FISHERY RESEARCH

PARENTAGE BASED TAGGING OF SNAKE RIVER HATCHERY STEELHEAD AND CHINOOK SALMON

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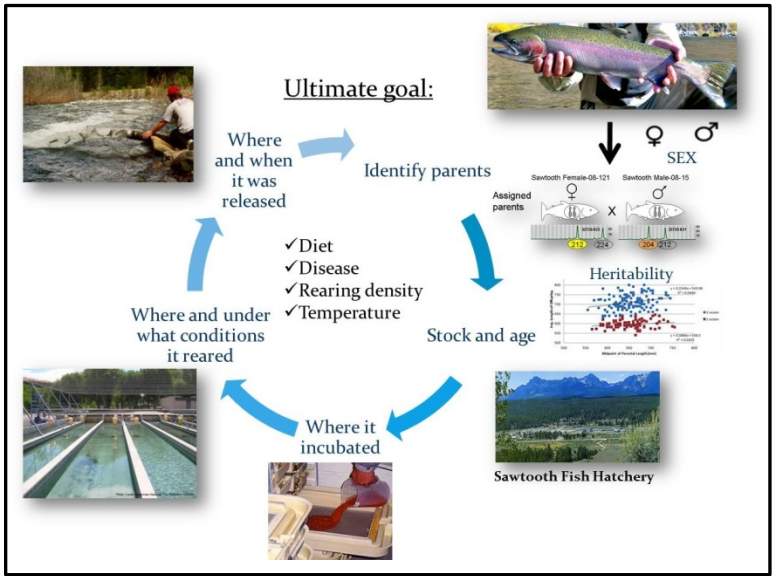

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Parentage Based Tagging of Snake River Hatchery Steelhead and Chinook Salmon

Project Progress Report

2015 Annual Report

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TABLE OF CONTENTS

Page

Table of Contents, continued.

Page

Table of Contents, continued.

Page

LIST OF TABLES

List of Tables, continued.

Page

LIST OF FIGURES

Page

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 July 1, 2014 to June 30, 2015 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 (~5,500 individuals annually), spring/summer Chinook Salmon (~8,000 individuals annually) and Fall Chinook Salmon (~2,700) 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 spring/summer Chinook Salmon broodstocks sampled in the Snake River basin from spawn year (SY) 2014 (Objective 2). In addition, summary data for Chinook Salmon broodstocks from SY2014 are presented. 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 spring/summer 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 2014 (SY2015), 2.) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2014 (SY2015), 3) Origin of samples from sport fisheries in the lower Snake River in migration years 2013 and 2014 (SY2014 and SY2015), 4) Origin of samples from various sport fisheries in Idaho in migration year 2013 (SY2014), 5) Parentage of SY2015 Upper Salmon B-run broodstock for real-time management of spawning, and 6) Correction of PIT expansions for SY2014 Sawtooth broodstock.

For Chinook Salmon, the PBT baselines were used to determine: 1) Parentage of returning Jacks (SY2015) for a density rearing study at Dworshak National Fish Hatchery, 2) Origin of samples from various sport fisheries in Idaho in (SY2014), 3) Parentage of Clearwater broodstock for real-time management of spawning, and 4) Age composition of SY2014 broodstocks.

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 not genotyped as they do not contribute to the offspring.

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 2015, we have collected and inventoried approximately 5,500 genetic samples from the steelhead broodstock (Table 1) spawned in the Snake River basin during spawn year (SY) 2014, and approximately 9,000 samples (Table 2) from spring/summer Chinook Salmon broodstock spawned in the Snake River basin during SY2014. We also report on fall Chinook collected from the Lyons Ferry and Nez Perce Tribal Fish hatcheries for SY2011 – SY2014 (N = ~10,000; 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 SY2014.

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 2014. Primer and probe sequence information for these markers are available on www.FishGen.net: CRITFC/IDFG Chinook Salmon 96 PBT v5.1 and CRITFC/IDFG Steelhead 96 PBT v5.1.

Beginning in SY2015, our lab will be adopting Genotyping-in-Thousands (GT-seq) protocols developed by the CRITFC genetics lab (Campbell et al. 2014) to genotype PBT baselines. 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 will sequence 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 will continue 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.

During the fifth year of this project, IDFG and CRITFC labs extracted and genotyped all samples for steelhead and Chinook Salmon broodstocks (~8,000 IDFG, ~8,000 CRITFC = ~16,000 total samples).

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 and amplification and SNP genotyping using multiplex 5'-nuclease reactions followed the methods described in Matala et al. (2011). DNA was extracted using the Nexttec Genomic DNA Isolation Kit from XpressBio (Thurmont, Maryland) or Qiagen DNeasy (Valencia, California). Prior to DNA amplification of SNP loci using primer-probe sets (fluorescent tags), an initial polymerase chain reaction (PCR) "pre-amp" was implemented using whole genomic DNA to jumpstart SNP amplification via increased copy number of target DNA regions. The PCR conditions for the pre-amp step were as follows: an initial mixing step of 95°C for 15 min, followed by 14 cycles of 95°C for 15 seconds and 60°C for four minutes, ending with a final 4°C dissociation step. For steelhead, all individuals were genotyped at 95 SNPs and a Y-specific allelic discrimination assay that differentiates sex. For Chinook Salmon, all individuals were genotyped at 95 SNPs (including one mtDNA SNP) and a Y-specific allelic discrimination assay that differentiates sex. Genotyping was performed using Fluidigm 96.96 Dynamic Array

IFCs (chips). For each genotyping run, 96 samples (including an extraction negative control, a PCR negative control, and a PCR positive control) and 96 TaqMan SNP assays were either hand-pipetted or auto-pipetted onto the 96.96 chips. Sample cocktail and SNP assay cocktail recipes are available by request from mike.ackerman@idfg.idaho.gov. Each 96.96 chip was pressurized to load the DNA and SNP assays into the array using a Fluidigm IFC Controller HX. SNP amplification on the 96.96 chips were performed using either an Eppendorf Stand-Alone Thermal Cycler (protocol: thermal mixing step of 50°C for 2 min, 70°C for 30 min, and 25°C for 10 min, a hot-start step of 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec and 60°C for 60 sec, and a final cool-down step of 25°C for 10 min) or a Fluidigm FC1 Fast-cycler (protocol: thermal mixing step of 70°C for 30 min and 25°C for 10 min, a hot-start step of 95°C for 60 sec, followed by 50 cycles of 95°C for 5 sec and 25°C for 25 sec, and a final cool-down step of 25°C for 10 min). Chips were imaged on a Fluidigm EP1 system and analyzed and scored using the Fluidigm SNP Genotyping Analysis Software version 3.1.1.

Standardized parental genotypes were stored on a Progeny database server housed at 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 small P-value suggests that there is a deficiency of heterozygotes which may be due to null alleles at the locus. For loci that exhibited a p-value less than 0.004 (corrected), the same program was used to estimate the proportion of null alleles per locus. Significance thresholds were adjusted using the modified B-Y Method proposed by Narum (2006). Basic diversity indices were calculated for the brood years. This included estimates of genetic diversity from minor allele frequency (MAF), and average observed (H_O) and expected (H_E) heterozygosity using Microsatellite Toolkit v.3.1.1 (Park 2001), 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 $\alpha = 0.05$.

This is the first year that we report on the genotyping of fall Chinook Salmon broodstock at the Lyons Ferry and Nez Perce Tribal fish hatcheries. PBT baselines are now complete for both hatcheries for spawn years 2011 – 2014. We report basic diversity indices for these populations/years. We also evaluate the accuracy of our current panel of SNP markers for PBT for these two fall Chinook Salmon stocks. This was accomplished using a simulated offspring/juvenile dataset produced using the software Mykiss (Kalinowski 2009) and the parentage software program Cervus 3.07 (Kalinowski 2007).

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 the parent's 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. For spawn year 2014, average realized PBT tagging rates at the level of release site were approximately 90%.

RESULTS

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 SY2014, all 5,626 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 5,626 samples, 5,588 (99.3%) were genotyped with an acceptable level of missing data (Table 4). In this final SY2014 PBT baseline comprising the remaining 5,588 samples, there were just 2,543 missing genotypes due to SNP failure out of a possible 530,860 genotypes. This resulted in missing data for just 0.5% of the genotypes.

For spring/summer Chinook Salmon SY2014, all 9,949 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 9,949 samples, 9,850 (99.0%) were genotyped with an acceptable level of missing data (Table 5). In this final SY2014 PBT baseline comprising the remaining 9,850 samples, there were just 3,199 missing genotypes due to SNP failure out of a possible 935,750 genotypes. This resulted in missing data for just 0.3% of the genotypes.

For fall Chinook Salmon SY2010 - 2014, all 10,123 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 10,123 samples, 9,956 (99.0%) were genotyped with an acceptable level of missing data (Table 6). In this final SY2010 - 2014 PBT baseline comprising the remaining 9,956 samples, there were just 6,138 missing genotypes due to SNP failure out of a possible 945,820 genotypes. This resulted in missing data for just 0.6% of the genotypes.

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 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: Catherine Creek = 88.2%; Lostine = 73.6%, and Tucannon = 89.1%. These three stocks represent less than 2.5% 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 Spawn Years 2011 – 2014, expect for SY2011 Nez Perce which was tagged at a rate of 89.2%.

Poor Performing Loci

Of the samples that genotyped with <10 missing SNPs, most SNPs had very high genotyping success. For SY2014 steelhead, only one locus failed to genotype at >3% of samples (Omy_105385-406 = 27.7%). For SY2014 spring/summer Chinook Salmon, there was also only a single locus that failed at >3% of the samples (Ots_110495-380 = 27.8%). For SY2010 – SY2014 Fall Chinook, there were six loci that failed at >3% of the samples (Ots_OTALDBINT1-SNP1 = 3.5%; Ots_101704-143 = 10.2%; Ots_112301-43 = 8.2%; Ots_GCSH-A1 = 4.7%; Ots_pigh-105-A1 = 5.7%; and Ots_u07-17.135 = 4.7%).

Error Rate (Quality Control)

For steelhead SY2014, a subset of 214 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 20,330 rerun genotypes being compared to the original PBT genotypes. Of these genotypes, 274 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 20,056 genotypes with 271 discrepancies between the original and samples and a genotyping error rate of 0.014%.

For Chinook Salmon SY2014, a subset of 482 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 45,790 rerun genotypes being compared to the original PBT genotypes. Of these genotypes, 221 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 45,596 genotypes with 86 discrepancies between the original and samples and a genotyping error rate of 0.002%.

Null Alleles

For steelhead SY2014, 31 of the 95 PBT loci were found to exhibit a deficiency of heterozygotes in at least one population, but none of these loci had null frequencies estimated >5% (Table 7).

For spring/summer Chinook Salmon SY2014, 29 of the 95 PBT loci were found to exhibit a deficiency of heterozygotes in at least one population, but only one locus had a null frequency estimated >5% (Table 8).

For fall Chinook Salmon SY2010 - 2014, 34 of the 95 PBT loci were found to exhibit a deficiency of heterozygotes in at least one population (Table 9). Ten loci exhibited a deficiency of heterozygotes in 4 or more of the 8 fall Chinook sample groups and five loci had null alleles estimated at a frequency of >5%.

Sex Markers

The sex-specific assay for steelhead matched phenotypic sex in 99.2% of the samples (Table 10). For instances in which genetically-determined sex did not correspond to the phenotypic sex, the majority were cases in which phenotypic females were misidentified by genotype as males. The assay either failed to genotype or provided ambiguous results for only 0.6% of the samples.

The sex-specific assay for spring/summer Chinook Salmon matched phenotypic sex in 99.4% of the samples (Table 11). The majority of discrepancies were phenotypic males genetically identified as female. 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 18.7% of samples.

The sex-specific assay for fall Chinook Salmon matched phenotypic sex in 98.3% of the samples (Table 12). For instances in which the genetically-determined sex did not correspond to the phenotypic sex, the majority were cases in which phenotypic males were misidentified by genotype as females. 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 18.8% of samples.

Average Minor Allele Frequency and Average Heterozygosity

The average minor allele frequency (MAF) for all steelhead broodstocks combined was >0.300, with individual MAF ranging from 0.299 in S.F. Clearwater to 0.342 in Pahsimeroi (Table 13). The average MAF for all spring/summer Chinook salmon broodstocks combined was ~0.250, with individual MAF ranging from 0.237 in Johnson Creek to 0.261 in Catherine Creek (Table 14). The average MAF for fall Chinook salmon broodstocks was ~0.212, with minimal difference observed across years or between the two stocks (Table 15). Levels of observed heterozygosity within steelhead broodstocks was ~0.40 for all hatcheries broodstocks (Table 13). For Chinook salmon, levels of observed heterozygosity was ~0.35 in spring/summer stocks (Table 14) and lower (~0.29) in fall stocks across the four years (SY2010 – 2014) (Table 15).

Population Structure

Pairwise F_{ST} was calculated among the steelhead SY2014 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 SF Clearwater and Little Sheep Creek stocks. All tests of genetic differentiation among stocks were significant.

For spring/summer Chinook Salmon SY2014 pairwise F_{ST} values ranged from a low of 0.010 between the Nez Perce Tribal Hatchery and the Dworshak stock to a high of 0.027

between the Sawtooth and Tucannon stocks (Table 17). All tests of genetic differentiation among stocks were significant. We observed no significant genetic differentiation among the two fall Chinook salmon stocks, nor did we observe any significant temporal differentiation ($F_{ST} \leq 0.010$).

Effective Population Size

Effective population size (N_e) for steelhead hatchery broodstock in SY2014 ranged from a low of 41.3 for the Upper Salmon B-run broodstock to a high of 257.6 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 SY2014 ranged from a low of 98.7 for Powell to a high of 525.5 for Rapid River (Table 19). Effective population size for the two fall Chinook Salmon hatchery broodstocks were large with the Nez Perce stock ranging from 925 – 1,457 and the Lyons Ferry stock ranging from 1,006 – 1,499 (Table 20).

Evaluating the SNP marker set for PBT of fall Chinook Salmon in the Snake River basin

To evaluate the 95 SNP marker set that we have previously used for PBT of spring/summer Chinook salmon in fall Chinook Salmon in the Snake River basin, we generated simulated parents (100 females and 100 males) and simulated offspring (N = 1,000) from those parents using allele frequencies observed in SY2010 – 2014 Lyons Ferry broodstock. We then tested the accuracy of parentage assignments using those parents and "known" offspring in Cervus parentage software. Cervus indicated that the current SNP panel is powerful enough to avoid false negatives (failure to assign the two correct parents when they are within the parent dataset). It correctly identified the two "known" parents for all 1,000 offspring (data not shown). We then removed the "known" 100 female parents and included 100 additional simulated non-parent females. Results from this run indicated that the current marker set is powerful enough to avoid false positives (assignment to two incorrect parents) when one parent is not in the parent database. Cervus did not identify any assignments with zero mismatches (Table 21).

There are situations where a parent may be unsampled within the hatchery or is sampled but fails to genotype. Currently, families in which one or both parents are not genotyped are "untagged" (we cannot assign parentage to their offspring). It is possible that, with a sufficiently powerful set of SNP markers, we could perform single parent assignments. We have previously tested this in spring/summer stocks and concluded that our current PBT panel of 95 SNPs does not yield sufficient accuracy (high false positive error; IDFG unpublished data). Similar to spring/summer stocks, our simulations with fall Chinook indicate that our current PBT SNP panel is too small to avoid false positives for single parent assignments. Of the 1,000 simulated offspring, 119 (11.9%) incorrectly assigned to a non-parent with zero mismatches. Encouragingly, we simulated another parent/offspring dataset using 190 SNPs with 300 "known" males and 200 non-parent females against 1,000 offspring. In this simulation, Cervus correctly identified the single parent of all 1,000 offspring (data not shown).

DISCUSSION

We have demonstrated 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.

Sex Markers

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

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.

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 ≥97.0%.

Poor Performing Loci

Our SNP locus panels for steelhead and spring/summer Chinook salmon have been genotyped on seven years' worth of hatchery broodstock in the Snake River basin (>100,000 samples). We observe very few loci that are not easy to score and that do not exhibit high genotyping success. This is the first year that we have summarized genotyping success rate for our Chinook SNP panel in fall Chinook salmon. We observed a higher, but modest, number of loci that failed at >3% of the samples. These loci will be closely evaluated during our transition to GT-seq genotyping methodologies. If these loci continue to exhibit lower genotyping success rates we will probably recommend their replacement in the new GT-seq SNP panels.

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

We have used the SNP panels for steelhead and spring/summer Chinook salmon for seven years. We have generally found that while null alleles are probably present within certain stocks, the frequency of these alleles is low and they have minimal impact on the accuracy of parentage assignment. We observed similar results in SY2014 samples with only one locus estimated with a null frequency >5% in the steelhead and spring/summer Chinook salmon stocks that were screened. We did observe more loci that exhibited null alleles at frequencies >5% in Snake River fall Chinook salmon stocks. This is the first year that we have summarized genotyping data for Snake River fall Chinook salmon. Loci that exhibit high levels of null alleles in these two stocks may benefit from revised scoring rules. Another consideration is that we will be switching to GT-seq genotyping methods for SY2015 samples and we may be able to reduce null alleles by redesigning primers for these loci. Alternatively, because we will be screening larger numbers of loci with GT-seq, any locus exhibiting significant null alleles may be dropped.

Minor Allele Frequency and Average Heterozygosity

Minor allele frequency (MAF) is simply the frequency at which the less common allele occurs in a given population. While a locus is most informative for parentage analyses when its alleles are equifrequent (MAF = 0.5), Anderson and Garza (2006) demonstrated through simulations that 60 – 100 SNPs with an average MAF of 0.2 or greater should allow accurate pedigree reconstruction, even in situations involving thousands of potential mothers, fathers, and offspring. Average MAF observed for our 95 SNP sets used for PBT of steelhead and Chinook salmon in the Snake River basin were greater than 0.2. The highest average MAFs were observed in steelhead (0.324) and the lowest were observed in fall Chinook broodstocks (0.212). A similar pattern was observed with expected heterozygosity. The average expected heterozygosity was high and uniform across both steelhead hatchery stocks (~0.40) and Chinook Salmon (~0.35). Average expected heterozygosity in fall Chinook salmon was lower (~0.25). Overall results demonstrate that the degree of variability in these SNP sets is sufficient for accurate parentage analysis of hatchery stocks throughout the Snake River basin.

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/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 all extirpated following the construction of Lewiston Dam in 1927. Present day stocks were all derived predominantly from Rapid River origin broodstock.

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 less than 50 (41.3), 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.

In upcoming years we will calculate direct measurements of N_E through parental assignments of subsequent generations to previous generations of broodstock. This observed value of N_e through pedigree reconstruction can then be compared to estimates of N_E from various software programs (e.g. NeEstimator, Colony) that are derived using genetic data from a single generation.

Evaluating the Chinook Salmon SNP marker set for PBT of fall Chinook Salmon in the Snake River basin

The SNP panel that we have been using for spring/summer Chinook Salmon exhibits less variation in our fall Chinook salmon stocks, but still performed accurately in assigning simulated juveniles back to parents. We observed no evidence of false negatives or false positives in our parent/offspring datasets produced from the allele frequencies observed in the Lyons Ferry broodstock. Similar to spring/summer Chinook Salmon, the current marker set consisting of 95 SNPs is not powerful enough for single parent assignments. We expect that the increase of our PBT SNP panels from 95 to ~192 - 300 will allow assignment of single parents in situations where one parent was either inadvertently not sampled or not successfully genotyped.

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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 2014 (SY2015), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2014 (SY2015). 3) Origin of samples from sport fisheries in the lower Snake River in migration year 2014 (SY2015), 4) Origin of samples from various sport fisheries in Idaho in migration year 2013 (SY2014), 5) Parentage of SY2015 Upper Salmon B-run broodstock for real-time management of spawning, 6) Age composition and origin of the SY2014 broodstocks, 7) Correction of PIT expansions for SY2014 Sawtooth broodstock, and 8) stock composition of returning adults at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2014), 2) Age composition and origin of SY2014 broodstocks, 3) PBT-determined rearing location for returning offspring raised in 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 2014 (SY2015). A total of 1,597 samples (1,105 from Zones 1-5 and 492 from above Bonneville Dam) were processed for PBT assignment. An example of the methods used for this annual sampling can be found in Byrne et al. (2015).

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) 2014 (e.g. spawn year 2015). A total of 1,168 samples from clipped steelhead were analyzed. An example of the methods used for this annual sampling can be found in Byrne et al. (2015).

Steelhead Sport Fisheries in Lower Snake River

Washington Department of Fish and Wildlife (WDFW) collected samples of steelhead harvested in the SY2015 lower Snake River sport fishery from the mouth of the Snake to the Idaho/Washington border. A total of 720 samples were processed for PBT assignment. An example of the methods used for this annual sampling can be found in Byrne et al. (2015).

Steelhead Sport Fisheries in Idaho

IDFG collected samples of steelhead harvested in the SY2014 sport fishery from various river systems including the Clearwater and Salmon. A total of 1,618 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 SY2015 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.

Correction of PIT Expansions in Steelhead

This project was conducted to estimate the combined effects of tag loss, differential mortality, and tag malfunction for PIT tags and to provide a correction factor for PIT-tag detections of SY2014 Sawtooth broodstock. PBT was used to assign Sawtooth broodstock from SY2014 back to the SY2009, SY2010, and SY2011 cohorts. Once the cohort of origin for each sample was determined, the proportion of PIT-tagged and non-PIT-tagged assignments in each cohort were then compared to the expected proportions of PIT-tagged and non-PIT-tagged fish for each cohort (based on the PIT-tag rate of the smolts for that year). The difference was used to correct the PIT expansions.

Age Composition of SY2014 Steelhead Broodstock

PBT was used to determine age composition of steelhead broodstocks in Idaho by assigning the SY2014 broodstocks back to the SY2009–SY2011 broodstocks, thereby identifying the age of each fish. A total of 5,457 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 2014 and spring of 2015 (Warren et al. *In prep*) and 1,023 samples were analyzed with PBT.

Chinook Salmon Sport Fishery in Idaho

Fisheries managers within IDFG implemented PBT sampling of Chinook Salmon harvested in the sport fishery in SY2014. A total of 1,395 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 SY2014 Chinook Salmon Broodstock

PBT was used to determine age composition of Chinook Salmon broodstocks in Idaho by assigning the SY2014 broodstocks back to the SY2008–SY2010 broodstocks, thereby identifying the age of each fish. A total of 9,248 hatchery-origin broodstock samples from ten different broodstocks were analyzed with PBT.

Evaluation of Increased Rearing Density to Increase Adult Returns of Spring Chinook Salmon to Dworshak National Fish Hatchery

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 could yield increased adult returns. To evaluate this, the hatchery created replicate normal and high density groups in BY2012. The first returning offspring (n = 97) reared under these conditions were sampled as jacks in SY2015.

RESULTS

Steelhead Sport Fisheries in Columbia River

Of the 1,597 samples analyzed, 1,051 assigned to the PBT baseline. After expanding by PBT rates, 59% of the sport samples in Zones 1-5 and 77% of the sport samples above Bonneville Dam assigned to hatcheries in the Snake River basin. A detailed breakdown of stock composition in these fisheries is presented in Byrne et al. (In Prep.).

Steelhead Tribal Fisheries in Zone 6 of Columbia River

Of the 1,025 samples analyzed from adipose-clipped fish, 924 assigned to the PBT baseline. After expanding by PBT rates, 93% 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 720 samples analyzed, 686 assigned to the PBT baseline. After expanding by PBT rates, the origin of 97% 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 1,618 samples analyzed, 1,453 assigned. After expanding by PBT rates, 98% 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

Six 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.

Correction of PIT Expansions in Steelhead

Cohort membership was determined through PBT for 99.1% of the SY2014 Sawtooth broodstock. Differences between the observed and expected PIT rates for each cohort yielded a correction factor to be applied to PIT detections. Results will appear in upcoming IDFG technical reports.

Stock Composition of Adult Steelhead at Lower Granite Dam

One of the 1,023 samples failed to genotyped and was omitted from analysis. PBT assigned 965 (94.4%) to the baseline. After expanding by the tag rates, the origin of 992 (97.1%) samples could be accounted for, suggesting that the stock-specific tag rates that we applied were very accurate. A summary of stock composition and age will be provided in an upcoming IDFG technical report (Warren et al. In prep).

Age Composition of SY2014 Steelhead Broodstock

Of the 5,457 samples analyzed with PBT 4,957 assigned (90.8%) to the baseline. After expanding by the tag rates, the origin of 5,242 (96.1%) samples could be accounted for, which suggests that the stock-specific tag rates that we applied were very accurate. 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 1,395 samples analyzed, 6 were omitted because they failed to genotype adequately or were determined to be duplicate samples from the same fish. After expanding by PBT rates, the origin of 99.3% 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 SY2014 Chinook Salmon Broodstock

Of the 9,248 hatchery-origin broodstock samples analyzed, 9,039 assigned (97.7%) to the PBT baseline. After expanding by the tag rates, the origin of 9,170 (99.2%) samples could be accounted for, suggesting that the stock-specific tag rates that we applied were very accurate. 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 Salmon to Dworshak National Fish Hatchery

Samples were collected from 97 jack-sized Chinook salmon returning to Dworshak Hatchery. Samples were assigned to their parents using the Snake River basin PBT baseline. Results are intended to provide information on return rates from experimental rearing conditions including rearing in high-density, burrows ponds, and A-bank ponds.

All 97 samples assigned to parents, but 11 samples assigned to broodstock from SY2011 rather than SY2012, indicating that these samples were not jacks but small 4-year-olds. (Interestingly, six of these small 4-year-olds are siblings.) Ten additional samples originated from either the Powell broodstock or from non-experimental Dworshak-origin broodstock.

Using the genetic ID of the PBT-determined mother, the rearing location of the sample was determined. All experimental rearing conditions were represented (High/Low density, Burrows ponds, A-bank ponds) and replicates (A-1 – A-14) 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 Zones 1–6 of 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 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 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.*).

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*).

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.

Correction of PIT-tag Expansions in Steelhead

One advantage of genetically marking hatchery fish through PBT is that the "mark" cannot be shed and that genetically marked fish have no differential mortality compared to unmarked fish. This is not always the case for fish marked with physical tags. In this case

managers knew that PIT-tag detections were underrepresenting returning hatchery adults and were likely caused by shedding of the PIT tag or differential mortality. To determine a correction factor for PIT-tag detections, managers needed to unambiguously determine the age composition of a broodstock in order to compare the observed and expected PIT-tag rates for each cohort. The implementation of PBT allowed managers the opportunity to independently assess and correct PIT-tag rates for the broodstock of interest.

Chinook Salmon Sport Fishery in Idaho

This effort represents one 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 SY2014 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 SY2014 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 Salmon to Dworshak National Fish Hatchery

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 feasibility 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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TABLES

Table 1. Total steelhead broodstock genetically sampled in SY2014 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	693
E.F. Salmon River	27
Pahsimeroi	1,320
Upper Salmon R. B-run	144
Oxbow	334
Dworshak	1,925
S.F. Clearwater	148
Little Sheep Cr.	132
Tucannon R.	62
Touchet R.	31
Cottonwood Cr.	324
Wallowa	486
Total	**5,626**

Table 2. Total spring/summer Chinook Salmon broodstock sampled in SY2014 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	1,224
Dworshak	1,746
Kooskia	247
Johnson Cr.	64
Imnaha	274
Catherine Cr.	98
Lostine	141
Grande Ronde	142
Lookingglass Cr.	159
Tucannon	126
S.F. Salmon	541
Nez Perce Tribal FH	353
Pahsimeroi	772
Powell	619
Rapid River	2,571
Sawtooth	872
Total	**9,949**

Table 3. Total fall Chinook Salmon broodstock sampled in SY2014 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
SY2011 Nez Perce	651
SY2012 Nez Perce	793
SY2013 Nez Perce	827
SY2014 Nez Perce	706
SY2011 Lyons Ferry	1,761
SY2012 Lyons Ferry	1,755
SY2013 Lyons Ferry	1,949
SY2014 Lyons Ferry	1,681
Total	**10,123**

Table 4. Sample sizes and genotyping completion rate of SY2014 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.

	2014		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
Sawtooth	693	691 (99.7%)	99.4%
E.F. Salmon River	27	27 (100%)	100.0%
Pahsimeroi	1,320	1,318 (99.8%)	99.7%
Upper Salmon R. B-run	144	144 (100%)	100.0%
Oxbow	334	329 (98.5%)	97.0%
Dworshak	1,925	1,903 (98.9%)	97.7%
S.F. Clearwater	148	147 (99.3%)	98.7%
Little Sheep Cr.	132	129 (97.7%)	95.5%
Tucannon R.	62	61 (98.4%)	96.8%
Touchet R.	31	31 (100%)	100.0%
Cottonwood Cr.	324	323 (99.7%)	99.4%
Wallowa	486	485 (99.8%)	99.6%
Total	**5,626**	**5,588 (99.3%)**	**98.7%**

Table 5. Sample sizes and genotyping completion rate of SY2014 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.

	2014		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
S.F. Clearwater	1,224	1,204 (98.4%)	96.8%
Dworshak	1,746	1,740 (99.7%)	99.3%
Kooskia	247	243 (98.4%)	96.8%
Johnson Cr.	64	64 (100%)	100.0%
Imnaha	274	261 (95.3%)	90.7%
Catherine Cr.	98	92 (93.9%)	88.1%
Lostine	141	121 (85.8%)	73.6%
Grande Ronde	142	140 (98.6%)	97.2%
Lookingglass Cr.	159	152 (95.6%)	91.4%
Tucannon	126	119 (94.4%)	89.2%
S.F. Salmon	541	535 (98.9%)	97.8%
Nez Perce Tribal FH	353	346 (98.0%)	96.1%
Pahsimeroi	772	771 (99.9%)	99.7%
Powell	619	619 (100%)	100.0%
Rapid River	2,571	2,571 (100%)	100.0%
Sawtooth	872	872 (100%)	100.0%
Total	**9,949**	**9,850 (99.0%)**	**98.0%**

Table 6. Sample sizes and genotyping completion rate of SY2011 – SY2014 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.

	2011 - 2014		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
SY2011 Nez Perce	651	615 (94.5%)	89.2%
SY2012 Nez Perce	793	788 (99.4%)	98.7%
SY2013 Nez Perce	827	821 (99.3%)	98.6%
SY2014 Nez Perce	706	700 (99.2%)	98.3%
SY2011 Lyons Ferry	1,761	1,736 (98.6%)	97.2%
SY2012 Lyons Ferry	1,755	1,714 (97.7%)	95.4%
SY2013 Lyons Ferry	1,949	1,920 (98.5%)	97.0%
SY2014 Lyons Ferry	1,681	1,662 (98.9%)	97.8%
Total	**10,123**	**9,956 (98.4%)**	**96.7%**

Table 7. Ranked estimates of null allele frequencies (NullFreq) for the 31 loci in which 1 or more loci exhibited a deficiency of heterozygotes (H_ED) across steelhead hatchery populations in SY2014.

SNP Name	H_ED	NullFreq
Omy_105385-406-A1	5	0.025
Omy_Il1b-198-A1	3	0.042
M09AAJ.163-A1	3	0.028
Omy_99300-202-A1	3	0.027
Omy_hsf2-146-A1	2	0.031
Omy_111383-51-A1	2	0.029
Omy_crb-106-A1	2	0.027
OMS00077-A1	2	0.024
Omy_redd1-410-A1	2	0.019
OMS00072-A1	2	0.018
OMY1011SNP-A1	2	0.015
Omy_114315-438-A1	1	0.028
OMS00106-A1	1	0.021
OMS00101-A1	1	0.020
OMS00111-A1	1	0.020
Omy_107806-34-A1	1	0.019
Omy_Il-1b_.028-A1	1	0.018
Omy_metA-161-A1	1	0.017
Omy_oxct-85-A1	1	0.016
Omy_cd59-206-A1	1	0.015
OMS00002-A1	1	0.014
Omy_IL17-185-A1	1	0.013
Omy_rbm4b-203-A1	1	0.013
Omy_128923-433-A1	1	0.012
Omy_nkef-241-A1	1	0.011
Omy_129870-756-A1	1	0.011
Omy_108007-193-A1	1	0.011
Omy_Ogo4-212-A1	1	0.011
Omy_u09-53.469-A1	1	0.010
Omy_NaKATPa3-50-A1	1	0.010
Omy_105105-448-A1	1	0.006

Table 8. Ranked estimates of null allele frequencies (NullFreq) for the 29 loci in which one or more loci exhibited a deficiency of heterozygotes (H_ED) across spring//summer Chinook salmon hatchery populations in SY2014.

SNP Name	H_ED	NullFreq
Ots_MHC1-A1	9	0.113
Ots_OTALDBINT1-SNP1-A1	5	0.033
Ots_110495-380-A1	5	0.008
Ots_u6-75-A1	4	0.018
Ots_u4-92-A1	3	0.007
Ots_parp3-286-A1	2	0.015
Ots_TGFB-A1	2	0.007
Ots_GCSH-A1	1	0.024
Ots_unk526-A1	1	0.015
Ots_101554-407-A1	1	0.015
Ots_HSP90B-100-A1	1	0.015
Ots_MHC2-A1	1	0.015
Ots_94857-232R-A1	1	0.015
Ots_112820-284-A1	1	0.015
Ots_105105-613-A1	1	0.014
Ots_110064-383-A1	1	0.014
Ots_vatf-251-A1	1	0.012
Ots_u07-18.378-A1	1	0.012
Ots_105407-117-A1	1	0.011
Ots_hsc71-3'-488-A1	1	0.011
Ots_ppie-245-A1	1	0.010
Ots_TAPBP-A1	1	0.010
Ots_113242-216-A1	1	0.009
Ots_117432-409-A1	1	0.008
Ots_ARNT-A1	1	0.008
Ots_Prl2-A1	1	0.008
Ots_110689-218-A1	1	0.008
Ots_102801-308-A1	1	0.006
Ots_u1002-75-A1	1	0.006

Table 9. Ranked estimates of null allele frequencies (NullFreq) for the 34 loci in which 1 or more loci exhibited a deficiency of heterozygotes ($H_E D$) across Fall Chinook salmon hatchery populations in SY2010 – SY2014.

SNP Name	$H_E D$	NullFreq
Ots_101704-143-A1	8	0.049
Ots_112301-43-A1	8	0.054
Ots_IGF-I.1-76-A1	8	0.005
Ots_NOD1-A1	7	0.059
Ots_OTALDBINT1-SNP1-A1	6	0.027
Ots_u07-25.325-A1	6	0.051
Ots_Ikaros-250-A1	5	0.000
Ots_txnip-321-A1	4	0.018
Ots_u07-17.135-A1	4	0.021
Ots_ppie-245-A1	4	0.030
Ots_TGFB-A1	2	0.010
Ots_101554-407-A1	2	0.012
Ots_105385-421-A1	2	0.010
Ots_MHC1-A1	2	0.068
Ots_GCSH-A1	2	0.007
Ots_mapKpr-151-A1	1	0.009
Ots_Prl2-A1	1	0.014
Ots_110689-218-A1	1	0.010
Ots_u07-49.290-A1	1	0.015
Ots_redd1-187-A1	1	0.012
Ots_102414-395-A1	1	0.011
Ots_123921-111-A1	1	0.019
Ots_ARNT-A1	1	0.000
Ots_u211-85-A1	1	0.005
Ots_AsnRS-60-A1	1	0.007
Ots_128757-61R-A1	1	0.009
Ots_pigh-105-A1	1	0.003
Ots_u6-75-A1	1	0.007
Ots_SClkF2R2-135-A1	1	0.008
Ots_u4-92-A1	1	0.013
Ots_115987-325-A1	1	0.006
Ots_CirpA-A1	1	0.005
Ots_ntl-255-A1	1	0.005
Ots_u07-18.378-A1	1	0.010

Table 10. Results of comparisons between phenotypic sex and genetically determined sex using the sex-specific assay for SY2014 steelhead (Omy1_2SEXY).

	Total Samples	Missing Genetic Data	Total Successful Genotypes	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Sawtooth	693	8 (1.2%)	685 (98.8%)	667 (97.4%)	18 (2.6%)	0	18	349 (50.4%)	344 (49.6%)
E.F. Salmon	27	0 (0%)	27 (100%)	27 (100%)	0 (0%)	0	0	14 (51.9%)	13 (48.1%)
Pahsimeroi	1,320	2 (0.2%)	1,318 (99.8%)	1,318 (100%)	0 (0%)	0	0	660 (50.0%)	660 (50.0%)
Upper Sal. B	144	5 (3.5%)	139 (96.5%)	139 (100%)	0 (0%)	0	0	57 (39.6%)	87 (60.4%)
Oxbow	334	1 (0.3%)	333 (99.7%)	327 (98.2%)	6 (1.8%)	3	3	167 (50.0%)	167 (50.0%)
Dworshak	1,925	16 (0.8%)	1,909 (99.2%)	1,901 (99.6%)	8 (0.4%)	4	4	871 (45.2%)	1,054 (54.8%)
S.F. Clearwater	148	1 (0.7%)	147 (99.3%)	147 (100%)	0 (0%)	0	0	71 (48.0%)	77 (52.0%)
Little Sheep Cr.	132	3 (2.3%)	129 (97.7%)	124 (96.1%)	5 (3.9%)	5	0	66 (50.0%)	66 (50.0%)
Tucannon R.	62	0 (0%)	62 (100%)	61 (98.4%)	1 (1.6%)	1	0	33 (53.2%)	29 (46.8%)
Touchet R.	31	0 (0%)	31 (100%)	31 (100%)	0 (0%)	0	0	16 (51.5%)	15 (48.4%)
Cottonwood Cr.	324	0 (0%)	324 (100%)	322 (99.4%)	2 (0.6%)	2	0	163 (50.3%)	161 (49.7%)
Wallowa	486	0 (0%)	486 (100%)	482 (99.2%)	4 (0.8%)	0	4	243 (50.0%)	243 (50.0%)
Total	**5,626**	**36 (0.6%)**	**5,590 (99.4%)**	**5,546 (99.2%)**	**44 (0.8%)**	**15**	**29**	**2,710 (48.2%)**	**2,916 (51.8%)**

Table 11. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for spring/summer Chinook Salmon (IDFG-OTS-SEX) from the SY2014 broodstocks.

	Total Samples	Missing Genetic Data	Total Successful Genotypes	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
S.F. Clearwater	1,224	875 (71.5%)	359 (28.5%)	349 (100%)	0 (0%)	0	0	522 (42.6%)	702 (57.4%)
Dworshak	1,746	21 (1.2%)	1,725 (98.8%)	1,723 (99.9%)	2 (0.1%)	0	2	664 (38.0%)	1,082 (62.0%)
Kooskia	247	6 (2.4%)	241 (97.6%)	238 (98.8%)	3 (1.2%)	0	3	141 (57.1%)	106 (42.9%)
Johnson Cr.	64	60 (93.8%)	4 (6.2%)	4 (100%)	0 (0%)	0	0	32 (50.0%)	32 (50.0%)
Imnaha	274	1 (0.4%)	273 (99.6%)	268 (98.2%)	5 (1.8%)	5	0	136 (49.6%)	138 (50.4%)
Catherine Cr.	98	0 (0%)	98 (100%)	91 (92.9%)	7 (7.1%)	7	0	55 (56.1%)	43 (43.9%)
Lostine	141	0 (0%)	141 (100%)	129 (91.5%)	12 (8.5%)	12	0	70 (49.6%)	71 (50.4%)
Grande Ronde	142	0 (0%)	142 (100%)	138 (97.2%)	4 (2.8%)	2	2	75 (52.8%)	67 (47.2%)
Lookingglass Cr.	159	2 (1.3%)	157 (98.7%)	149 (94.9%)	8 (5.1%)	8	0	77 (48.4%)	82 (51.6%)
Tucannon	126	0 (0%)	126 (100%)	123 (97.6%)	3 (2.4%)	1	2	60 (47.6%)	66 (52.4%)
S.F. Salmon	541	0 (0%)	541 (100%)	538 (99.4%)	3 (0.6%)	3	0	269 (49.7%)	272 (50.3%)
Nez Perce FH	353	308 (87.3%)	45 (12.7%)	45 (100%)	0 (0%)	0	0	165 (46.7%)	188 (53.3%)
Pahsimeroi	772	0 (0%)	772 (100%)	772 (100%)	0 (0%)	0	0	386 (50.0%)	386 (50.0%)
Powell	619	591 (95.5%)	28 (4.5%)	28 (100%)	0 (0%)	0	0	273 (44.1%)	346 (55.9%)
Rapid River	2,571	1 (0.1%)	2,570 (99.9%)	2,570 (100%)	0 (0%)	0	0	1,129 (43.9%)	1,442 (56.1%)
Sawtooth	872	0 (0%)	872 (100%)	869 (99.7%)	3 (0.3%)	3	0	441 (50.6%)	431 (49.4%)
Total	**9,949**	**1,865 (18.7%)**	**8,084 (81.3%)**	**8,034 (99.4%)**	**50 (0.6%)**	**41**	**9**	**4,495 (45.2%)**	**4,590 (50.9%)**

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 SY2014 broodstocks. (NOTE: No phenotypic sex data available for SY2012 and SY2013 from Nez Perce FH. Total percentages for corresponding and non-corresponding sex calls are calculated omitting these two broodstocks)

Stock	Total Samples	Missing Genetic Data	Total Successful Genotypes	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
SY2011 Nez Perce	651	3 (0.5%)	648 (99.5%)	644 (99.4%)	4 (0.6%)	3	1	214 (32.9%)	437 (67.1%)
SY2012 Nez Perce	793	8 (1.0%)	785 (99.0%)	N/A	N/A	N/A	N/A	N/A	N/A
SY2013 Nez Perce	827	16 (1.9%)	811 (98.1%)	N/A	N/A	N/A	N/A	N/A	N/A
SY2014 Nez Perce	706	1 (0.1%)	705 (99.9%)	701 (99.4%)	4 (0.6%)	3	1	307 (43.5%)	399 (56.5%)
SY2011 Lyons Ferry	1,761	78 (4.4%)	1,683 (95.6%)	1,652 (98.2%)	30 (1.8%)	15	15	471 (26.8%)	1,289 (73.2%)
SY2012 Lyons Ferry	1,755	689 (39.3%)	1,066 (60.7%)	1,048 (98.3%)	17 (1.6%)	16	1	571 (32.6%)	1,183 (67.4%)
SY2013 Lyons Ferry	1,933	1,071 (55.4%)	862 (44.6%)	824 (95.6%)	37 (4.3%)	28	9	685 (35.5%)	1,243 (64.5%)
SY2014 Lyons Ferry	1,681	37 (2.2%)	1,644 (97.8%)	1,624 (98.8%)	18 (1.1%)	3	15	503 (30.0%)	1,176 (70.0%)
Total	**10,107**	**1,903 (18.8%)**	**8,204 (81.2%)**	**6,493 (98.3%)**	**110 (1.7%)**	**68**	**42**	**2,751 (32.4%)**	**5,727 (67.6%)**

Table 13. Average minor allele frequency (MAF) and average observed and expected heterozygosity with associated standard deviation of hatchery steelhead stocks for SY2014.

Stock	MAF	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
Sawtooth	0.331	0.424	0.007	0.423	0.002
EF Salmon	0.321	0.420	0.009	0.428	0.010
Upper Salmon B	0.304	0.431	0.007	0.429	0.001
Oxbow	0.337	0.396	0.010	0.404	0.004
Pahsimeroi	0.342	0.426	0.008	0.434	0.003
Dworshak	0.300	0.393	0.010	0.391	0.001
SF Clearwater	0.299	0.394	0.010	0.388	0.004
Little Sheep Ck	0.331	0.420	0.009	0.422	0.004
Tucannon	0.333	0.425	0.009	0.416	0.007
Touchet	0.322	0.421	0.010	0.417	0.009
Cottonwood Ck	0.334	0.427	0.007	0.429	0.003
Wallowa	0.335	0.428	0.007	0.426	0.002

Table 14. Average minor allele frequency (MAF) and average observed and expected heterozygosity with associated standard deviation of hatchery spring/summer Chinook Salmon stocks in SY2014.

Stock	MAF	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
S.F. Clearwater	0.250	0.338	0.001	0.341	0.013
Dworshak	0.243	0.336	0.001	0.335	0.013
Kooskia	0.251	0.350	0.003	0.344	0.013
Johnson Cr.	0.237	0.315	0.006	0.325	0.015
Imnaha	0.251	0.336	0.003	0.342	0.013
Catherine Cr.	0.261	0.362	0.005	0.354	0.013
Lostine	0.246	0.338	0.004	0.337	0.014
Grande Ronde	0.253	0.346	0.004	0.345	0.013
Lookingglass Cr.	0.260	0.361	0.004	0.354	0.012
Tucannon	0.252	0.358	0.005	0.342	0.014
S.F. Salmon	0.241	0.331	0.002	0.331	0.014
Nez Perce Tribal FH	0.250	0.343	0.003	0.343	0.013
Pahsimeroi	0.240	0.335	0.002	0.332	0.013
Powell	0.256	0.337	0.002	0.342	0.014
Rapid River	0.248	0.337	0.001	0.338	0.013
Sawtooth	0.246	0.334	0.002	0.334	0.014

Table 15. Average observed and expected heterozygosity with associated standard deviation of hatchery spring/summer Chinook Salmon stocks in SY2014.

Stock	MAF	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
SY2011 Nez Perce	0.214	0.285	0.001	0.287	0.018
SY2012 Nez Perce	0.214	0.287	0.001	0.289	0.018
SY2013 Nez Perce	0.215	0.289	0.001	0.289	0.018
SY2014 Nez Perce	0.211	0.287	0.001	0.288	0.018
SY2011 Lyons Ferry	0.215	0.282	0.002	0.285	0.018
SY2012 Lyons Ferry	0.216	0.283	0.002	0.286	0.018
SY2013 Lyons Ferry	0.216	0.288	0.002	0.287	0.018
SY2014 Lyons Ferry	0.216	0.280	0.002	0.283	0.018

Table 16. Population structure (F_{ST}) (lower left) among steelhead hatchery stocks sampled in SY2014. 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	Wallowa
Dworshak	0.025	---	*	*	*	*	*	*	*	*	*
EF Salmon	0.018	0.018	---	*	*	*	*	*	*	*	*
Oxbow	0.012	0.026	0.017	---	*	*	*	*	*	*	*
Little Sheep	0.014	0.035	0.026	0.012	---	*	*	*	*	*	*
Pahsimeroi	0.011	0.027	0.016	0.003	0.012	---	*	*	*	*	*
Sawtooth	0.011	0.027	0.016	0.004	0.015	0.004	---	*	*	*	*
SF Clearwater	0.025	0.001	0.018	0.026	0.036	0.027	0.027	---	*	*	*
Touchet	0.015	0.028	0.024	0.014	0.017	0.015	0.016	0.029	---	*	*
Tucannon	0.009	0.023	0.017	0.011	0.012	0.011	0.012	0.023	0.009	---	*
Wallowa	0.003	0.025	0.019	0.010	0.014	0.010	0.009	0.025	0.013	0.009	---
Up Sal B	0.024	0.011	0.022	0.026	0.036	0.027	0.027	0.012	0.031	0.025	0.025

Table 17. Population structure (F_{ST}) (lower left) among spring/summer Chinook Salmon hatchery stocks sampled in SY2014. 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	0.007	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.009	0.009	---	*	*	*	*	*	*	*	*	*	*	*	*
Imnaha	0.008	0.009	0.010	---	*	*	*	*	*	*	*	*	*	*	*
Johnson Cr.	0.020	0.019	0.019	0.019	---	*	*	*	*	*	*	*	*	*	*
Kooskia	0.007	0.003	0.008	0.009	0.018	---	*	*	*	*	*	*	*	*	*
Lookingglass	0.004	0.006	0.007	0.007	0.018	0.006	---	*	*	*	*	*	*	*	*
Lostine	0.017	0.018	0.018	0.014	0.032	0.019	0.015	---	*	*	*	*	*	*	*
Nez Perce TFH	0.006	0.003	0.007	0.006	0.018	0.002	0.005	0.018	---	*	*	*	*	*	*
Pahsimeroi	0.018	0.017	0.020	0.016	0.024	0.017	0.017	0.022	0.016	---	*	*	*	*	*
Powell	0.008	0.004	0.010	0.008	0.019	0.004	0.007	0.019	0.005	0.018	---	*	*	*	*
Rapid River	0.010	0.011	0.011	0.007	0.023	0.011	0.008	0.020	0.006	0.023	0.011	---	*	*	*
Sawtooth	0.015	0.014	0.017	0.014	0.020	0.015	0.015	0.022	0.013	0.013	0.014	0.019	---	*	*
S.F. Clearwater	0.006	0.003	0.006	0.006	0.016	0.004	0.006	0.016	0.003	0.017	0.004	0.006	0.013	---	*
S.F. Salmon	0.011	0.010	0.011	0.009	0.009	0.011	0.010	0.018	0.010	0.016	0.010	0.015	0.011	0.008	---
Tucannon	0.014	0.014	0.019	0.016	0.028	0.015	0.014	0.024	0.016	0.027	0.016	0.022	0.027	0.015	0.019

Table 18. Estimates of effective population size (N_E) and 95% confidence intervals for steelhead hatchery stocks in SY2014.

Stock	N_E	95% CI
Sawtooth	198.1	183.2 - 214.5
E.F. Salmon River	114.7	65.8 - 361
Pahsimeroi	202.1	190 - 214.9
Upper Salmon R. B-run	41.3	38 - 45
Oxbow	143.2	129.9 - 158.3
Dworshak	257.6	242.4 - 273.7
S.F. Clearwater	135.7	116 - 161.3
Little Sheep Cr.	138.8	116.4 - 169.2
Tucannon R.	226.2	145 - 472
Touchet R.	488.5	131.5 - Infinite
Cottonwood Cr.	68.5	63.7 - 73.6
Wallowa	157.4	144.8 - 171.5

Table 19. Estimates of effective population size and 95% confidence intervals for SY2014 spring/summer Chinook Salmon hatchery stocks.

Stock	Ne	95% CI
S.F. Clearwater	260.1	242.2 - 279.5
Dworshak	260.6	245.1 - 277.2
Kooskia	160.5	141.6 - 183.5
Johnson Cr.	197.3	131.9 - 365.3
Imnaha	311.9	258 - 387.7
Catherine Cr.	223.6	161.5 - 348.4
Lostine	170.3	135.9 - 222.8
Grande Ronde	162	134.1 - 201
Lookingglass Cr.	180.6	149.3 - 224.6
Tucannon	377.5	253.1 - 696.8
S.F. Salmon	391.5	337.5 - 460.5
Nez Perce Tribal FH	231	203.7 - 264.1
Pahsimeroi	160.5	149.4 - 172.5
Powell	98.7	92.4 - 105.4
Rapid River	525.5	488.5 - 565.7
Sawtooth	211.6	195.2 - 229.5

Table 20. Estimates of effective population size and 95% confidence intervals for SY2010 - 2014 Fall Chinook Salmon hatchery stocks.

Stock	Ne	95% CI
SY2011 Nez Perce	924.5	803.7 - 1073
SY2012 Nez Perce	995.9	861.4 - 1163.2
SY2013 Nez Perce	944.4	831 - 1080.7
SY2014 Nez Perce	1456.9	1206.4 - 1801.5
SY2011 Lyons Ferry	1301.6	909.7 - 2155.4
SY2012 Lyons Ferry	1498.5	1077 - 2341
SY2013 Lyons Ferry	1006.7	795.4 - 1333.2
SY2014 Lyons Ferry	1114.3	834.4 - 1611.1

Table 21. Number of loci mismatching (N_M) and counts (C_{TM}) observed for 1000 simulated juveniles observed in a simulated parentage dataset. The simulated parentage dataset created 100 “known” males and 100 non-parent females with 95 SNP genotypes as inputs in the parentage software program Cervus. Results indicated low false assignment error, with none of the 1000 simulated juveniles assigning to a parent pair with no mismatches.

N_M	C_{TM}
1	13
2	81
3	152
4	223
5	235
6	157
7	76
8	45
9	12
10	6

FIGURES

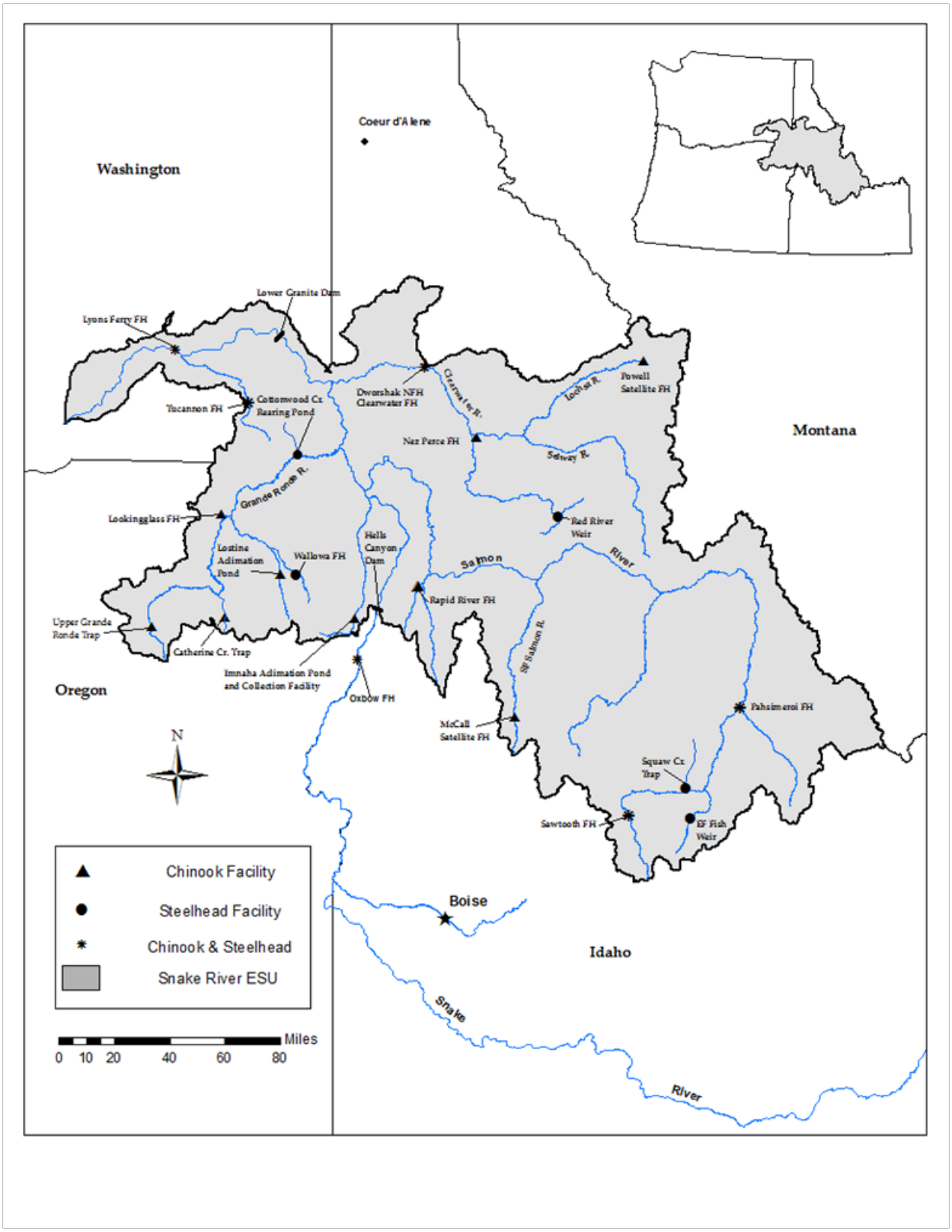

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

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