FISHERY RESEARCH

PARENTAGE BASED TAGGING OF SNAKE RIVER HATCHERY STEELHEAD AND CHINOOK SALMON

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Project Progress Report

2014 Annual Report

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

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, 2013 to June 30, 2014 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) and spring/summer Chinook Salmon (~8,000 individuals annually). 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) 2013 (Objective 2). In addition, summary data for Chinook Salmon broodstocks from SY2013 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, thus far, 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 Hatchery Chinook Salmon and Steelhead Broodstock

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 and spring/summer 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 2013 (SY2014), 2.) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2013 (SY2014), 3.) Origin of samples from sport fisheries in the lower Snake River in migration years 2012 and 2013 (SY2013 and SY2014), 4.) Origin of samples from various sport fisheries in Idaho in migration year 2012 (SY2013), 5.) Parentage of SY2014 Upper Salmon B-run broodstock for real-time management of spawning, and 6.) Correction of PIT expansions for SY2013 Sawtooth broodstock.

For Chinook Salmon, the PBT baselines were used to determine: 1.) Origin of carcasses encountered in spawning-ground surveys on the South Fork Salmon River in SY2013, 2.) Origin of samples from various sport fisheries in Idaho in (SY2013), 3.) Parentage of Clearwater broodstock for real-time management of spawning, 4.) Age composition of SY2013 broodstocks, and 5.) Origin of "escaped" fingerlings sampled from headboxes of Dworshak raceways.

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 sample 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 or operculum punches, and stored either in 2 ml vials of 200-proof non-denatured ethanol or on absorbent sheets of Whatman 3mm chromatography paper (LaHood et al. 2008). The samples are then 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 2014, we have collected and inventoried approximately 5,000 genetic samples from the steelhead broodstock (Table 1) spawned in the Snake River basin during spawn year (SY) 2013, and approximately 9,000 samples (Table 2) from Chinook Salmon broodstock spawned in the Snake River basin during SY2013. 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 SY2013.

INTRODUCTION

A set of PBT single nucleotide polymorphism (SNPs) was identified for steelhead and Chinook Salmon, and it was demonstrated that the selected SNPs would provide sufficient resolving power (Steele et al. 2011). These markers were used to genotype broodstock samples collected in 2013 (Table 1 and 2).

During the fourth year of this project (FY2013), IDFG and CRITFC labs extracted and genotyped all samples for steelhead and Chinook Salmon broodstocks (~7,000 IDFG, ~7,000 CRITFC = ~14,000 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.

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 identify loci with null alleles and 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 average observed (H_o) and expected (H_e) heterozygosity using ARLEQUIN (Excoffier and Lischer 2010), estimates of relatedness between stocks through genetic structure (F_{st}) using GENEPOP (Rousset 2008), and effective population size (N_e) using LDNE (Waples and Do 2008).

Sex Locus

The accuracy of the sex-determining SNP assay for steelhead and Chinook Salmon was evaluated for hatchery stocks spawned in SY2013; 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

Because genotypes from 100% of the broodstock were not always obtained for all hatchery stocks, this resulted in a small portion of hatchery-origin offspring that were genetically "untagged." 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 3 and 4).

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 years 2012 and 2013, average realized PBT tagging rates at the level of release site were 89.1% and 91.8%, respectively.

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 SY2013, all 5,023 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 5,023 samples, 4,948 (98.5%) were genotyped with an acceptable level of missing data (Table 3). In this final SY2013 PBT baseline comprising the remaining 4,948 samples, there were just 2,786 missing genotypes due to SNP failure out of a possible 470,060 genotypes. This resulted in missing data for just 0.6% of the genotypes.

For Chinook Salmon SY2013, all 9,024 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 9,024 samples, 8,924 (98.9%) were genotyped with an acceptable level of missing data (Table 4). In this final SY2013 PBT baseline comprising the remaining 8,924 samples, there were just 2,595 missing genotypes due to SNP failure out of a possible 847,780 genotypes. This resulted in missing data for just 0.3% of the genotypes.

Tagging Rate

Overall tagging rates were very high for both steelhead (Table 3) and Chinook Salmon (Table 4). All stock-level tag rates were greater than 90% except for a single steelhead broodstock (Little Sheep Creek) and a single Chinook Salmon broodstock (Johnson Creek).

Poor Performing Loci

Of the samples that genotyped with <10 missing SNPs, poor performing SNP assays were identified within the 95 PBT SNP panel.

For SY2013 steelhead, two loci failed to genotype at >3% of samples. Locus Omy_99300-202 failed at 354 (7.2%) of the samples, OMS00039 failed to genotype 151 (3.1%) of the samples.

For SY2013 Chinook Salmon, there were two loci that failed at >3% of the samples. Ots_ppie-245 failed at 263 (3.0%) and Ots_pigh-105 failed at 295 (3.3%) samples.

Error Rate (Quality Control)

For steelhead SY2013, a subset of 166 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 15,770 rerun genotypes being compared

to the original PBT genotypes. Of these genotypes, 141 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 15,629 genotypes with 49 discrepancies between the original and samples and a genotyping error rate of 0.31%.

For Chinook Salmon SY2013, a subset of 365 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 34,675 rerun genotypes being compared to the original PBT genotypes. Of these genotypes, 368 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 34,307 genotypes with 71 discrepancies between the original and samples and a genotyping error rate of 0.21%.

Null Alleles

For steelhead SY2013, 35 of the 95 PBT loci were found to have a frequency of null alleles greater than zero, but only three loci had frequencies >5% (Table 5).

For Chinook Salmon SY2013, 36 of the PBT loci were found to have a frequency of null alleles greater than zero, but none had a frequency >5% (Table 6).

Sex Markers

The sex-specific assay for steelhead matched phenotypic sex in 99.6% of the samples (Table 7). For instances in which genetically-determined sex did not correspond to the phenotypic sex, all but five were cases in which phenotypic females were misidentified by genotype as males. The assay either failed to genotype or provided ambiguous results for 5.9% of the samples.

The sex-specific assay for Chinook Salmon matched phenotypic sex in 97.3% of the samples (Table 8). The majority of discrepancies were phenotypic females genetically identified as male. The assay produced ambiguous results, or failed to genotype, 1.5% of samples.

Average Heterozygosity

Levels of observed heterozygosity within steelhead broodstocks was ~0.4 for all hatcheries broodstocks (Table 9). Levels of observed heterozygosity tended to be lower in Chinook Salmon (~0.35) in all stocks (Table 10).

Population Structure

Pairwise F_{st} was calculated among the steelhead SY2013 hatchery broodstock (Table 11). Values ranged from a low of -0.002 between the Touchet and Tucannon stocks. A high of 0.065 was observed between the SF Clearwater stock and Little Sheep Creek. All F_{st} values among stocks were significant.

For Chinook Salmon SY2013 pairwise F_{st} values ranged from a low of 0.001 at the Nez Perce Tribal Hatchery and the Dworshak stock to a high of 0.046 between Sawtooth and Tucannon (Table 12). All F_{st} values among stocks were significant within each year.

Effective Population Size

Effective population size (N_e) and 95% CI for each steelhead hatchery broodstock in SY2013 ranged from a low of 27.6 for the Upper Salmon B-run broodstock to a high of 258.4 for Dworshak National Fish Hatchery (DNFH) production broodstock (Table 13). Reliable estimates could not be made for the EF Salmon, Touchet, or Tucannon broodstocks as indicated by infinitely larger CIs or negative values of N_e. Infinite estimates and negative N_e values 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 and 95% CI for each Chinook Salmon hatchery broodstock in SY2013 ranged from a low of 98.3 for Grande Ronde to a high of 581.6 for Rapid River (Table 14).

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 sex marker performed well for all stocks. Most of the discrepancies involved the Oxbow stock. Discrepancies were traced to scoring errors of the genotypes. This error arose simply because a portion of genotyped samples contained only one sex, thereby producing only one cluster in the scatterplot of SNP genotypes. Without the other sex included in these samples, there was not a second cluster of genotypes to provide perspective and some samples were mis-scored. To minimize this type of error in the future, we initiated an additional procedure in our QA/QC protocol where phenotypic sex is checked against genotypic sex for all samples prior to closing the project. This additional step would have identified the scoring errors for the Oxbow stock. When these scoring errors were corrected the overall rate of correspondence between phenotypic and genetic sex was 99.8%.

The Chinook Salmon sex marker also performed well in all stocks. Most of the discrepancies involved Rapid River stock. In this case the discrepancies were also traced to scoring errors of the genotypes. As described above, this error arose simply because a portion of samples contained only one sex, thereby producing only one cluster of genotypes making the samples prone to mis-scoring. When these scoring errors were corrected the overall rate of correspondence between phenotypic and genetic sex was also 99.8%.

The results are encouraging in that these assays can provide an accurate and nonlethal 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

Broodstocks with small sample sizes are vulnerable to having a lower tag rate because each ungenotyped individual represents a larger proportion of the population, and thus a larger proportion of untagged offspring, than broodstocks with larger sample sizes. The broodstocks with the lowest tag rates in SY2013 (Little Sheep Ck. = 72.9% and Johnson Ck. = 89.9%) are stocks with small numbers of spawners (Table 3 and 4). Despite lower rates for these two stocks the overall tag rate for each species was ≥97.0%.

Poor Performing Loci

Both of the poor-performing steelhead loci (Omy_99300-202 and OMS00039) in SY2013 are known to have null alleles. To prevent null allele genotypes from being included in the database we have adopted scoring rules for these loci. If genotyping patterns for samples at these loci suggest the presence of null alleles then the genotypes are manually 'no called', meaning that the genotype is not scored nor included in the data to minimize including null allele genotypes. The high proportion of failed samples at these loci is likely due to conservative scoring of genotypes.

Both of the poor-performing Chinook Salmon loci (Ots_ppie-245 and Ots_pigh-105) in SY2013 are also known to have either null alleles or poor clustering patterns. The high proportion of failed samples at these loci is also likely due to samples being manually 'no called' because of the suspected presence of null alleles.

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

The three steelhead PBT loci that had the highest frequencies (>5%) of null alleles (OMS00118, OMS00070, Omy_113490159) consistently have had high levels of null alleles in previous broodstock collections. These loci may need to be reevaluated or scoring rules for the loci may need to be modified to account for null alleles.

No loci within the Chinook Salmon SNP panel had a null allele frequency >5%. Locus Ots_OTALDBINT1SNP1 had the highest null allele frequency of 3.6%. This locus was identified as having a null allele frequency >5% for SY2008, SY2009, and SY2010 and was also ~5% in SY2011 and SY2012. This locus may need to be re-evaluated because of consistent presence of null alleles.

Average Heterozygosity

The average expected heterozygosity was high and uniform across both steelhead hatchery stocks (~0.40) and Chinook Salmon (~0.35) demonstrating that the degree of

variability in these SNP sets makes them useful for 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 population histories, such as the Oxbow, Sawtooth, and Pahsimeroi stocks. Low divergence among Oxbow, Sawtooth, and Pahsimeroi reflect their shared history of being 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).

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). This perhaps reflects the current management practice of managing all Chinook Salmon in the Clearwater drainage as a single stock in which broodstock are moved among the different locations.

Effective Population Size

Effective population sizes generally corresponded to size of broodstock. Larger hatchery programs (e.g., steelhead stocks at Dworshak, Oxbow, Pahsimeroi, Sawtooth, and Wallowa or Chinook Salmon stocks at Clearwater and Rapid River) tended to have larger N_e, while programs with smaller broodstocks (steelhead stocks of Upper Salmon B-run and EFSR or Chinook Salmon stocks of the Grande Ronde) had a smaller N_e. Several steelhead stocks, including Touchet, Tucannon, and EF Salmon, had an unrealistic estimate of N_e as indicated by either a negative N_e estimate and/or an infinitely large CI. Both indicate that an accurate estimate could not be made due to either small sample size of lack of linkage disequilibrium in the samples.

Sampling broodstock for PBT provides a unique opportunity to test the accuracy of N_e estimation methods. A direct measurement of N_e can be obtained 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. LDNE, Colony) that are derived using genetic data from a single generation. Future research efforts will more thoroughly explore the correlations between these methods.

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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 2013 (SY2014), 2.) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2013 (SY2014), 3.) Origin of samples from sport fisheries in the lower Snake River in migration years 2012 and 2013 (SY2013 and SY2014), 4.) Origin of samples from various sport fisheries in Idaho in migration year 2012 (SY2013), 5.) Parentage of SY2014 Upper Salmon B-run broodstock for real-time management of spawning, and 6.) Correction of PIT expansions for SY2013 Sawtooth broodstock.

For Chinook Salmon, the PBT baselines were used to determine: 1.) Origin of carcasses encountered in spawning-ground surveys on the South Fork Salmon River in SY2013, 2.) Origin of samples from various sport fisheries in Idaho in (SY2013), 3.) Parentage of Clearwater broodstock for real-time management of spawning, 4.) Age composition of SY2013 broodstocks, and 5.) Origin of "escaped" fingerlings sampled from headboxes of Dworshak raceways.

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 Zones 1–6 of Columbia River

IDFG coordinated the sampling of steelhead harvested in the lower Columbia River sport fishery (river Zones 1–6) in 2013 (SY2014). A total of 1,248 samples (1,070 from Zones 1-5 and 178 from Zone 6) were processed for PBT assignment. A more detailed description of this project is presented 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) 2013 (e.g. spawn year 2014). A total of 1,025 samples from clipped steelhead were analyzed. A more detailed description of this project is also presented in Byrne et al. (*In Prep.*).

Steelhead Sport Fisheries in Lower Snake River

Washington Department of Fish and Wildlife (WDFW) collected samples of steelhead harvested in the SY2014 lower Snake River sport fishery from the mouth of the Snake to the Idaho/Washington border. A total of 712 samples were processed for PBT assignment. A more detailed description of this project is in Byrne et al. (*In Prep*).

Steelhead Sport Fisheries in Idaho

IDFG collected samples of steelhead harvested in the SY2013 sport fishery from various river systems including the Clearwater and Salmon. A total of 1,860 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 SY2014 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 SY2013 Sawtooth broodstock. PBT was used to assign Sawtooth broodstock from SY2013 back to the SY2008, SY2009, and SY2010 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 SY2013 Steelhead Broodstock

PBT was used to determine age composition of steelhead broodstocks in Idaho by assigning the SY2013 broodstocks back to the SY2008–SY2010 broodstocks, thereby identifying the age of each fish. A total of 4,784 samples from seven different broodstocks were analyzed with PBT.

Chinook Salmon Carcasses in the South Fork of the Salmon River

Chinook Salmon carcasses encountered during spawning-ground surveys of the South Fork Salmon River were sampled in SY2013 to estimate the proportion of hatchery-origin summer Chinook Salmon on the spawning grounds (pHOS). A total of 141 carcasses were sampled and analyzed with PBT. Study rationale and design is more thoroughly presented in Hinrichsen et al. (*In Prep*.).

Chinook Salmon Sport Fishery in Idaho

Fisheries managers within IDFG implemented PBT sampling of Chinook Salmon harvested in the sport fishery in 2013. A total of 837 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.*).

Chinook Salmon Broodstock Management at Clearwater Hatchery

Summer Chinook Salmon from releases in Crooked River that strayed into the DNFH adult trap in 2014 presented a unique opportunity to utilize PBT as a real-time broodstock management tool. The summer Chinook Salmon could not be visually distinguished from spring Chinook Salmon because there are several release groups in the Clearwater River basin that are released without adipose fin clips, but all had coded wire tags. All unclipped hatchery Chinook Salmon (identified by the presence of a coded wire tag) that were encountered at DNFH had a tissue sample removed to be used for PBT analysis, a PIT tag was inserted, and fish were transferred to a holding pond at Clearwater Hatchery until results from the PBT analysis could be obtained. The PIT tag and tissue samples were tracked together, and when PBT results were available they were paired with the PIT tag number to allow sorting of individuals based on spring or summer origin. The week following tissue sampling, the fish were sorted into spring and summer Chinook Salmon holding ponds by scanning for PIT tags which identified the fish's origin based on the paired PIT and PBT data. This process was executed for three weeks during the 2014 trapping season.

Age Composition of SY2013 Chinook Salmon Broodstock

PBT was used to determine age composition of Chinook Salmon broodstocks in Idaho by assigning the SY2013 broodstocks back to the SY2008–SY2010 broodstocks, thereby identifying the age of each fish. A total of 7,183 samples from seven different broodstocks were analyzed with PBT.

Origin of Chinook Salmon "Escapees"

Fingerlings were discovered in the headboxes of Chinook Salmon raceways at DNFH. Managers wished to identify whether fish were escaping from onsite raceways or were pumped into the hatchery through the intake system. A total of 28 samples were processed and analyzed with PBT.

RESULTS

Steelhead Sport Fisheries in Zones 1–6 of Columbia River

Of the 1,248 samples analyzed, 13 were omitted from the analysis because they failed to meet genotyping criteria or were determined to be duplicate samples from the same fish. After expanding by PBT rates, 66% of the sport harvest in Zones 1-5 and 82% of the sport harvest in Zone 6 of the lower Columbia River 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, 12 samples were omitted from the analysis because they failed to meet genotyping criteria. After expanding by PBT rates, 81%

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 712 samples collected, two were omitted from the analysis because they failed to meet genotyping criteria or were determined to be duplicate samples from the same fish. After expanding by PBT rates, the origin of 95% of samples could be accounted for. A breakdown of stock and cohort proportions will be presented in Byrne et al. (*In Prep.*).

Steelhead Sport Fisheries in Idaho

Of the 1,860 samples analyzed, 24 were omitted from the analysis because they failed to meet genotyping criteria or were duplicates. After expanding by PBT rates, 100% 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 eggs from the inbred crosses were excluded from future production.

Correction of PIT Expansions in Steelhead

Cohort membership was determined through PBT for 95.8% of the SY2013 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.

Age Composition of SY2013 Steelhead Broodstock

Of the 4,784 samples analyzed with PBT 4,452 assigned (93.1%) to the baseline. After expanding by the tag rates, the origin of 4,628 (96.7%) 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 Carcasses in the South Fork Salmon River

Of the 153 carcass samples 40 failed to genotype adequately, likely due to DNA degradation in the carcass, and were removed from analysis. Using PBT assignments (n = 43) expanded by the appropriate PBT rate and the maximum likelihood estimator presented in Hinrichsen et al. (*In Prep.)*, the proportion of hatchery-origin fish on the spawning grounds of the South Fork Salmon River was 40.1% (SE = 4.1%) which could be broken into 3-year-old jacks (3.6%), 4-year-olds (29.1%), and 5-year-olds (7.4%). CWT recoveries (n = 7) provided similar estimates for the cohorts of hatchery-origin fish; however, the standard errors of estimates were smaller when the PBT data were used. Results are more thoroughly presented in Hinrichsen et al. (*In Prep*.).

Chinook Salmon Sport Fishery in Idaho

Of the 837 samples genotyped, 13 were omitted from the analysis 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 100% 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.*).

Chinook Salmon Broodstock Management at Clearwater Hatchery

Of the 82 samples analyzed, all but one assigned back to broodstock tagged with PBT. Of the 81 samples that received a parentage assignment eight were identified as summer Chinook Salmon from Crooked River releases. The 74 spring Chinook Salmon and eight summer Chinook Salmon were sorted into the appropriate brood holding ponds based on the paired PIT tag and PBT information.

Age Composition of SY2013 Chinook Salmon Broodstock

Of the 7,183 samples analyzed with PBT, 6,542 assigned (91.1%) to the baseline. After expanding by the tag rates, the origin of 7,164 (99.7%) 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.

Origin of Chinook Salmon "Escapees"

Of the 28 samples analyzed, 13 were determined to be steelhead and the remaining 15 did not assign with PBT. It was deduced that because the samples comprised a mixture of steelhead and Chinook Salmon and because none of the Chinook Salmon samples received a PBT assignment, the fingerlings in the headbox did not escape from on-site raceways but rather were wild juveniles that entered through the intake system.

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. Implications of the results are more thoroughly explored in Byrne et al. (*In Prep.*).

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 Carcasses in the South Fork Salmon River

This project demonstrated that PBT provides similar, and slightly more precise, estimates of pHOS than CWT. Results will be used to design an efficient sampling protocol using PBT to determine pHOS in spawning grounds that have multiple contributing stocks with different marking rates (Hinrichsen et al., *In Prep*.).

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*.).

Chinook Salmon Broodstock Management at Clearwater Hatchery

Timing is critical when spawning broodstock and managers increasingly desire information about their broodstocks before it begins. We have demonstrated that genetic samples from unspawned broodstock can be genotyped and analyzed with PBT to help manage spawning.

Age Composition of SY2013 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 SY2013 broodstock. Determining age composition of the broodstock through PBT is another benefit of implementing the technology.

Origin of Chinook Salmon "Escapees"

Novel solutions are often needed for unexpected problems that arise at hatcheries. The discovery of fingerlings in the headboxes of Chinook Salmon raceways at DNFH indicated that either hatchery fish were escaping out of raceways or wild fish were being pumped into the hatchery. While PBT was conceived as a means for determining stock composition of mixed stock fisheries, situations like this demonstrate how versatile a tool PBT can be.

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TABLES

Table 1. Total steelhead broodstock genetically sampled in SY2013 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	576
E.F. Salmon River	25
Oxbow	208
Pahsimeroi	1,080
Upper Salmon R. B-run	365
Dworshak	1,792
Wallowa	505
Little Sheep Ck	130
Tucannon	45
Touchet	18
Cottonwood	279
Total	**5,023**

Table 2. Total Chinook Salmon broodstock sampled in SY2013 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
Rapid River	2,023
Dworshak	1,858
Powell	224
SF Clearwater	979
Sawtooth	948
Pahsimeroi	447
Tucannon	149
S.F. Salmon	1,053
Imnaha	189
Lostine	123
Catherine Ck	86
Grande Ronde	364
Lookingglass Ck	119
Nez Perce Tribal Hatchery (NPTH)	385
Johnson Ck	77
Total	**9,024**

Table 3. Sample sizes and genotyping completion rate of SY2013 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.

	2013		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
Sawtooth	576	575 (99.8%)	99.7%
E. Fk. Salmon R	25	24 (96.0%)	92.2%
Upper Salmon B-run	365	364 (99.7%)	99.5%
Oxbow	208	206 (99.0%)	98.1%
Pahsimeroi	1,080	1,071 (99.2%)	98.3%
Dworshak	1,642	1,624 (98.9%)	97.8%
S.F. Clearwater	150	149 (99.3%)	98.7%
Little Sheep Ck	130	111 (85.4%)	72.9%
Tucannon	45	45 (100.0%)	100.0%
Touchet	18	18 (100.0%)	100.0%
Cottonwood Ck	279	279 (100.0%)	100.0%
Wallowa	505	482 (95.5%)	91.1%
Total	**5,023**	**4,948 (98.5%)**	**97.0%**

Table 4. Sample sizes and genotyping completion rate of SY2013 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.

	2013		
Snake River Hatchery Stocks	**Samples**	**Genotyped (%)**	**Tagging Rate**
Rapid River	2,023	2,007 (99.2%)	98.4%
Dworshak	1,858	1,815 (97.7%)	95.4%
Powell	224	222 (99.1%)	98.2%
SF Clearwater	979	967 (98.8%)	97.6%
Sawtooth	948	946 (99.8%)	99.6%
Pahsimeroi	447	446 (99.8%)	99.6%
Tucannon	149	149 (100.0%)	100.0%
SF Salmon	1,053	1,046 (99.3%)	98.7%
Lookingglass	119	116 (97.5%)	95.0%
Imnaha	189	189 (100.0%)	100.0%
Lostine	123	120 (97.6%)	95.2%
Catherine Ck	86	84 (97.7%)	95.4%
Grande Ronde	364	359 (98.6%)	97.2%
Nez Perce Tribal Hatchery	385	385 (100.0%)	100.0%
Johnson Ck	77	73 (94.8%)	89.9%
Total	**9,024**	**8,924 (98.9%)**	**97.8%**

Table 5. Ranked estimates of null allele frequencies for 35 loci from the combined steelhead SY2013 PBT broodstock.

SNP Name	Frequency of Null Allele	SNP Name	Frequency of Null Allele
OMS00039	0.002	Omy_11138351	0.024
Omy_g1282	0.013	Omy_IL6320	0.024
Omy_metA161	0.014	Omy_b1266	0.025
OMS00101	0.016	Omy_colla1525	0.025
Omy_Il1b_028	0.016	OMS00062	0.026
OMS00002	0.018	Omy_ntl27	0.026
Omy_stat3273	0.018	Omy_108007193	0.027
OMS00024	0.019	Omy_anp17	0.027
Omy_crb106	0.019	OMS00089	0.032
Omy_hsc71580	0.019	OMS00180	0.038
OMY1011SNP	0.019	OMS00064	0.042
Omy_104519624	0.020	OMS00053	0.044
Omy_109243222	0.020	Omy_vatf406	0.046
OMS00175	0.021	Omy_u0954311	0.049
Omy_rbm4b203	0.022	OMS00070	0.054
Omy_Il1b198	0.023	Omy_113490159	0.063
M09AAJ163	0.024	OMS00118	0.070
OMS00106	0.024		

Table 6. Ranked estimates of null allele frequencies for 36 loci from the combined Chinook Salmon SY2013 PBT broodstock.

SNP Name	Frequency of Null Allele	SNP Name	Frequency of Null Allele
Ots_u0717135	0.008	Ots_9490399R	0.018
Ots_129458451	0.010	Ots_Prl2	0.018
Ots_96500180	0.011	Ots_102414395	0.018
Ots_115987325	0.013	Ots_HSP90B100	0.020
Ots_110689218	0.013	Ots_RAG3	0.021
Ots_112876371	0.014	Ots_P53	0.021
Ots_TGFB	0.014	Ots_NOD1	0.022
Ots_112820284	0.014	Ots_TAPBP	0.022
Ots_105105613	0.015	Ots_117432409	0.022
Ots_ppie245	0.015	Ots_FGF6B_1	0.022
Ots_11820561	0.015	Ots_u0725325	0.023
Ots_hsc713488	0.015	Ots_IGFI176	0.026
Ots_mybp85	0.015	Ots_101704143	0.027
Ots_GCSH	0.016	Ots_E2275	0.029
Ots_txnip321	0.016	Ots_TLR3	0.029
Ots_105407117	0.017	Ots_u675	0.031
Ots_GPH318	0.018	Ots_MHC2	0.031
Ots_vatf251	0.018	Ots_OTALDBINT1SNP1	0.036

Table 7. Results of comparisons between phenotypic sex and genetically determined sex using the sex-specific assay for SY2013 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	576	5 (0.9%)	571 (99.1%)	571 (100%)	0 (0%)	0 (0%)	0 (0%)	288 (50.0%)	288 (50.0%)
E. F. Salmon	25	5 (20.0%)	20 (80.0%)	20 (100%)	0 (0%)	0 (0%)	0 (0%)	13 (52.0%)	12 (48.0%)
Oxbow	208	7 (3.4%)	201 (96.6%)	190 (94.5%)	11 (5.5%)	1 (9.1%)	10 (90.9%)	104 (50.0%)	104 (50.0%)
Pahsimeroi	1,080	8 (0.7%)	1,072 (99.3%)	1,070 (99.8%)	2 (0.2%)	1 (50.0%)	1 (50.0%)	540 (50.0%)	540 (50.0%)
Upper Sal. B	365	5 (1.4%)	360 (98.6%)	358 (99.4%)	2 (0.6%)	0 (0%)	2 (100%)	139 (38.1%)	226 (61.9%)
Dworshak	1,642	80 (4.9%)	1,562 (95.1%)	1,560 (99.9%)	2 (0.1%)	1 (50.0%)	1 (50.0%)	712 (43.4%)	930 (56.6%)
SF Clearwater	150	6 (4.0%)	144 (96.0%)	143 (99.3%)	1 (0.7%)	1 (100%)	0 (0%)	70 (46.7%)	80 (53.3%)
Wallowa	505	88 (17.4%)	417 (82.6%)	416 (99.8%)	1 (0.2%)	1 (100%)	0 (0%)	252 (49.9%)	253 (50.1%)
Little Sheep Ck	130	35 (26.9%)	95 (73.1%)	94 (98.9%)	1 (1.1%)	0 (0%)	1 (100%)	64 (49.2%)	66 (50.8%)
Tucannon	45	0 (0%)	45 (100%)	45 (100%)	0 (0%)	0 (0%)	0 (0%)	20 (44.4%)	25 (55.6%)
Touchet	18	4 (22.2%)	14 (77.8%)	14 (100%)	0 (0%)	0 (0%)	0 (0%)	8 (44.4%)	10 (55.6%)
Cottonwood	279	53 (19.0%)	226 (81.0%)	226 (100%)	0 (0%)	0 (0%)	0 (0%)	135 (48.4%)	144 (51.6%)
Total	**5,023**	**296 (5.9%)**	**4,727 (94.1%)**	**4,707 (99.6%)**	**20 (0.4%)**	**5 (25.0%)**	**15 (75.0%)**	**2345 (46.7%)**	**2678 (53.3%)**

Table 8. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for Chinook Salmon (IDFG-OTS-SEX) from the SY2013 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
Rapid River	2,023	16 (0.8%)	2,007 (99.2%)	1,914 (95.4%)	93 (4.6%)	0 (0.0%)	93 (100%)	997 (49.3%)	1026 (50.7%)
Dworshak	1,858	48 (2.6%)	1810 (97.4%)	1,801 (99.5%)	9 (0.5%)	1 (11.1%)	8 (72.7%)	891 (48.0%)	967 (52.0%)
Powell	224	6 (2.7%)	218 (97.3%)	218 (100%)	0 (0%)	0 (0%)	0 (0%)	113 (50.4%)	111 (49.6%)
SF Clearwater	979	22 (2.2%)	957 (97.8%)	956 (99.9%)	1 (0.1%)	0 (0%)	1 (100%)	495 (50.6%)	484 (49.4%)
Sawtooth	948	7 (0.7%)	941 (99.3%)	941 (100%)	0 (0%)	0 (0%)	0 (0%)	474 (50.0%)	474 (50.0%)
Pahsimeroi	447	2 (0.4%)	445 (99.6%)	445 (100%)	0 (0%)	0 (0%)	0 (0%)	223 (49.9%)	224 (50.1%)
Tucannon	149	0 (0%)	149 (100%)	149 (100%)	0 (0%)	0 (0%)	0 (0%)	71 (47.7%)	78 (52.3%)
SF Salmon	1,053	10 (0.1%)	1,043 (99.1%)	1,043 (100%)	0 (0%)	0 (0%)	0 (0%)	527 (50.0%)	526 (50.0%)
Imnaha	189	0 (0%)	189 (100%)	189 (100%)	0 (0%)	0 (0%)	0 (0%)	87 (46.0%)	102 (54.0%)
Lostine	123	3 (2.4%)	120 (97.6%)	120 (100%)	0 (0%)	0 (0%)	0 (0%)	54 (43.9%)	69 (56.1%)
Catherine Ck	86	2 (2.3%)	84 (97.7%)	84 (100%)	0 (0%)	0 (0%)	0 (0%)	37 (43.0%)	49 (57%)
Grande Ronde	364	7 (1.9%)	357 (98.1%)	349 (97.8%)	8 (2.2%)	8 (100%)	0 (0%)	178 (48.9%)	186 (51.1%)
Lookingglass	119	4 (3.4%)	115 (96.6%)	115 (100%)	0 (0%)	0 (0%)	0 (0%)	52 (43.7%)	67 (56.3%)
Nez Perce	385	2 (0.5%)	383 (99.5%)	383 (100%)	0 (0%)	0 (0%)	0 (0%)	192 (49.9%)	193 (50.1%)
Johnson Ck	77	7 (9.1%)	70 (90.9%)	70 (100%)	0 (0%)	0 (0%)	0 (0%)	43 (55.8%)	34 (44.2%)
Total	**9,024**	**136 (1.5%)**	**8,888 (98.5%)**	**8,777 (97.3%)**	**111 (1.2%)**	**9 (8.1%)**	**102 (91.9%)**	**4,434 (49.1%)**	**4,590 (50.9%)**

Table 9. Average observed and expected heterozygosity with associated standard deviation of hatchery steelhead stocks for SY2013.

Stock	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
Sawtooth	0.430	0.077	0.427	0.070
EF Salmon	0.411	0.123	0.416	0.089
Upper Salmon B	0.413	0.113	0.399	0.097
Oxbow	0.431	0.081	0.431	0.072
Pahsimeroi	0.432	0.083	0.427	0.077
Dworshak	0.396	0.010	0.397	0.098
SF Clearwater	0.393	0.103	0.394	0.095
Little Sheep Ck	0.428	0.108	0.423	0.093
Tucannon	0.411	0.106	0.426	0.079
Touchet	0.411	0.124	0.424	0.091
Cottonwood Ck	0.435	0.086	0.429	0.076
Wallowa	0.435	0.078	0.424	0.074

Table 10. Average observed and expected heterozygosity with associated standard deviation of hatchery Chinook Salmon stocks in SY2013.

Stock	Avg. het. (Obs)	SD	Avg. het. (Exp)	SD
Rapid River	0.338	0.129	0.337	0.128
Dworshak	0.345	0.123	0.344	0.123
Powell	0.350	0.122	0.348	0.120
SF Clearwater	0.343	0.124	0.345	0.123
Sawtooth	0.338	0.140	0.337	0.138
Pahsimeroi	0.336	0.142	0.334	0.135
Tucannon	0.340	0.137	0.340	0.137
McCall	0.331	0.136	0.330	0.135
Lookingglass	0.352	0.130	0.353	0.122
Imnaha	0.348	0.137	0.342	0.127
Lostine	0.327	0.145	0.322	0.140
Catherine Ck	0.359	0.136	0.353	0.126
Grande Ronde	0.328	0.130	0.336	0.130
Nez Perce Tribal Hatchery	0.344	0.124	0.344	0.123
Johnson Ck	0.331	0.143	0.332	0.136

Table 11. Population structure (F_{ST}) among steelhead hatchery stocks sampled in SY2013. Asterisks (*) indicate that F_{ST} values were significantly different from zero ($p < 0.01$).

Population	EF Salmon	Sawtooth	Pahsimeroi	Up Sal B	Dworshak	SF Clearwater	Cottonwood	Touchet	Tucannon	Little Sheep	Oxbow	Wallowa
EF Salmon	---	*	*	*	*	*	*	*	*	*	*	*
Sawtooth	0.017	---	*	*	*	*	*	*	*	*	*	*
Pahsimeroi	0.016	0.005	---	*	*	*	*	*	*	*	*	*
Up Sal B	0.035	0.042	0.046	---	*	*	*	*	*	*	*	*
Dworshak	0.030	0.048	0.052	0.032	---	*	*	*	*	*	*	*
SF Clearwater	0.036	0.049	0.054	0.038	0.002	---	*	*	*	*	*	*
Cottonwood	0.026	0.018	0.021	0.045	0.045	0.048	---	*	*	*	*	*
Touchet	0.025	0.023	0.022	0.062	0.056	0.059	0.024	---	*	*	*	*
Tucannon	0.020	0.021	0.020	0.047	0.045	0.048	0.016	-0.002	---	*	*	*
Little Sheep	0.026	0.026	0.024	0.051	0.062	0.065	0.025	0.024	0.019	---	*	*
Oxbow	0.021	0.007	0.006	0.048	0.053	0.055	0.022	0.024	0.021	0.026	---	*
Wallowa	0.024	0.018	0.021	0.043	0.045	0.048	0.006	0.019	0.012	0.024	0.023	---

Table 12. Population structure (F_{st}) among SY2013 Chinook Salmon hatcheries. Asterisks (*) indicate F_{st} values are significantly different from zero ($p < 0.01$).

	Clearwater	**Dwor.**	**Johnson**	**Tucann.**	**McCall**	**NPTH**	**Pahsim.**	**Powell**	**Rapid**	**Sawt.**	**Looking.**	**Imnaha**	**Lostine**	**Catherine**	**Grande Ronde**
Clearwater	---	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Dworshak	0.003	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Johnson Ck	0.024	0.023	---	*	*	*	*	*	*	*	*	*	*	*	*
Tucannon	0.028	0.024	0.040	---	*	*	*	*	*	*	*	*	*	*	*
McCall	0.018	0.017	0.013	0.035	---	*	*	*	*	*	*	*	*	*	*
NPTH	0.001	0.001	0.023	0.023	0.018	---	*	*	*	*	*	*	*	*	*
Pahsimeroi	0.032	0.031	0.042	0.045	0.030	0.031	---	*	*	*	*	*	*	*	*
Powell	0.006	0.004	0.029	0.029	0.022	0.005	0.037	---	*	*	*	*	*	*	*
Rapid River	0.008	0.017	0.035	0.044	0.029	0.013	0.043	0.018	---	*	*	*	*	*	*
Sawtooth	0.023	0.022	0.030	0.046	0.019	0.022	0.025	0.025	0.030	---	*	*	*	*	*
Lookingglass	0.006	0.008	0.025	0.028	0.021	0.006	0.029	0.011	0.015	0.024	---	*	*	*	*
Imnaha	0.012	0.013	0.026	0.023	0.015	0.013	0.029	0.017	0.018	0.024	0.012	---	*	*	*
Lostine	0.020	0.022	0.044	0.034	0.029	0.020	0.037	0.025	0.032	0.036	0.021	0.020	---	*	*
Catherine Ck	0.010	0.010	0.027	0.027	0.023	0.009	0.033	0.013	0.019	0.025	0.006	0.016	0.024	---	*
Grande Ronde	0.010	0.011	0.029	0.041	0.023	0.010	0.036	0.015	0.019	0.029	0.011	0.021	0.026	0.016	---

Table 13 Estimates of effective population size (N_e) and 95% confidence intervals for steelhead hatchery stocks and release groups in SY2013.

Stock	N_e	95% CI
EF Salmon	457.9	110.4 – Infinity
Sawtooth	216.5	198.0 – 237.2
Pahsimeroi	210.4	196.8 –225.0
Up Sal B	27.6	26.0 – 29.1
Dworshak	258.4	242.8 – 275.0
SF Clearwater	56.6	51.5 – 62.3
Cottonwood	51.1	47.7 – 54.8
Touchet	-91.0	-625.7 – Infinity
Tucannon	577.0	202.6 – Infinity
Little Sheep	160.5	128.7 – 208.4
Oxbow	120.0	106.8 –135.6
Wallowa	186.3	169.9 – 204.7

Table 14. Estimates of effective population size and 95% confidence intervals for SY2013 Chinook Salmon hatchery stocks.

Stock	Ne	95% CI
Clearwater	267.3	247.6 – 288.9
Dworshak	648.4	595.1 – 707.9
Grande Ronde	98.3	91.0 – 106.4
Catherine Ck	195.8	143.1 – 298.1
Imnaha	243.6	200.3 – 305.2
Lostine	117.2	99.4 – 140.6
Lookingglass	123.4	103.7 – 150.0
Johnson Ck	445.3	236.6 – 2,499.8
Nez Perce FH	342.8	295.5 – 403.4
SF Salmon	307.0	283.8 – 332.4
Pahsimeroi	205.6	186.2 – 228.0
Powell	100.1	90.6 – 111.1
Rapid	581.6	538.1 – 629.4
Sawtooth	324.2	298.0 – 353.4
Tucannon	306.8	232.7 – 437.0

FIGURES

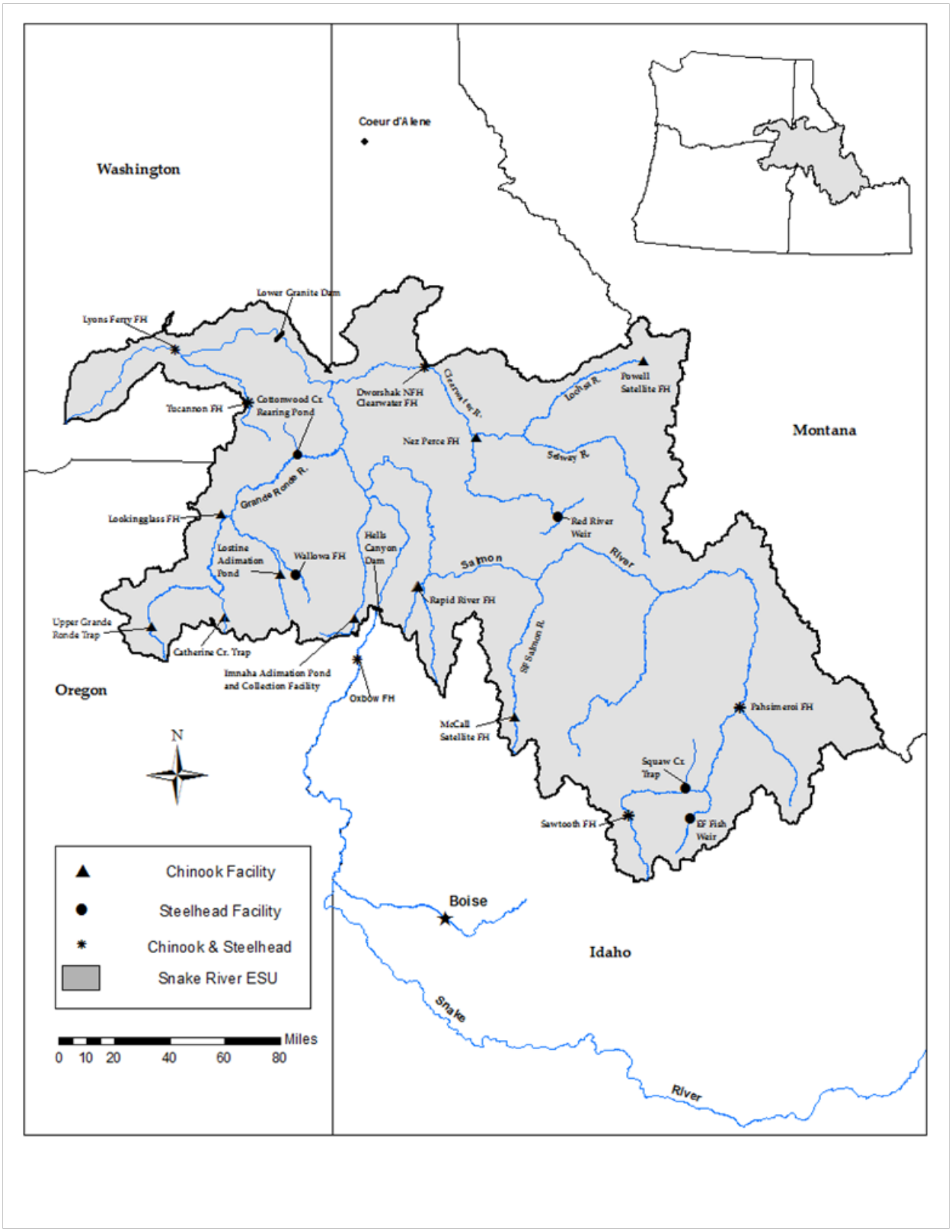

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

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