%)
Nez Perce	1270.6	1048.1 - 1593.1
Lyons Ferry	1642.6	1485.1 - 1829.3

Table 18. Average observed and expected heterozygosity of hatchery steelhead stocks for SY2019 at 92 loci comparing the originally targeted SNPs with the loci treated as microhaplotypes.

	SNP				Microhaplotype			
Pop	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**
Dworshak	0.227	0.019	0.220	0.019	0.290	0.020	0.288	0.020
E.F. Salmon R.	0.256	0.018	0.254	0.020	0.326	0.020	0.321	0.021
Little Sheep Cr.	0.245	0.019	0.246	0.020	0.322	0.020	0.325	0.021
Oxbow	0.265	0.017	0.261	0.017	0.341	0.019	0.345	0.020
Pahsimeroi	0.256	0.017	0.249	0.017	0.333	0.018	0.332	0.018
S.F. Clearwater	0.228	0.019	0.221	0.019	0.291	0.020	0.288	0.020
Sawtooth	0.249	0.018	0.243	0.017	0.326	0.019	0.327	0.019
Touchet R.	0.240	0.018	0.235	0.018	0.309	0.019	0.303	0.020
Tucannon R.	0.249	0.018	0.240	0.018	0.324	0.019	0.324	0.020
Up. Sal. R. B-run	0.229	0.019	0.228	0.019	0.289	0.020	0.291	0.021
Wallowa	0.257	0.018	0.248	0.017	0.336	0.019	0.332	0.019

Table 19. Average observed and expected heterozygosity of hatchery spring/summer Chinook Salmon stocks for SY2019 at 53 loci comparing the originally targeted SNPs with the loci treated as microhaplotypes.

	SNP				Microhaplotype			
Pop	Avg. Het. (Exp)	SE	Avg. Het. (Obs)	SE	Avg. Het. (Exp)	SE	Avg. Het. (Obs)	SE
Catherine Cr.	0.255	0.025	0.251	0.024	0.340	0.026	0.340	0.026
Dworshak	0.247	0.024	0.246	0.024	0.333	0.027	0.333	0.027
Grande Ronde	0.243	0.025	0.242	0.025	0.333	0.026	0.334	0.027
Kooskia	0.247	0.024	0.245	0.024	0.327	0.027	0.329	0.028
Lookingglass Cr.	0.248	0.024	0.253	0.025	0.331	0.027	0.342	0.028
Lostine	0.257	0.025	0.260	0.026	0.324	0.028	0.333	0.029
Nez Perce FH	0.230	0.025	0.224	0.026	0.257	0.033	0.321	0.049
Pahsimeroi	0.225	0.025	0.224	0.025	0.308	0.027	0.308	0.027
Powell	0.244	0.024	0.243	0.024	0.330	0.027	0.333	0.028
Rapid River	0.240	0.025	0.240	0.025	0.331	0.026	0.336	0.027
S.F. Clearwater	0.249	0.025	0.246	0.024	0.341	0.027	0.350	0.029
S.F. Salmon	0.238	0.024	0.239	0.025	0.320	0.027	0.322	0.027
Sawtooth	0.231	0.026	0.231	0.026	0.317	0.028	0.321	0.028
Tucannon	0.249	0.023	0.261	0.026	0.325	0.027	0.341	0.029
Imnaha	0.260	0.024	0.257	0.024	0.346	0.025	0.346	0.025
Johnson Cr.	0.237	0.025	0.243	0.026	0.317	0.027	0.319	0.028

Table 20. Average observed and expected heterozygosity of hatchery fall Chinook Salmon stocks for SY2018 adults at Lower Granite Dam at 53 loci comparing the originally targeted SNPs with the loci treated as microhaplotypes.

	SNP				Microhaplotype			
Pop	Avg. Het. (Exp)	SE	Avg. Het. (Obs)	SE	Avg. Het. (Exp)	SE	Avg. Het. (Obs)	SE
Nez Perce	0.257	0.010	0.242	0.010	0.328	0.021	0.329	0.021
Lyons Ferry	0.256	0.010	0.241	0.010	0.335	0.021	0.337	0.023

FIGURES

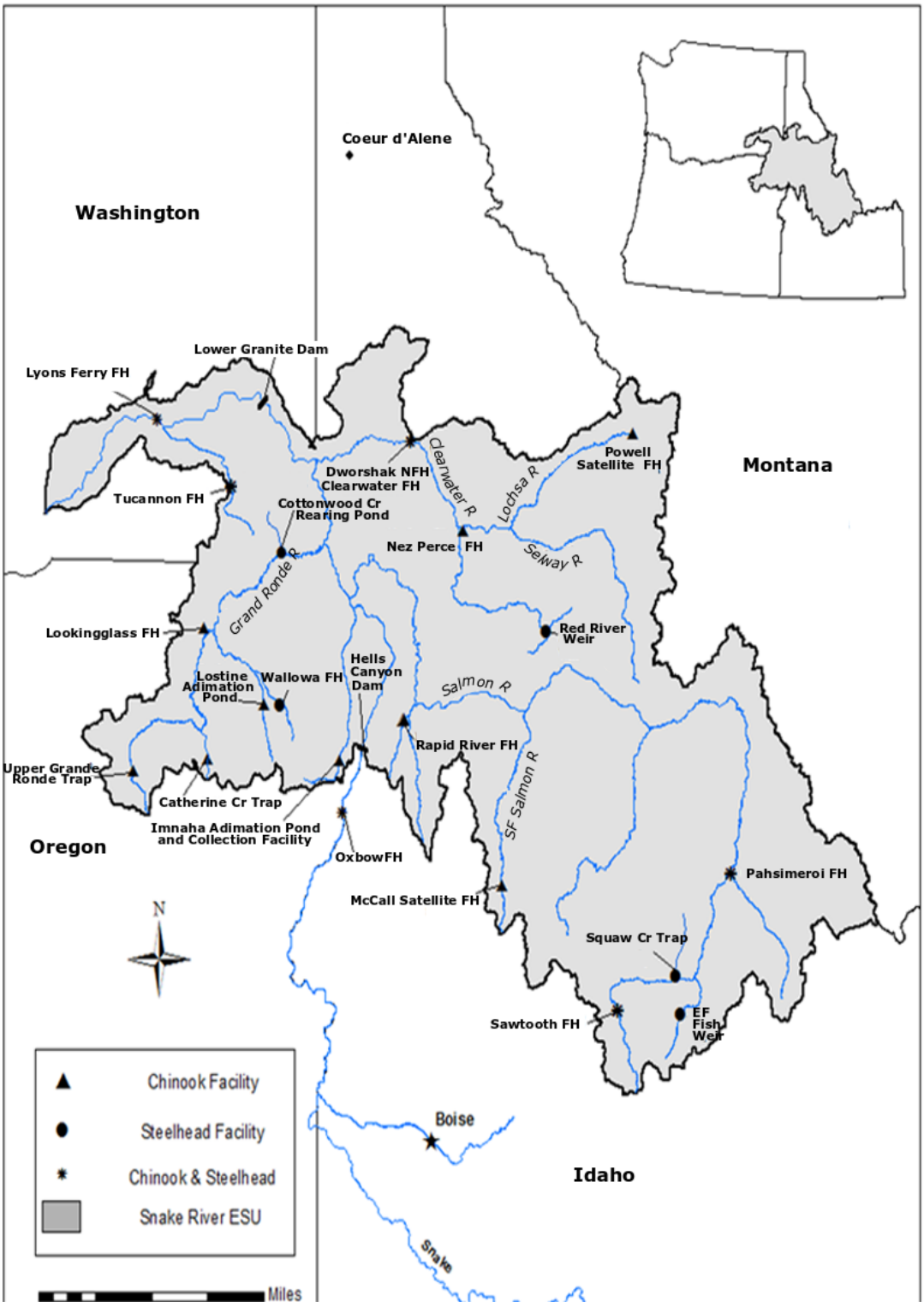

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

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