FISHERY RESEARCH

GENETIC MONITORING OF SNAKE RIVER SALMONIDS

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Genetic Monitoring of Snake River Salmonids

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ABSTRACT

This report summarizes the application of genetic techniques to the management and conservation of anadromous salmonids (steelhead *Oncorhynchus mykiss* and spring-summer Chinook Salmon *O. tshawytscha*) in the Snake River basin. In 2010, Idaho Department of Fish and Game, in collaboration with the Columbia River Inter-Tribal Fish Commission, initiated two BPA-funded projects (2010-026-00 and 2010-031-00) to test and implement genetic monitoring programs for Snake River basin steelhead and spring-summer Chinook Salmon utilizing two genetic technologies that identify origins of hatchery and wild fish. The first technology, called parentage-based tagging (PBT), involves annually sampling and genotyping all hatchery broodstock which are added to a genetic baseline of candidate parents. The genotyping of broodstock permanently genetically "tags" all of their offspring. A non-lethal tissue sample from any offspring of these broodstock can be genotyped and analyses can be completed to assign parentage, thereby identifying hatchery of origin and age. The second technology is called genetic stock identification (GSI), and involves creating a reference genetic baseline from all contributing wild stocks. Wild fish of unknown origin can then be sampled, genotyped, and assigned to a stock of origin via assignment testing. Over the last decade, these projects have demonstrated the accuracy, efficiency, and utility of these technologies for monitoring both wild and hatchery stocks throughout the Snake River and Columbia River basins. For hatchery stocks, PBT addresses objectives established by the Bonneville Power Administration (BPA) Fish and Wildlife Program which involves marking hatchery stocks, conducting hatchery evaluations and reform, and enforcing salmonid fishery management measures. For wild stocks, GSI provides unprecedented tools for monitoring wild stock abundance, productivity, and genetic diversity, which are required for NOAA ESA status assessments. These two projects were combined by BPA in 2021 (2010-031-00), with ongoing goals of keeping PBT and GSI baselines up-to-date in the Snake River basin, to maintain and enhance SNP genetic marker panels, and to continue projects that use these PBT and GSI baselines to address conservation and management issues of importance to the Council and state, tribal and federal fisheries managers. Combined there are eight objectives addressed in this report: 1) the maintenance and evaluation of single nucleotide polymorphism (SNP) panels for high-throughput genotyping of steelhead and Chinook Salmon in the Snake and Columbia river basins; 2) the updating, maintenance, and testing of SNP baselines to describe genetic variation and for use as a reference in conducting GSI for both species; 3) annual sampling of hatchery broodstock and creation of genetic parental databases; 4) utilization of PBT and GSI baselines to estimate genetic stock composition and life history diversity of steelhead and spring-summer Chinook Salmon passing Lower Granite Dam (LGR); 5) application of PBT baselines to estimate the stock composition of steelhead in the Columbia and Snake River tribal and sport fisheries; 6) the monitoring of integrated hatchery programs for Chinook Salmon; 7) the summarization of life history and genetic diversity information for steelhead and spring-summer Chinook Salmon detected at PIT tag detection systems; and 8) the development and application of grandparentage technology for use in the Snake River basin.

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INTRODUCTION

Over the last century, populations of wild salmon and steelhead on the west coast of the United States have experienced significant declines in abundance. During this same time period, the use of hatcheries as a management tool has increased. From a fisheries management perspective, there is a need to track the abundance of both wild and hatchery-origin Chinook Salmon *Oncorhynchus tshawytscha* and steelhead *O. mykiss* to address a suite of distinct but interconnected research and management goals.

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

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

Parentage-based genetic tagging (described in Anderson and Garza 2005, 2006), a technological alternative to CWT, was proposed as an alternative tag not subject to the same limitation of small sample sizes. Parentage-based tagging (PBT) involves annual sampling and genotyping of hatchery broodstock and creating a database of parental genotypes. Progeny from any of these parents (collected either as juveniles or adults), can be non-lethally sampled and, if genotyped, be assigned back to their parents, thus identifying their hatchery of origin and their exact brood year. The exceptional advantage that PBT has over CWT technology is increased sample size. By genotyping all parental broodstock, every juvenile is genetically “tagged”.

Snake River Chinook Salmon and Steelhead Parentage Based Tagging (BPA project 2010-031-00) was initiated in 2010, partly in response to Independent Scientific Review Panel (ISRP) and Independent Scientific Advisory Board (ISAB) recommendations that proof-of-concept trials be completed on Parentage Based Tagging (PBT) technology (ISRP/ISAB 2009). The technology involves the annual sampling and genotyping of all hatchery broodstock and creating a genetic database of parental genotypes. The project’s initial accomplishments were detailed in Steele et al. (2013a) in which the number of genetic markers needed for PBT was empirically confirmed, the power of microsatellite markers and single nucleotide polymorphisms (SNPs) was compared, and the ability to match assignments made with PBT and coded-wire tags was demonstrated. Since the publication of Steele et al. (2013a), the primary objectives of this project have been to oversee the sampling and genotyping of parental broodstock at all Snake River hatcheries, and to genotype these samples with powerful genetic marker panels that are standardized between our main collaborating lab (Columbia River Inter-Tribal Fish Commission) and other salmon genetic labs in the Pacific Northwest. In addition, we help organize and summarize projects that use the Snake River PBT baseline, along with offspring recoveries, to address conservation and management issues of importance to the Council and state and tribal fisheries managers. A review of PBT-related accomplishments over the last 10 years was published in 2019 (Steele et al. 2019), and provides details on how PBT is used to track family

groups, the estimation of PBT tag rates, how PBT baseline data are stored and where they can be accessed by the public.

In addition to efforts to efficiently tag and track hatchery-origin Chinook Salmon and steelhead, there is a pressing need to monitor the abundance, distribution, and diversity of wild Chinook Salmon and steelhead. To this end, a second genetic technique, known as genetic stock identification (GSI), can be used to assign a wild fish of unknown origin to a reference population. GSI uses multilocus genotype data from reference populations (representing the contributing stocks) as a baseline and complimentary genotype data from mixtures of fish of unknown origin to estimate stock proportions within the mixture (Shaklee et al. 1999; Anderson et al. 2008).

Evaluating the status and recovery of wild stocks of Chinook Salmon and steelhead populations in the Columbia River basin is dependent upon accurate estimates of abundance (i.e. number of adults on spawning grounds) (McElhany et al. 2000). Stock-specific abundance estimates can be further broken down into sex and age composition over time, which allows for estimation of productivity (e.g. recruits-per-female). Both abundance and productivity metrics provide indicators of population resiliency and allow assessments of extinction risk. Estimates of these metrics at the population or major population group (MPG) scale is information that fisheries managers can use to achieve sustainable harvest of larger populations, while protecting weaker stocks and the genetic diversity present within them.

The use of genetic techniques to infer population abundance and productivity is particularly helpful in the Snake River basin for several reasons. For example, population-level parameters (e.g., length, age, sex-ratios) have been difficult to obtain because steelhead and Chinook Salmon are widely distributed, occur in remote areas that are difficult to access, and spawn at a time when environmental conditions prevent the use of traditional counting methodologies (weirs and redd count surveys). Monitoring of Snake River steelhead can be hampered due by high turbidity and changing flow conditions during the time of spawning (Thurow 1985). This is less of a problem for spring-summer Chinook Salmon, although turbid water conditions resulting from storms and forest fires have at times impacted the ability to estimate adult abundance using redd-based surveys in the Middle Fork and South Fork Salmon rivers (Thurow 2000). As a result, escapement estimates (and other demographic information) have not been available for most Snake River populations (Busby et al. 1996; Good et al. 2005) until recently. The collection of biological data at Lower Granite Dam in conjunction with GSI methods has enabled managers to generate population-level parameters for many populations of steelhead and Chinook in the Snake River basin for which these data were formerly unknown.

Genetic stock identification has been used extensively to understand and manage mixed stock fisheries for a variety of Pacific salmonids including Chinook Salmon (Smith et al. 2005), Sockeye Salmon (Habicht et al. 2010), Coho Salmon (Beacham et al. 2001), and steelhead (Beacham et al. 2000). In the Snake River basin, studies have indicated that both steelhead and Chinook Salmon exhibit significant genetic structuring at the watershed (or subbasin) level (Moran 2003; Narum et al. 2007; Nielsen et al. 2009; Matala et al. 2014). Previously, researchers have made use of this genetic structure to identify the genetic stock origin of kelt steelhead at Lower Granite Dam (Narum et al. 2008) and to estimate the stock composition of natural-origin and hatchery Chinook Salmon (Smith 2007) and natural-origin steelhead and Chinook Salmon (Ackerman et al. 2012; Campbell et al. 2012; Camacho et al. 2017; Camacho et al. 2018a; Camacho et al. 2018b) at LGR. Recently, work has shown the combined application of PBT and GSI to Chinook Salmon and steelhead at LGR can significantly reduce bias associated with estimation of wild escapement (Hargrove et al. 2021a).

REPORT STRUCTURE

The first section reports efforts to evaluate and maintain the use of single nucleotide polymorphisms (SNPs) for use in genetic monitoring. The second section covers the maintenance and expansion of genetic baselines for genetic stock identification (GSI) work. The third section details the maintenance of parentage-based tagging (PBT) baselines. The fourth section details monitoring of wild and hatchery steelhead and Chinook Salmon at Lower Granite Dam. The fifth section covers the stock composition of steelhead in the Columbia River and Snake River tribal and sport fisheries. The sixth section details monitoring efforts associated with integrated hatchery efforts. The seventh section summarizes the genetic diversity and life history characteristics of adults detected at passive integrated transponder (PIT) arrays in the Snake River basin. The eighth section covers the development of grandparentage technology for use in the Snake River basin.

In this report, we refer to adult steelhead and Chinook Salmon migrating past LGR using spawn years (SY). For steelhead, a spawn year refers to adults that migrate upstream past LGR during the fall of the previous calendar year and the spring of the current calendar year (e.g., SY2021 steelhead are adults that migrated past LGR between 7/1/20 – 6/30/21 and spawned in spring of 2021). For spring-summer Chinook Salmon, a spawn year refers to adults that migrate past LGR prior to August 17 and spawn that same fall. We refer to juveniles of both species migrating past LGR using migratory years (MY). A migratory year refers to juveniles migrating downstream past LGR from the end of March to the end of July that year.

OBJECTIVES

This report is divided into eight sections, one for reach of the objectives for this reporting period. For this performance period, the Snake River PBT project includes the following objectives:

Objective 1: Evaluate and maintain SNP marker panels

Completion of this objective demonstrates the maintenance and evaluation of single nucleotide polymorphism (SNP) panels for high-throughput genotyping of steelhead and Chinook Salmon in the Snake and Columbia river basins.

Objective 2: GSI Baseline maintenance and expansion

Completion of this objective details the results associated with updating, maintaining, and testing of SNP baselines to describe genetic variation and for use as a reference in conducting genetic stock identification (GSI) for steelhead and Chinook Salmon.

Objective 3: PBT Baseline maintenance

Completion of this objective is achieved by sampling all broodstocks, genotyping samples, and creating a database of parental genotypes for each spawn year (SY) of steelhead, spring-summer Chinook Salmon, and fall Chinook Salmon.

Objective 4: Lower Granite Dam wild and hatchery stock monitoring

Completion of this objective details the results of implementing GSI to estimate genetic stock composition and life history diversity of steelhead and spring-summer Chinook Salmon passing Lower Granite Dam. Additionally, 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 is demonstrated.

Objective 5: Steelhead and Chinook Salmon stock composition in the Columbia and Snake River Tribal and Sport Fisheries

Completion of this objective details the results of "back end" projects that use the PBT baselines to assign parentage to samples of unknown origin. We demonstrate the versatility of PBT by summarizing several projects.

For steelhead, the PBT baselines were used to determine: 1) Origin of samples from sport fisheries in the Columbia River during migration year 2020 (SY2021), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2020 (SY2021), 3) Origin of samples from various sport fisheries in Idaho in migration year 2020 (SY2021), 4) Age composition and origin of the SY2020 broodstocks, and 5) stock composition of returning adults during SY2021 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2020), 2) Age composition and origin of SY2020 broodstocks, 3) Stock composition of returning adults during SY2020 at Lower Granite Dam.

Objective 6: Integrated Hatchery Monitoring

Completion of this objective involves addressing four key research questions: 1) what is the proportionate natural influence (PNI) in the supplemented populations, 2) how does survival compare between integrated broodstock (IB) and segregated stock (SS) hatchery programs, 3) can we alter the spawning distribution of IB adults, and 4) what is the replacement rate of natural (NP) and IB spawners. Results for SY2021 will be presented in a companion report titled "Integrated Broodstock Evaluation".

Objective 7: Genetic diversity and life history characteristics of adults detected at PIT tag array

Completion of this objective summarizes the life history and genetic diversity information for steelhead and spring-summer Chinook Salmon detected at PIT tag detection systems throughout the Snake River basin. Hargrove and others will present results for SY2021 in a companion report titled "Abundance, life history, and genetic diversity of natural-origin steelhead and spring-summer Chinook Salmon detected at instream PIT tag detection systems in the Snake River basin".

Objective 8: Development of Grandparentage Technology in the Snake River Basin

Completion of this objective summarizes progress made to identify marker panels of suitable size and power to resolve grandparent-grandchildren relationships, develop new SNP marker panels for grandparentage analysis, and genotype collections of broodstock for use in grandparentage analysis.

SECTION 1: EVALUATE AND MAINTAIN SNP MARKER PANELS

INTRODUCTION

The conclusion of calendar year 2021 marks our sixth full year of genotyping using the Genotyping-in-Thousands (GT-seq) platform for GSI and PBT applications. The current GT-seq marker panel consists of all markers developed on the previous Fluidigm platform as well as SNPs newly developed in 2017 and 2018. Both of the original Chinook Salmon and steelhead GT-seq panels consisted of 95 PBT loci, 96 GSI loci, and 1 sex marker. In 2017, in collaboration with the Columbia River Inter-Tribal Fish Commission (CRITFC) Hagerman Genetics Lab, the Chinook Salmon and steelhead GSI/PBT GT-seq panels were expanded to consist of 299 markers for Chinook Salmon and 269 markers for steelhead. Beginning in 2018, again in collaboration with CRITFC Hagerman Genetics Lab, an expanded SNP panel was developed for Chinook Salmon and steelhead. The latest SNP panel for Chinook Salmon includes 343 loci and the SNP panel for steelhead has 368 loci. We have adopted the use of expanded marker panels for PBT and GSI work in 2017 (299 and 269 marker sets for Chinook Salmon and steelhead) and again in 2020 (343 and 368 marker sets for Chinook Salmon and steelhead).

Data for the SNP marker panels described above can be accessed via the FishGen webpage (https://www.fishgen.net/Home.aspx). Once a user account has been set up with FishGen, the details of these panels can be accessed with the following link: (https://www.fishgen.net/WebPages/CustomMarkerSet/MarkerExport.aspx). Metadata for each marker include synonym of species, Vic probe, Vic allele, Fam probe, Fam allele, forward primer, and reverse primer. The new larger Chinook Salmon panel known as 'CRITFC IDFG Chinook GTseq v4.0 343' consists of 95 PBT loci, 96 GSI loci , 1 sex marker, and 151 additional SNP markers. The new larger steelhead panel known as 'CRITFC/IDFG Steelhead GTseq v5.0 368' consists of 95 PBT loci, 96 GSI loci, 1 sex marker, and 176 additional SNP markers.

In an effort to expand the information content of our current GT-seq panels, we have modified the bioinformatics pipeline used to call SNP variants to extract additional information from available sequence data. Microhaplotype (hereafter microhap) is a term used to describe a combination of two or more physically linked variants within a small genomic region (Leitwein et al. 2020). The current GT-seq approach generates short sequence reads that include a SNP of interest and surrounding nucleotides (flanking regions). In 2021, we discovered microhaps for steelhead by searching the flanking regions of existing SNPs for additional SNP variants at appreciable frequency across individuals and populations. Because microhaps are multi-SNP haplotypes, they can provide a greater amount of information than a stand-alone SNP marker (Oldoni et al. 2019). Microhaps have shown promise in a number of fisheries related studies (e.g., Baetscher et al. 2018; May et al. 2020), and for GSI the additional genetic variation contained within microhaps can allow for greater discrimination among populations and increase the accuracy of GSI assignments (McKinney et al. 2020). In addition to potentially increasing GSI accuracy, these data are generated at no additional cost.

To date, microhaps have been discovered for steelhead from the Snake River basin and their characteristics were described by Hargrove et al. (2021b). Briefly, 92 microhaps were identified for steelhead displaying up to 6 alleles per locus, affording increased resolution for GSI purposes. Microhaps have also been discovered for Chinook Salmon and will be characterized in next year's report in conjunction with the description of a new GSI baseline for that species.

DISCUSSION

Marker panels have continued to expand since their inception in an effort to maximize the resolution of genetic data used in genetic stock identification and parentage-based tagging. For this reporting period, we have initiated work to characterize microhaps for use with Chinook Salmon. Importantly, we have modified existing bioinformatic pipelines to extract microhaps data, which provides additional information relative to bi-allelic SNPs. The resultant increased levels of variability associated with microhaps can directly benefit GSI and PBT efforts. Future work will include describing microhaps for Chinook Salmon and developing new marker panels with even greater numbers of microhaps (Section 8).

Because new panels include all previously used markers, the expansion of our marker panels does not affect backwards compatibility of our GSI/PBT work. In other words, direct comparisons can be made between currently analyzed samples and historic ones.

SECTION 2: GSI BASELINE MAINTENANCE AND EXPANSION

INTRODUCTION

Genetic data helps address an array of conservation and management needs including the description of genetic diversity across the landscape, identification of management units, prioritization of populations for conservation, and an evaluation of restoration efforts. In the Snake River basin, GSI techniques have been used in a conservation and management framework since 2009 to address an array of potential issues (Ackerman et al. 2012). Currently, SNP baselines for steelhead and Chinook Salmon in the Snake River are used to monitor genetic structure and diversity of natural-origin Snake River populations both spatially and temporally. Additionally, these same baselines serve as a reference for GSI work at LGR, which allows fisheries managers to understand the composition of adult escapement returning to the Snake River basin.

The monitoring of genetic structure over time and space provides insight regarding gene flow, both historic and contemporary, from natural (successful straying) and manmade (i.e. out-of-basin hatchery stocking) causes. Monitoring genetic diversity of populations provides information about gain or loss in genetic diversity over time and provides insight into the adaptive potential of populations. Historically, our GSI baselines provide genetic structure and diversity information for 23 extant steelhead National Marine Fisheries Service (NMFS 2017) populations and 28 extant Chinook Salmon populations throughout the Snake River basin to aid in viable salmonid population (VSP; McElhany et al. 2000) monitoring of the Snake River steelhead distinct population segment (DPS) and spring-summer Chinook Salmon evolutionarily significant unit (ESU).

The Snake River SNP baselines serve as a reference for GSI conducted at LGR to estimate genetic stock composition of out-migrating smolts (e.g., Stark et al. 2016) and returning adults (e.g. Lawry et al. 2020). Genetic stock composition estimates of adults and juveniles at LGR, combined with sex and age data, will allow us to estimate abundance, productivity, and life history diversity of genetic stocks over time for VSP monitoring. For GSI, our objective is to periodically update and maintain the SNP baselines to accurately estimate contemporary allele frequencies (genetic structure) of natural-origin populations throughout the Snake River contributing to production at LGR.

Maintaining and updating genetic baselines for GSI is critical to the power and accuracy of GSI. Both biological and analytical factors can affect the ability for GSI methodologies to assign fish accurately to their genetic stock of origin. Biological factors such as straying rates, whereby fish reproduce in populations near to, but not in, their natal river or stream can lower levels of genetic differentiation between populations and decrease the accuracy of genetic stock assignments (e.g., Vähä et al. 2016). In the Snake River basin (and elsewhere) there are several instances where nearby populations exhibit low levels of differentiation relative to nearby collections and the probabilities of assignment associated with GSI are relatively low (Hargrove et al. 2019). One approach to increase the accuracy of assignments in such cases is to increase the number of molecular markers used for GSI assignments (McKinney et al. 2017; Powell et al. 2018). Advancements in sequencing technology (e.g., GT-seq; Campbell et al. 2015) make it possible to genotype multiple SNPs within a given locus, which contrasts with earlier approaches (e.g., TaqMan assays) which were restricted to calling a single SNP per locus. Microhaplotypes (hereafter microhaps) refer to a combination of two or more physically linked variants within a small genomic region (Section 1; Leitwein et al. 2020), and the inclusion of these additional variants has been shown to increase the accuracy of GSI (McKinney et al. 2020). In 2021, we reported the construction of a new GSI baseline for steelhead which included 334 SNP markers,

92 of which were multi-allelic SNPs, or microhaps. We are currently developing a new baseline for Chinook Salmon which includes 53 microhaps. A description of these markers and baseline will be presented in next year's report.

DISCUSSION

For the current reporting period we worked on the development of a new genetic baseline (v4) for Chinook Salmon. The characteristics of this baseline including a comparison of its performance relative to previous iterations will be presented in forthcoming reports. For this year, we used baselines v3.1 to determine the source of origin for Snake River steelhead and Chinook Salmon at LGR. Briefly, the current Chinook Salmon baseline consists of 4,356 samples from 30 populations genotyped at 173 loci (Powell et al. 2018). These collections represent 31 of 41 Technical Recovery Team (TRT) populations and all five major population groups (MPG). The GSI baseline v3.1 for steelhead consists of all 23 TRT populations and five major population groups. Currently, there are 5,967 samples from 45 populations included in the steelhead individuals genotyped at 179 loci (Hargrove et al. 2021b; Appendix A).

SECTION 3: PBT BASELINE MAINTENANCE

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. Additionally, a summary is provided for genetic data collected from steelhead and Chinook Salmon broodstocks in SY2020.

Previously, sets of 96 single nucleotide polymorphism (SNP) markers were identified for steelhead and Chinook Salmon, and the selected SNPs provided sufficient resolving power for dual-parentage assignments (Steele et al. 2011). These sets of markers were again used to genotype broodstock samples collected in 2020. Primer and probe sequence information for these markers are available on http://www.FishGen.net: CRITFC/IDFG Chinook Salmon 96 PBT v5.1 and CRITFC/IDFG Steelhead 96 PBT v5.1.

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

Beginning in SY2015, our laboratory adopted Genotyping-in-Thousands (GT-seq) protocols developed by the CRITFC genetics lab (Campbell et al. 2015) to genotype PBT baselines (also see https://www.monitoringmethods.org/; *SNP genotyping using Genotyping in Thousands (GT-seq) on Illumina Sequencer platform v1.0*, *Method ID# 5446*). This technology utilizes a next-generation DNA sequencing instrument (Illumina NextSeq). This instrument was purchased in September 2015 via a grant from the Pacific Coast Salmon Recovery Fund. It sequences multiplexed PCR products to genotype samples with a minimum of 192 SNP loci at reduced consumable costs. The screening of additional numbers of SNPs for this project continues to allow the two labs (IDFG and CRITFC) to remain standardized and may allow the assignment of single parents in situations where one parent was either inadvertently not sampled or not successfully genotyped. Including the vast majority of the original 96 markers for each species, Chinook Salmon were genotyped at a total of 299 or 343 markers, and steelhead were genotyped at a total of 368 markers. The only Chinook Salmon collection genotyped with 299 markers was the Lyon's Ferry fall Chinook, and so statistics calculated for the fall Chinook populations are restricted to the markers overlapping between the 299 and 343 marker panels.

The 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

Broodstock Sampling

The overall goal is to obtain high quality tissue samples and accurate biological data from every adult that contributes to spawning. This includes species, sex, hatchery/stock, date sampled/spawned, tag information, and markings. Hatcheries also record length and cross information whenever possible. Tissue samples are collected in the form of fin tissue stored on

absorbent sheets of Whatman 3mm chromatography paper (LaHood et al. 2008; and see https://www.monitoringmethods.org/ *Genetic sampling and storage using chromatography filter paper v1.0, Method ID# 4087*). The samples are shipped to the IDFG genetics lab in Eagle, Idaho. Care is taken to avoid contamination during sampling by rinsing scissors or hole-punch tools in water or ethanol and wiping with a paper towel between each tissue sample.

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

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

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

Laboratory Protocol for Creation of Genetic Databases

Genomic DNA extraction followed the methods described in Matala et al. (2011) and was extracted using the Nexttec Genomic DNA Isolation Kit from XpressBio (Thurmont, Maryland) or Qiagen DNeasy (Valencia, California). Campbell et al. (2015) describes protocols of library preparation for GT-seq. Library preparation begins with an initial multiplex PCR reaction that is used to ligate a pair of sequencing primers to the target sequences which contain a known single-nucleotide polymorphism (SNP). In a subsequent PCR reaction the sample is “barcoded” by ligating an additional sequence to the target that identifies the sample’s tray of origin (i7 barcode) and its position on the tray (i5 barcode). After barcoding, the quantity of DNA must be normalized for each sample. A SequalPrep™ Normalization Plate Kit (Applied Biosystems) is used to bind a standard amount of amplicon product and normalize concentrations. All 96 samples are then pooled into a single ‘plate library’. All plate libraries are quantified by a Qubit fluorometer (Invitrogen), and concentrations are normalized again before being pooled. Loci are genotyped by sequencing the target location on the Illumina NextSeq. A bioinformatics pipeline is used to assign resulting sequences and the genotypes back to individual samples using the unique combination of i5 and i7 barcodes.

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

Data quality was inferred from estimates of completion rate, missing data, poor performing loci, and error rates. Basic diversity indices were calculated for the brood years which included average observed (H_O) and expected (H_E) heterozygosity. We also generated estimates of differentiation among stocks through estimates of pairwise F_{ST} and tests of allelic differentiation using Genepop (Rousset 2008). Effective population size (N_E) was estimated using NeEstimator v.2 (Do et al. 2014) assuming a random-mating model and minimum allele frequency of 0.05. We visualized broodstocks using neighbor-joining dendrograms and discriminant analysis of principle components (DAPC; Jombart et al. 2010). Unrooted neighbor-joining dendrograms were generated from Cavalli-Sforza genetic chord distances in PHYLIP (Felsenstein 2005) with 1,000 bootstrap replicates. *A priori* populations (i.e., broodstocks) were used as genetic clusters in DAPC, and the number of principle components retained was optimized using the a-score optimization procedure.

Sex Markers

The accuracy of the sex-determining SNP assay for steelhead and Chinook Salmon was evaluated for hatchery stocks spawned in SY2020; comparisons were made between the phenotypic sex of samples, which was determined at time of spawning, and the genetically determined sex of samples.

Tagging Rate

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

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

Microhaplotype Comparisons

Previous work identified 92 microhaplotypes in the current steelhead marker panel and 53 loci in the Chinook Salmon panel (Delomas et al. 2021). We calculated expected and observed heterozygosity with the microhaplotypes and compared it to those calculated using just the SNPs in those loci targeted by GT-seq. For the steelhead, spring-summer Chinook Salmon, and fall Chinook Salmon stocks, SY2020 broodstock were used to calculate heterozygosity.

Adaptive Haplotype Frequencies

In recent years, researchers have discovered adaptive genetic markers associated with phenotypes that managers often use to categorize steelhead and Chinook Salmon stocks, such as migration timing and age-at-maturity (Hess et al. 2016; Narum et al. 2018; Micheletti et al. 2018). Current GT-seq panels for steelhead include 13 candidate markers on chromosome 28 associated with migration timing (Collins et al. 2020) and 10 candidate markers on chromosome 25 associated with age-at-maturity (Willis et al. 2020). For Chinook Salmon, current GT-seq panels include 28 candidate markers on chromosome 28 associated with migration timing (Willis et al. 2021). To summarize information about these candidate regions in hatchery broodstocks, we calculated haplotype frequencies for chromosome 28 (both steelhead and Chinook Salmon) and chromosome 25 (steelhead only) using the haplo.stats package in R (Sinnwell and Schaid 2021). Haplotype frequencies were calculated both within broodstocks and overall, though we only present distinct haplotypes that had overall frequencies >0. Because the Lyons Ferry fall Chinook Salmon hatchery broodstock is genotyped at an older PBT SNP panel that does not include chromosome 28 markers, we were unable to calculate haplotype frequencies for this broodstock.

RESULTS

Broodstock Sampling

During SY2020, we collected and inventoried approximately 4,621 genetic samples from steelhead broodstock (Table 1) and approximately 9,066 samples (Table 2) from spring-summer Chinook Salmon broodstock spawned in the Snake River basin. We also reported on fall Chinook Salmon collected from the Lyons Ferry and Nez Perce Tribal Fish hatcheries for SY2020 (N = 2,476; 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.

Genetic Database Completion Rate and Missing Data

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

For steelhead SY2020, all samples were extracted and genotyped with the expanded panel of 368 SNPs which includes a sex-identification assay. Of the 4,621 total samples collected, 4,583 (99.1%) were genotyped with an acceptable level of missing data (Table 1).

For spring-summer Chinook Salmon SY2020, all samples were extracted and genotyped with the expanded panel of 343 PBT SNPs which includes a sex-identification assay. Of the 9,066 total samples collected, 8,977 (99.0%) were genotyped with an acceptable level of missing data (Table 2).

For fall Chinook Salmon SY2020, all Lyons Ferry FH samples were genotyped with an older expanded panel of 298 PBT SNPs and the sex-identification assay, while the Nez Perce Tribal FH samples were genotyped with a newer expanded panel of 342 SNPs and the sex-identification assay. Of the 2,476 total samples collected, 2,441 (98.6%) were genotyped with an acceptable level of missing data (Table 3).

Poor Performing Loci

Most SNPs in the samples that passed the genotyping threshold had high genotyping success. For SY2020 steelhead, 27 loci failed to genotype at >5% of samples (Table 4). For SY2020 spring-summer Chinook Salmon, 26 loci failed at >5% of the samples (Table 5). For SY2020 fall Chinook Salmon, 12 loci failed at >5% of the samples (Table 6).

Error Rate (Quality Control)

For steelhead SY2020, a subset of 54 samples were rerun and the resulting 19,303 genotypes (limited to non-missing genotypes in both runs) were checked for discrepancies. Of these genotypes, there were 3 discrepancies, excluding any SNP failures in either the original or the rerun genotype, which resulted in a genotype error rate of 0.02%.

For spring-summer Chinook Salmon SY2020 genotyped at EFGL, a subset of 173 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 56,738 rerun genotypes being compared to the original genotypes. This resulted in 4 discrepancies, excluding any SNP failures in either the original or the rerun genotype, between the original and samples and a genotyping error rate of 0.01%.

Average Heterozygosity

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

Population Structure

Pairwise F_{ST} was calculated among the steelhead SY2020 hatchery broodstocks (Table 13). Values ranged from a low of -0.0007 between the Touchet R. and Tucannon R. stocks, and a high of 0.0683 between the Little Sheep Creek and Dworshak stocks. All tests of genetic differentiation among stocks were significant ($p < 0.01$), except between the Touchet R. and Tucannon R. stocks. The DAPC and neighbor-joining dendrogram both show the same broad patterns as pairwise F_{ST}, with broodstocks of shared ancestry clustering together (Figure 2; Figure 3).

For spring-summer Chinook Salmon SY2020, pairwise F_{ST} values ranged from a low of 0.0013 between the Dworshak and the Nez Perce FH stocks and a high of 0.0531 between the Tucannon and Sawtooth stocks (Table 14). All tests of genetic differentiation were statistically

significant ($p < 0.01$). Differentiation among the two fall Chinook Salmon stocks was very low ($F_{ST} = 0.0045$) and genotypic differentiation was not statistically significant. The DAPC and neighbor-joining dendrogram for spring-summer Chinook Salmon show similar patterns as pairwise F_{ST}, with clustering of broodstocks by shared ancestry (Figure 4; Figure 5). In addition, a DAPC plot of both spring-summer stocks and fall stocks combined shows a large degree of separation by run timing (Figure 6).

Effective Population Size

Effective population size (N_E) for steelhead hatchery broodstock in SY2020 ranged from a low of 57.9 for the Up. Sal. R. B-run broodstock to a high of 2,364.2 (for the Touchet R. broodstock; Table 15). Previous research has indicated that when sample sizes are less than the true effect size, the accuracy and precision of N_E estimates decrease (Ackerman et al 2017). Three stocks exhibited either wide or infinite confidence bounds (E.F. Salmon River, 127.8 - 756.1; Tucannon R., 178.8 – Infinite; and Touchet R., 182.2 – Infinite). We suggest caution in interpreting N_E estimates for these three stocks.

Effective population size for spring-summer Chinook Salmon hatchery broodstock in SY2020 ranged from a low of 74.1 for Lookingglass Cr. to a high of 549.3 for Rapid River (Table 16). Effective population size for the two fall Chinook Salmon hatchery broodstocks were large with the Nez Perce stock estimated at 1,258.5 and the Lyons Ferry stock estimated at 1,256.2 (Table 17).

Sex Markers

The sex-specific assay for steelhead matched phenotypic sex in 99.8% of the samples (Table 7). In the instances (n = 9) in which genetically-determined sex did not correspond to phenotypic sex, it was slightly more likely that phenotypic males were misidentified as females than the opposite. The assay either failed to genotype or provided ambiguous results for 0.04% of the samples.

The sex-specific assay for spring-summer Chinook Salmon matched phenotypic sex in 99.9% of the samples (Table 8). In the instances (n = 13) in which genetically-determined sex did not correspond to phenotypic sex, it was slightly more likely that phenotypic males were misidentified as females than the opposite. The assay either failed to genotype or provided ambiguous results for 0.4% of the samples.

The sex-specific assay for fall Chinook Salmon matched phenotypic sex in 99.8% of the samples (Table 9). In the instances (n = 5) in which genetically-determined sex did not correspond to phenotypic sex, it was slightly more likely that phenotypic females were misidentified as males than the opposite. The assay either failed to genotype or provided ambiguous results for 1.1% of the samples.

Tagging Rate

Overall tagging rates were very high for steelhead (Table 1), spring-summer Chinook Salmon (Table 2), and fall Chinook Salmon stocks (Table 3). All stock-level tag rates met or exceeded 90% in all but two steelhead broodstocks. Stock-level tag rates were greater than 90% in all but one of the spring-summer Chinook Salmon broodstocks. Both Nez Perce and Lyons Ferry fall Chinook Salmon hatchery broodstocks were tagged at 95% or greater for SY2020.

Microhaplotype Comparisons

Heterozygosity of the microhaplotypes was higher than that of just the corresponding SNPs. In steelhead, expected heterozygosity of the microhaplotypes and SNPs had range of 0.29-0.34 and 0.23-0.26, respectively (Table 18). In spring-summer Chinook Salmon, expected heterozygosity of the microhaplotypes and SNPs had range of 0.31-0.35 and 0.23-0.27 respectively (Table 19). In fall Chinook Salmon, expected heterozygosity in both stocks of the microhaplotypes and SNPs was 0.34 and 0.26, respectively (Table 20).

Adaptive Haplotype Frequencies

In steelhead hatchery broodstocks, 33 distinct haplotypes on chromosome 28 and 26 distinct haplotypes on chromosome 25 were inferred from GT-seq genotypes (Table 21; Table 22). Many of these haplotypes and their relative frequencies align with previous estimates from interior-lineage steelhead sampled at Bonneville Dam (Willis et al. 2020). We also inferred 62 distinct haplotypes on chromosome 28 in spring-summer Chinook Salmon broodstocks (Table 23; Table 24) and 57 in the Nez Perce fall Chinook Salmon broodstock (Table 25).

DISCUSSION

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

Completion Rate and Missing Data

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

Poor Performing Loci

Our panels of SNP loci for steelhead and spring-summer Chinook Salmon have been genotyped on 13 years' worth of hatchery broodstock in the Snake River basin (>100,000 samples). We observe very few loci that do not exhibit high genotyping success. Transitioning to GT-seq has helped reduce the number of poor performing loci. The GT-seq protocol uses an automated procedure to score loci, thereby removing inconsistency in the scoring. While it is interesting to identify loci that have differential genotyping success rates, we have decided that it is not necessary that these loci be replaced in any of the SNP panels, especially since the PBT panel has been expanded using GT-seq. The number of SNP loci in the PBT panel is close to or above 300 markers for steelhead and Chinook Salmon and the presence of several poorly genotyping loci is not critical for accurate parentage analysis given the remaining number of successfully genotyping loci in the panel.

Error Rate (Quality Control)

To minimize false negatives in parentage assignments, genetic markers need to exhibit low genotyping error rates and researchers should accommodate estimated error rates during data analysis (Kalinowski et al. 2007). Genotyping error rates for SNPs vary depending on the technique used to genotype them. For methods that rely on genotyping-by-sequencing, error rates are also dependent upon the sequencing depth. With genotyping-by-sequencing, SNP genotyping error rates have been estimated at less than 1% with depths greater than 30 reads per locus (Fountain et al. 2016). With our GT-seq panels, mean depth is typically ~150 reads per locus, suggesting that our error rates should be lower. For the parentage software programs CERVUS and SNPPIT, the default error rate used is 1%. We consistently observed error rates ≤1% for both the steelhead and Chinook Salmon PBT panels of SNPs across several years.

Population Structure

Within steelhead, the highest pairwise F_{ST} values are seen between the Dworshak Hatchery stock (and its derivatives such as the Upper Salmon B-run stock and SF Clearwater stock) and other locations. The Dworshak, Upper Salmon B-run, and SF Clearwater stocks also form a distinct clade or cluster separate from other stocks outside the Clearwater basin in both the neighbor-joining dendrogram and DAPC. The larger degree of divergence between Dworshak and 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. Similar patterns of shared ancestry are evident in the neighbor-joining dendrogram and DAPC for steelhead stocks.

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

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 N_E of all hatchery broodstocks using the commonly employed linkage disequilibrium estimator. Results indicate that while we observe variation in N_E between larger hatchery programs (e.g., Dworshak steelhead, Lyons Ferry fall Chinook Salmon) and smaller programs (e.g., Powell spring-summer Chinook Salmon), N_E is frequently fairly large (>150) for hatchery broodstock populations

spawned annually in the Snake River basin. No steelhead or Chinook Salmon broodstocks were estimated to have N_E <50, which based on genetic theory would put the population at risk of inbreeding (Franklin 1980).

Sex Markers

The steelhead and Chinook Salmon sex markers continue to provide an accurate (~99%) method of identifying phenotypic sex in both species.

Tagging Rates

This project continues to demonstrate that it is possible to achieve high PBT tagging rates even when tens of thousands of fish require tissue sampling and genotyping. The overall tag rate for the Snake River basin was 98.4% for steelhead, 98.0% for spring-summer Chinook Salmon, and 97.2% for fall Chinook Salmon.

Microhaplotype Comparisons

The added variation of microhaplotypes discovered in 2020 will improve accuracy of relationship inference and increase the opportunity to utilize single parent assignments in future analyses.

Adaptive Haplotype Frequencies

Recently discovered candidate markers in steelhead and Chinook Salmon provide a genetic tool to potentially predict phenotypes of great interest to managers, such as run timing and age-at-maturity (Willis et al. 2020; Collins et al. 2020; Willis et al. 2021). Haplotype frequencies of these candidate regions on chromosomes 25 (age-at-maturity in steelhead) and 28 (migration timing in both steelhead and Chinook Salmon) demonstrate the underlying genetic variation in hatchery broodstocks that may drive differences in run timing and age-at-maturity. Efforts are currently underway to compare historical and contemporary haplotype frequencies from candidate regions on chromosomes 25 and 28 for Dworshak National Fish Hatchery steelhead.

SECTION 4. LOWER GRANITE DAM WILD AND HATCHERY STOCK MONITORING

INTRODUCTION

The long-range goal of the Idaho Department of Fish and Game's anadromous fish program is to preserve Idaho's salmon and steelhead runs and recover them to benefit all users (IDFG 2018). This goal is consistent with basin-wide mitigation and recovery efforts. Fisheries management requires an understanding of how salmonid populations function as well as regular status assessments to document progress towards achieving these goals (McElhany et al. 2000). Estimates of abundance, combined with sex and age information over time, allow estimation of population growth rates; and both abundance and productivity metrics provide indicators of the resiliency and viability of populations. Estimates of these metrics at the genetic stock or MPG level can be used by fisheries managers to prescribe sustainable harvest rates for larger populations, while protecting weaker stocks and the genetic diversity within them.

However, population level or MPG assessments of abundance and productivity for ESA-listed Snake River steelhead and spring-summer Chinook Salmon can be particularly difficult (see Report Introduction). Specific data on Snake River steelhead and Chinook Salmon MPGs and populations are lacking, particularly key parameters such as population abundance, age composition, genetic diversity, recruits per spawner, and survival rates (ICTRT 2003). Genetic stock identification (GSI) is one potential means of estimating these parameters at a finer-scale (e.g., MPG, genetic stock [reporting group], or population). Genetic stock identification uses multilocus genotype data from reference populations (representing potential contributing stocks) as a baseline and a complimentary set of genotype data from mixtures of fish of unknown origin to estimate stock proportions within the mixture and to estimate stock of origin of individual fish (Shaklee et al. 1999). The SNP baselines used for GSI in the Snake River basin are described in Powell et al. (2018) and Hargrove et al. (2021b; Appendix A for steelhead). Here we use complementary sets of genotype data from adults sampled at the Lower Granite Dam adult trap and juveniles sampled at the LGR juvenile bypass facility to estimate the genetic stock of origin of upstream migrating adults and emigrating juveniles. We then provide life history diversity (sex, length, age, migration timing) information of individuals assigning to the various Snake River genetic stocks.

METHODS

Adult Trap Operations

Detailed methods for operation of the LGR adult trap can be found in Lawry et al. (2020) and citations within. Briefly, adult steelhead and spring-summer Chinook Salmon migrating upstream past LGR may be intercepted at a trapping facility, located on the adult fish ladder above the counting window, according to a predetermined sampling rate. Sample rates that achieve sample requirements for various projects while balancing fish handling concerns are determined by a committee of collaborating management agencies. Sample rates are typically 10–20%. The sample rate determines how long a trap gate remains open four times per hour; the trap is operational 24 hours per day from March 1 to approximately mid-November every year.

Juvenile Trap Operations

Detailed methods for operation of the LGR juvenile trap can be found in Camacho et al. (2018b) and citations within. The juvenile trap is located on the LGR juvenile bypass system. The

trap captures a systematic sample of fish by operating two trap gates according to a predetermined sample rate. The sample rate determines how long the trap gates remain open, up to six times per hour. The trap is operational 24 hours per day and fish are processed every morning. Sample rate is predetermined daily to collect 250–750 fish per day (all species combined) and is based on the expected number of fish entrained in the bypass system that day.

Fish Handling Protocols (Adults and Juveniles)

Fish handling procedures are detailed in Lawry et al. (2020) for adults and Camacho et al. (2018b) for juveniles (and citations within both reports). Fish captured at the LGR adult or juvenile trap are anesthetized; identified to species; examined for external marks, tags, and injuries; scanned for an internal CWT or PIT tag; and measured for fork length (FL, mm). All fish are examined for the presence (unclipped) or absence (clipped) of the adipose fin and classified to putative origin (hatchery or natural). All natural-origin fish have an unclipped adipose fin because they spend their entire life cycle in the natural environment. Most hatchery-origin fish have a clipped adipose fin. However, some hatchery fish may be released with an unclipped adipose fin for supplementation or tribal harvest opportunities. Thus, unclipped fish are also examined for a CWT or a PIT tag. The presence of a CWT definitively identifies an unclipped fish as hatchery origin. For unclipped adult steelhead, hatchery origin may also be determined by the presence of dorsal and/or ventral fin erosion, which is assumed to occur only in hatchery-reared steelhead (Latremouille 2003). Captured fish determined to be putatively natural-origin or unclipped hatchery with no CWT are sampled for scales (for age; except juvenile Chinook Salmon). Tissue from adults sampled at LGR for sex and genotype data are collected from fish with either an unclipped or a clipped adipose fin. For juveniles, fish bearing PIT tags and/or diseased or injured fish were omitted from the subsample, as were Chinook deemed to be yearling fall Chinook Salmon based on external morphology (Tiffan et al. 2000).

Scales were taken from above the lateral line and posterior to the dorsal fin. Samples were stored in coin envelopes for transport to the IDFG Nampa Research Anadromous Ageing Laboratory in Nampa, Idaho. Tissue samples were taken from a small clip of the anal fin. Tissues were stored on a Whatman sheet for transport to the IDFG Eagle Fish Genetics Laboratory. Gender was not visually determined at the trap, but was assessed using Y chromosome-specific genetic assays (Campbell et al. 2012). After processing, all fish were returned to the fish ladder to resume upstream migration (adults) or the bypass system to resume downstream migration (juveniles).

Scale Age Protocol

Protocols for determining a fish's age from scales are detailed in Wright et al. (2015).

Genetics Laboratory Protocol

Laboratory protocols for DNA extraction, amplification, and SNP genotyping are detailed in Section 2 of Vu et al. (2015). Juvenile MY2021 steelhead and Chinook Salmon juveniles and SY2021 adult steelhead and Chinook Salmon were processed at either IDFG's Eagle Fish Genetics Lab (EFGL) in Eagle, Idaho or the Columbia River Inter-Tribal Fish Commission Lab in Hagerman, Idaho.

Parentage-Based Tagging

Beginning in 2008, parentage-based tagging (PBT; Anderson and Garza 2005) has been used to genetically tag nearly all hatchery-origin steelhead in the Snake River basin (Steele et al. 2013a, 2013b). This genetic tagging technique is accomplished by genotyping all parental broodstock each spawn year, thereby allowing any offspring to be assigned back to their parents and identifying the hatchery of origin and age of offspring. The implementation of PBT provides an alternative to coded-wire tags (CWT) for identifying the origin and age of fish harvested in mixed-stock fisheries or that stray into natural spawning areas.

We conducted PBT analysis for all unclipped juvenile fish sampled in MY2021 and adult fish sampled in SY2021 using the software SNPPIT (Anderson 2010, available at: https://github.com/eriqande/snppit). In using PBT to evaluate all the fish, we are better able to identify putative natural-origin (unmarked, untagged) fish that are truly of hatchery origin. Any individuals identified as unmarked hatchery origin adults with a PBT were removed from the dataset before performing GSI and evaluating life history diversity of genetic stocks.

Genetic Stock Identification

Genetic stock identification is a complimentary genetic technique to PBT that seeks to identify the source of origin of wild fish. Briefly, this technique involves genotyping wild fish sampled on the landscape and using population-level allele frequencies to assign individual fish of unknown origin (adults sampled at LGR) to reporting groups (referred henceforth as genetic stocks). Genotypes were analyzed against genetic baseline populations to assign each individual to the genetic stock in which the probability of its genotype occurring was the greatest. Individuals were assigned to genetic stocks using the algorithms implemented in rubias (Moran and Anderson 2019) implemented in R. For Chinook Salmon, individual assignments for adult and juvenile samples were made using Snake River SNP baseline v3.1 developed by Powell et al. (2018; section 2) which is formally described in Appendix A. For steelhead, assignments for juvenile samples were made using the Snake River SNP baseline v4 described by Hargrove et al. (2021b). Adult steelhead were compared against Snake River SNP baseline v3.1 described by Hargrove et al. (2021b; Appendix A). Analysis of steelhead using two separate baselines occurred because the latest iteration (baseline v4) only became available after adult samples had been processed. Genetic stocks are assemblages of reference (baseline) populations grouped primarily by genetic and geographic similarities and secondarily by political boundaries and/or management units (Ackerman et al. 2011). Individual assignment (IA) procedures assign an individual's genotype to the reporting group from which it is most likely to have originated.

Ten genetic stocks were used for natural-origin steelhead IA analyses. Genetic stocks include: 1) UPSALM: upper Salmon River; 2) MFSALM: Middle Fork Salmon River (including Chamberlain and Bargamin creeks); 3) SFSALM: South Fork Salmon River; 4) LOSALM: lower Salmon River; 5) UPCLWR: upper Clearwater River (Lochsa and Selway rivers); 6) SFCLWR: South Fork Clearwater River (including Clear Creek); 7) LOCLWR: lower Clearwater River; 8) IMNAHA: Imnaha River; 9) GRROND: Grande Ronde River; and 10) LSNAKE: Asotin Creek and tributaries to the Snake River downstream of the Clearwater River confluence.

Seven natural-origin Chinook Salmon genetic stocks were used during IA analyses. Genetic stocks include: 1) UPSALM: upper Salmon River; 2) MFSALM: Middle Fork Salmon River; 3) CHMBLN: Chamberlain Creek; 4) SFSALM: South Fork Salmon River; 5) HELLSC: an aggregate reporting group that includes the Little Salmon, Clearwater, Grande Ronde, and Imnaha rivers; 6) TUCANO: Tucannon River, and 7) FALL: Snake River fall Chinook Salmon.

Three collections of Snake River fall Chinook Salmon (see Table 2 in Ackerman et al. 2012) are included in the SNP baselines (FALL genetic stock); we are able to identify fall Chinook within mixtures of spring-summer Chinook with 100% accuracy.

After performing IA, we estimated genetic stock compositions of all samples analyzed and evaluated life history diversity for each genetic stock. We summarize results for four sample groups:

- SY2021 steelhead adults
- MY2021 steelhead juveniles
- SY2021 Chinook Salmon adults
- MY2021 Chinook Salmon juveniles

RESULTS

We inventoried 8,981 samples (including both steelhead and Chinook Salmon) from SY2021 adults and MY2021 juveniles at LGR (Table 26). A total of 75 (0.8%) samples were not genotyped successfully (<90% complete). We assigned 1,857 (20.9%) samples to hatchery parents in our PBT baseline despite all samples being collected from fish with intact adipose fins. For the remaining 7,049 samples, we performed IA, which are summarized below and in Tables 27–30.

SY2021 Steelhead Adults

Of the 4,115 unclipped adult steelhead sampled in SY2021, 3,504 (85%) were phenotypically identified as natural-origin because they had no apparent dorsal or ventral fin erosion (Table 26). The remaining 611 (15%) fish were identified as hatchery origin due to the presence of dorsal and/or ventral fin erosion. A total of 4,074 (99%) of the queued samples were successfully genotyped. We assigned 362 (10%) phenotypically natural-origin fish to hatchery parents. Of the 607 fish that were successfully genotyped and phenotypically identified as hatchery origin fish, 565 individuals (93%) were assigned via PBT to hatchery parents. All remaining successfully genotyped, phenotypically natural-origin (3,105), and phenotypically hatchery (42) adult steelhead that failed to assign to hatchery parents were assigned to a genetic stock via IA.

Life history diversity information (sex, length, and ocean age) for the 3,105 unclipped, phenotypically wild adult steelhead sampled in SY2021 and assigned a genetic stock is summarized in Table 27. Steelhead were assigned to genetic stock in the following numbers, 1,049 (34%) assigned to GRROND, 385 (12%) to UPSALM, 345 (11%) to LSNAKE, 331 (11%) to UPCLWR, 233 (8%) to SFCLWR, 222 (7%) to MFSALM, 188 (6%) to IMNAHA, 182 (6%) to LOCLWR, 95 (3%) to SFSALM, and 75 (2%) to LOSALM.

MY2021 Steelhead Juveniles

A total of 1,338 (99%) of the 1,346 juveniles queued for genotyping were successfully amplified. Of these fish, 19 (1%) were assigned to hatchery parents. The remaining 1,319 (99%) were assigned a genetic stock via IA.

Life history diversity information for the 1,319 emigrating steelhead smolts that were assigned to a genetic stock is summarized in Table 28. Steelhead smolts were assigned to genetic stocks at the following rates, 442 (34%) assigned to GRROND, 238 (18%) to SFCLWR, 168 (13%)

to UPSALM, 165 (13%) to LSNAKE, 86 (7%) to IMNAHA, 82 (6%) to LOCLWR, 61 (5%) to UPCLWR, 47 (4%) to MFSALM, 19 (1%) to SFSALM, and 11 (1%) to LOSALM.

MY2021 Chinook Salmon Adults

Of the 2,676 unclipped adult Chinook Salmon sampled in SY2021, all but 3 (<1%) samples were genotyped successfully. We assigned 666 (25%) Chinook Salmon to hatchery parents. The remaining 2,007 Chinook Salmon were assigned to a genetic stock via IA.

Life history diversity information (sex, length, and ocean age) for the 2,007 unclipped, phenotypically wild adult Chinook Salmon sampled in SY2021 and assigned a genetic stock is summarized in Table 29. Of the 2,007 Chinook Salmon assigned a genetic stock, 668 (33%) assigned to HELLSC, 528 (26%) to SFSALM, 394 (20%) to UPSALM, 243 (12%) to MFSALM, 135 (7%) to FALL, 35 (2%) to CHMBLN, and 4 (<1%) to TUCANO.

MY2021 Chinook Salmon Juveniles

We queued 844 unclipped juvenile Chinook Salmon from MY2021 for genotyping, and 23 (3%) of samples failed to genotype (Table 26). Of the juveniles that were successfully genotyped, 245 (30%) were assigned back to hatchery parents and the remaining 576 (70%) were assigned a genetic stock via IA.

Life history diversity information for the 576 Chinook Salmon smolts assigned a genetic stock is summarized in Table 30. Of the smolts assigned a genetic stock, 392 (68%) assigned to HELLSC, 84 (15%) to SFSALM, 37 (7%) to MFSALM, 37 (6%) to UPSALM, 21 (4%) to FALL, 4 (<1%) to CHMBLN, and 0 to TUCANO.

DISCUSSION

Adult steelhead and spring-summer Chinook Salmon are intercepted at the LGR adult trapping facility at approximately a 10–20% trapping rate. Tissue samples are taken from trapped fish as part of this project to estimate abundance and life history diversity metrics at the genetic stock and/or MPG scale. This work allows estimation of abundance and productivity by the Idaho Department of Fish and Game for both steelhead and Chinook Salmon at the genetic stock scale across the entire Snake River basin. These metrics are critical components of VSP monitoring and are reported in the wild adult and juvenile steelhead and Chinook Salmon abundance and composition reports (e.g., Camacho et al. 2017; Camacho et al. 2018a; Camacho et al. 2018b).

Trapped adult fish are also PIT tagged by the Integrated Status and Effectiveness Monitoring Project (ISEMP; BPA Project 2003-017-00). Detection of these PIT-tagged fish throughout the Snake River basin are used in a Bayesian branching model to provide reliable and unbiased estimates of abundance at the tributary scale (QCI 2013; See et al. 2016). A multi-agency collaboration has been initiated to utilize information generated from SNP genotyping and PIT tag detections. The goal of this collaboration is to synthesize available data regarding abundance, life-history diversity, and genetic structure and diversity of Snake River steelhead and spring-summer Chinook Salmon. This information is available from the PIT tagging and biological sampling of adults at LGR and the subsequent detection of those adults throughout the Snake River basin. We discuss where the results of this collaboration are reported in Section 7.

The Columbia River Inter-Tribal Fish Commission conducts PBT and GSI of adult steelhead and Chinook Salmon at Bonneville Dam to estimate stock composition and abundance and to evaluate life history information for stocks migrating above Bonneville Dam. In the future, we intend to combine information from GSI at both LGR and Bonneville Dam to evaluate straying and survival between the two dams for both species. Further, we will evaluate adults captured in the Zone 6 fishery (between Bonneville Dam and McNary Dam) using a combination of PBT and GSI. The above information will also greatly assist run reconstruction efforts.

As part of ongoing advancements to increase the accuracy and utility of our genetic analyses, genetic markers are being continually discovered and added to existing marker panels. Similarly, new genetic GSI baselines are developed on a periodic basis to incorporate new markers, update allele frequencies, and ensure that fish populations throughout the basin are adequately represented (Hargrove et al. 2021b). As such, it is possible for different sets of samples to be analyzed at different marker/baseline combinations (e.g., SY2021 vs. MY2021 steelhead, this document). In order to avoid any biases associated with different marker/baseline combinations, we recommend reanalyzing samples using a common set of markers and common baseline on a periodic basis. For example, 5-year status reviews represent a good opportunity to ensure that all life-stage, run year combinations are analyzed using a common set of genetic markers and compared against a common baseline.

Parentage-based tagging is another important genetic technology implemented on all fish with intact adipose fins sampled at LGR prior to GSI. Using this technology, we can remove unmarked, untagged hatchery origin individuals from the natural-origin sample used to estimate abundance at the genetic stock, MPG, population, and/or subpopulation levels. Failing to remove these unidentified hatchery individuals will result in overestimating abundance of natural-origin stocks (Hargrove et al. 2021b). This overestimate is likely the largest potential source of bias in abundance estimation within the Snake River basin. We illustrate the importance of this filtering step with returning steelhead. Hatchery steelhead can potentially be identified through the presence of dorsal and/or ventral fin erosion that is assumed absent from natural-origin individuals (Latremouille 2003) in addition to any physical marks (e.g., coded wire tags, adipose fin clip) applied at the hatchery. Despite this additional “mark,” using PBT we identified an additional 10% of the phenotypically natural-origin steelhead trapped at LGR as hatchery origin in SY2021 (Table 26). Because there is no additional phenotypic hatchery “mark” for Chinook Salmon, the rate of adipose-intact hatchery fish identified via PBT-only is higher than for steelhead (in SY2021, 25% of adult Chinook Salmon that were phenotypically wild had hatchery-origin parents). Thus, the application of PBT is instrumental in accurately estimating abundance of natural-origin stocks in the Snake River basin.

Continuation of GSI efforts at LGR will allow us to 1) monitor genetic structure and diversity throughout the basin over time, and 2) estimate productivity parameters and related life history diversity information for genetic stocks throughout the Snake River basin.

SECTION 5: STEELHEAD AND CHINOOK SALMON STOCK COMPOSITION IN THE COLUMBIA AND SNAKE RIVER TRIBAL AND SPORT FISHERIES

INTRODUCTION

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

For steelhead, the PBT baselines were used to determine: 1) Origin of samples from sport fisheries in the Columbia River during migration year 2020 (SY2021), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2020 (SY2021), 3) Origin of samples from various sport fisheries in Idaho in migration year 2020 (SY2021), 4) Age composition and origin of the SY2020 broodstocks, and 5) stock composition of returning adults during SY2021 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2020), 2) Age composition and origin of SY2020 broodstocks, 3) stock composition of returning adults during SY2020 at Lower Granite Dam.

METHODS

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

Steelhead Sport Fisheries in Columbia River

IDFG coordinated the sampling of steelhead harvested in the lower Columbia River sport fishery in 2020 (SY2021). A total of 709 samples were processed for PBT assignment. An example of the methods used for this annual sampling and PBT assignment results can be found in Byrne et al. (In prep).

Steelhead Tribal Fisheries in Zone 6 of Columbia River

IDFG typically coordinates sampling of steelhead harvested in the tribal fishery between Bonneville Dam and McNary Dam (Zone 6). However, due to COVID-19, no Zone 6 samples were collected for collection year (CY) 2020 (i.e., spawn year 2021), but these analyses will be resumed in future years.

Steelhead Sport Fisheries in Idaho

IDFG collected samples of steelhead harvested in the SY2021 sport fishery from various river systems including the Clearwater and Salmon. A total of 3,155 samples were processed for PBT assignment. A more detailed description of this project is in LeCheminant et al. (In prep). Results from a previous year are available in Warren et al. (2018).

Age Composition of SY2020 Steelhead Broodstock

PBT was used to determine age composition of steelhead broodstocks in Idaho by assigning the SY2020 broodstocks back to all previously sampled broodstocks, thereby identifying the age of each fish. A total of 4,706 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 2020 and spring of 2021 (LeCheminant et al. In prep) and 2,574 samples were analyzed with PBT. Results from a previous year are available in Warren et al. (2018).

Chinook Salmon Sport Fishery in Idaho

Fisheries managers within IDFG continued PBT sampling of Chinook Salmon harvested in the sport fisheries in SY2020. A total of 445 samples representative of the various time strata and river sections were analyzed with PBT. Complete methodology and results are presented in Belnap et al. (In prep). Results for a previous year are available in Sullivan et al. (2018).

Age Composition of SY2020 Chinook Salmon Broodstock

PBT was used to determine age composition of Chinook Salmon broodstocks in Idaho by assigning the SY2020 broodstocks back to previously sampled broodstocks, thereby identifying the age of each fish. A total of 10,105 hatchery-origin broodstock samples were analyzed with PBT.

Stock Composition of Adult Chinook Salmon at Lower Granite Dam

Due to COVID-19, not enough representative samples of the adult Chinook Salmon run across Lower Granite Dam were collected in 2020, therefore no samples were analyzed with PBT.

RESULTS

Steelhead Sport Fisheries in Columbia River

Of the 690 samples analyzed after excluding duplicates and those that failed to genotype, 498 assigned to the PBT baseline. A detailed breakdown of stock composition in these fisheries is presented in Byrne et al. (In prep).

Steelhead Tribal Fisheries in Zone 6 of Columbia River

As noted above, because of COVID-19, no samples were collected or analyzed in 2020.

Steelhead Sport Fisheries in Idaho

Of the 3,067 samples analyzed after removing duplicates and genotyping failures, 2,858 (93%) assigned. A detailed breakdown of stock composition in this fishery is presented in LeCheminant et al. (In prep).

Age Composition of SY2020 Steelhead Broodstock

Of the samples collected, 4,556 were analyzed with PBT after excluding duplicate and un-genotyped samples. Of these, 4,208 (92%) assigned to the baseline. Age composition for 3-, 4-, and 5-year olds in each hatchery stock will be provided in upcoming IDFG technical reports.

Stock Composition of Adult Steelhead at Lower Granite Dam

Of the samples collected, 2,552 were analyzed after removing duplicated samples or samples that failed to genotype. Of these, 2,326 (91%) assigned to the baseline. A summary of stock composition and age will be provided in an upcoming IDFG technical report (LeCheminant et al. In prep).

Chinook Salmon Sport Fishery in Idaho

Of the samples collected, 407 were analyzed after removal of samples that failed to genotype. Of these, 279 (69%) received a PBT assignment. A detailed breakdown of stock and age composition of the harvest in this fishery is presented in Belnap et al. (In prep).

Age Composition of SY2020 Chinook Salmon Broodstock

Of the samples collected, 8,175 were analyzed after removing samples that failed to genotype and duplicate samples. Of these, 7,386 assigned (90.3%) to the PBT baseline. Age composition for 3-, 4-, and 5-year olds in each hatchery stock will be provided in upcoming IDFG technical reports.

Stock Composition of Adult Chinook Salmon at Lower Granite Dam

As noted above, because of COVID-19, no samples were analyzed in 2020.

DISCUSSION

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

Steelhead Sport Fisheries in Columbia River

This project represents some of the first comprehensive attempts to categorize the stock composition of the steelhead harvest in the Lower Columbia sport fishery. Results from this year's sampling (Byrne et al. In prep), as well as results from previous years (Byrne et al. 2015), will aid

in monitoring needs for the *U.S. v Oregon* Management Agreement and in the management of ESA-listed B-run steelhead that return to the Dworshak Fish Hatchery.

Steelhead Tribal Fisheries in Zone 6 of Columbia River

As noted above, because of COVID-19, no samples were collected or analyzed in 2020.

Steelhead Sport Fisheries in Idaho

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

Chinook Salmon Sport Fishery in Idaho

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

Age Composition of Broodstocks

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

SECTION 6: UTILIZATION OF PBT METHODS FOR INTEGRATED BROODSTOCK EFFECTIVENESS MONITORING

INTRODUCTION

Supplementation programs can be implemented in a variety of ways, but all use hatchery production to augment naturally spawning fish populations. Supplementation programs have been implemented throughout the Columbia River basin for several decades (ISAB 2003). Recently, integrated broodstock (IB) programs have been developed in an effort to minimize genetic divergence between hatchery donor stocks and recipient wild stocks. A properly integrated program is one where the natural environment drives the adaptation and fitness of a composite population of fish spawning in a hatchery and in the wild (HSRG 2009). This is hypothesized to minimize domestication selection in the hatchery was well as ecological risks to the natural population (HSRG 2009). Integrated broodstock programs may also be used to increase the abundance of natural populations (Sharma et al. 2006; Berejikian et al. 2008), provide harvest opportunities (Fast et al. 2015), minimize risks to wild populations from hatchery straying (Mobrand et al. 2005), provide genetic repositories for natural fish in the hatchery environment (Kline and Flagg 2014), and may expand the distribution of fish spawning in under-utilized habitats (Dittman et al. 2010; Venditti et al. 2018). The proportionate natural influence (PNI), based on Ford's (2002) gene flow model, was developed by the HSRG (2009) as a measure of integration between the hatchery and natural populations and to quantify the magnitude and direction of gene flow between hatchery and natural fish stocks in IB programs. The PNI is approximated as:

$$PNI \approx pNOB / (pNOB + pHOS)$$

where pNOB is the proportion of natural origin fish in the broodstock and pHOS is the proportion of hatchery origin fish spawning naturally. While the PNI concept has been adopted to guide the implementation of hatchery supplementation throughout the Pacific Northwest (HSRG 2014), few hatchery programs have formal monitoring programs in place to estimate PNI annually or measure the long-term influence that integration has on wild and hatchery survival, productivity and replacement rates.

One of the provisions in the 2008-2017 United States versus Oregon Management Agreement (US v. OR) was to initiate supplementation programs for Chinook Salmon in Idaho. In response, the IDFG transitioned a portion of the hatchery production at three hatcheries to IB programs in 2010. These include the Sawtooth Hatchery (SFH) on the upper Salmon River near Stanley, the Pahsimeroi Hatchery (PFH) on the Pahsimeroi River near Challis, and the McCall Hatchery (MFH) in McCall. The MFH operates a weir on the South Fork Salmon River (SFSR) near Warm Lake for broodstock collection.

There are currently two PNI objectives for these locations. The first applies to the IB and that portion of the population spawning naturally upstream of the hatchery weirs. The second is for the composite population and includes both components of the first plus that portion of the population spawning naturally downstream of the SFH and MFH weirs. The first PNI objective is to achieve an average PNI ≥ 0.67 for the SFH and MFH programs and an average PNI ≥0.80 for the PFH program. These measures of PNI are consistent with HSRG recommendations for "high priority" populations (HSRG 2009). The second PNI objective is to achieve an average PNI ≥0.50 for the composite populations in the upper Salmon and South Fork Salmon rivers (SFH and MFH, respectively).

For this performance period, we report on estimates of PNI for three integrated Chinook Salmon programs at the Sawtooth (Upper Salmon River), Pahsimeroi (Pahsimeroi River), and McCall (South Fork Salmon River) hatcheries for spawn year 2020.

METHODS

Detailed methods for this work are available in Venditti et al. (2020). Briefly, for each of these IB programs, PNI Is estimated for the portion of the population spawning upstream of the weir and for the entire population aggregate above and below the weir. To provide estimates of pHOS above the weir, all returning adults are examined for physical marks, enumerated, and genetically sampled for PBT analyses. To provide estimates of pHOS in the spawning population below the weir, spawning ground surveys are used to estimate spawner abundance (via redd counts) and composition (via carcass recoveries). Recovered carcasses are examined for physical marks, enumerated, and genetically sampled for PBT analyses. To provide estimates of pNOB in the hatchery, all fish used for as IB are examined for physical marks and genetically sampled for PBT analyses. Composition of IB spawners and individual cross information are recorded in the Fish Inventory System database (FINS; http://finsnet.org)

Field protocol

Methods for the collection of field samples can be found on https://www.monitoringmethods.org:

- Carcass tissue sampling - fin tissue for genetic analysis v1.0 (ID: 303) Published
- Tissue sampling for Parentage Based Tagging v1.0 (ID: 1432) Published
- Genetic sampling and storage using Chromatography filter paper v1.0 (ID: 4087) Published

Laboratory protocol

Methods for genomic DNA extraction and amplification and SNP genotyping using multiplex 5'-nuclease reactions can be found on https://www.monitoringmethods.org:

- Nexttec 96-Well Tray DNA Extraction Kit Protocol (ID: 1339) (Published)
- *O. mykiss and O. tshawytscha* SNP marker sets for PBT and GSI use in the Columbia River basin (ID: 1356) (Published)
- SNP genotyping using Genotyping in Thousands (GT-seq) on Illumina Sequencer platform v1.0 (ID: 5446) Published

Data Analysis/Interpretation

Methods for data quality checking and the estimation of genetic diversity, genetic structure, and effective population size of hatchery stocks can be found on https://www.monitoringmethods.org:

- Parentage assignments using SNPPIT software (ID: 1341) (Published)

RESULTS

In 2020, 886 Chinook Salmon DNA samples were collected and genotyped for this program. Of these, 373 were from adults collected for the SFH program, 393 for the MFH program, and 120 from the PFH program. Genotypes from these samples provide information to determine the parents and stock of origin for each individual as well as becoming the set of potential parents for the next generation. Parentage assignment rates were high (typically ≥95%) for hatchery origin (IB or segregated stock) adults at all locations. However, assignments for natural-origin adults were lower (40–60%) for the same locations and time, suggesting interchange between fish spawning above and below weirs, precocious male contribution, or both.

Estimated PNI values for the portion of Chinook Salmon populations spawning upstream of the hatchery weirs in 2020 were higher when based on mark than estimates made with origin-corrected PBT information. Estimates between the two methods had the highest concordance at the MFH, with a difference of 7 PNI units. The differences between methodologies at the other two facilities were similar at approximately 15 PNI units (Table 31). This year showed an upturn in PNI estimates based on PBT at all three hatcheries relative to 2019 (Table 31). PNI remained below target levels at the PFH. The target level was exceeded at the SFH and MFH. However, this was because only natural origin fish were available to pass for volitional spawning.

We did achieve target PNI goals upstream of the weir at the SFH and MFH by both estimation methods in 2020. Upstream of the weir at PFH, we achieved target PNI by mark, but not PBT. Natural-origin adult returns were insufficient to achieve targeted pNOB levels at SFH and MFH, but PFH did achieve a pNOB = 1 based on mark. At the SFH, IB adults were used in hatchery crosses within the IB instead of being released for volitional spawning. This resulted in higher PNI values but no supplementation. Long-term average PNIs (2014–2019) for those portions of the populations spawning upstream of the weirs remain below target levels. Over this period, PNI averaged 0.58 (mark) and 0.46 (PBT) at SFH, 0.68 (mark) and 0.56 (PBT) at PFH, and 0.51 (mark) and 0.39 (PBT) at MFH.

Composite PNI values for the SFSR and USR populations were variable, relative to the >0.5 target in 2020. The target was met in the SFSR, where the composite PNI was 0.53 (Table 31). However, composite PNI of 0.17 in the USR was well below this threshold (Table 31). The low PNI value in the USR was primarily driven by high proportions of segregated stock spawning downstream of weirs along with low numbers of natural-origin adults passed upstream of weirs and included in the IB broodstock.

DISCUSSION

PBT is an effective means of determining the origin of adults collected at hatchery weirs, and will be a useful tool to track PNI at facilities that maintain both mitigation and conservation programs over time. Effective mechanisms exist to track fish through the hatchery system to determine accurately whether an adult is part of the integrated or segregated stocks, which is crucial to long-term PNI monitoring. It is also critical to identify natural-origin fish as well, but, in this case, parentage is not necessarily required. Given the region-wide application of PBT marking and that we utilize the full regional suite of potential parents any adipose fin intact fish that does not get a PBT parentage assignment is almost certainly the product of natural production, albeit

with at least one un-sampled parent. However, since their DNA is collected when spawned or passed over weirs, their progeny will then be PBT marked, for future evaluation.

Low natural-origin and IB returns in 2020 were largely responsible for the PNI levels observed. Intentional retention of IB adults for use in the integrated hatchery program at the SFH increased PNI estimates but at the cost of supplementing the naturally spawning population. Sliding scales incorporated into Hatchery Genetic Monitoring Programs for each facility dictate how natural-origin adults are distributed between the IB and natural spawning over a range of returns. During years of low returns, additional integrated or, in extreme cases, segregated adults may be passed over the weir or spawned in the integrated broodstock. Until natural-origin returns improve, PNI levels will remain low.

SECTION 7: GENETIC DIVERSITY AND LIFE HISTORY CHARACTERISTICS OF ADULTS DETECTED AT PIT TAG ARRAYS

Hargrove and others are presenting this information for SY2021 in a companion report titled “Abundance, life history, and genetic diversity of natural-origin steelhead and spring-summer Chinook Salmon detected at instream PIT tag detection systems in the Snake River basin.”

SECTION 8: DEVELOPMENT OF GRANDPARENTAGE TECHNOLOGY IN THE SNAKE RIVER BASIN

INTRODUCTION

Fisheries managers have long used hatcheries to increase angling opportunity and to compensate for anthropogenic impacts that have decreased population sizes (Waples et al. 2007). In some situations, it has been observed that hatchery-origin fish have lower fitness in the wild relative to natural-origin conspecifics, potentially due to selection in the hatchery environment or the use of hatchery strains that are not locally adapted (Ford 2002; Miller et al. 2004; Araki et al. 2007; Christie et al. 2014). To prevent the negative effects of interbreeding between hatchery- and natural-origin fish, it has been recommended that hatcheries operate as either integrated or segregated programs (HSRG 2009). Integrated programs aim to balance the proportion of natural-origin fish in the hatchery broodstock and the proportion of hatchery-origin fish spawning naturally to minimize the effect of domestication (Goodman 2005). Segregated programs intend to minimize gene flow between hatchery- and natural-origin populations (Mobrand et al. 2005; HSRG 2009). In order to evaluate the efficacy of segregated hatchery programs and their ability to minimize unintended hatchery influence on the landscape, a method of monitoring gene flow from hatchery-origin fish to nearby natural-origin populations is needed.

Quantifying gene flow between hatchery- and natural-origin fish has been previously estimated in several ways, including recording the proportion of fish on the spawning grounds that are of hatchery origin through observation of marks and/or tags (Tattam and Ruzycki 2020), estimation of migration rates between hatchery and wild samples via genetic differentiation (van Doornik et al. 2013), or the presence of hatchery introgression via description of genetic structure (Lehnert et al. 2020). Importantly, each of these approaches have drawbacks. Observing the proportion of hatchery-origin fish on the spawning grounds, while suggestive, does not directly assess gene flow, as the reproductive success of hatchery origin fish is unknown. Methods utilizing genetic differentiation are not applicable to cases without sufficient differentiation between the hatchery- and natural-origin populations; such cases are common when hatchery stocks have been derived from nearby natural-origin populations.

An alternative technique uses genetic samples to infer relationships between hatchery broodstock and individuals sampled in the wild. Hatchery broodstock can be genetically sampled at the time of spawning, and their genotypes are later used to infer whether a given fish is a descendent of hatchery broodstock. Parentage-based tagging (PBT) uses this approach to identify offspring of the hatchery broodstock for monitoring and management of hatchery stocks (Anderson and Garza 2005, 2006). Parentage-based tagging has been implemented and validated on large and small scales for a variety of species (DeHaan et al. 2008; Denson et al. 2012; Bingham et al. 2018; Evans et al. 2018; Campbell et al. 2019; Vandeputte et al. 2021), most notably Pacific salmonids (Steele et al. 2013a, 2019; Beacham et al. 2019).

With appropriate methods for statistical inference, the general approach of PBT can be extended to identify grandchildren of hatchery broodstock. Genetic samples can be taken from hatchery broodstock, and samples from natural-origin fish can later be assessed to determine whether they are grandchildren of those broodstock (and therefore had an unsampled, hatchery-origin parent). The relationship being inferred is a grandparent–grandchild trio consisting of one grandchild and two grandparents on the same side (i.e., either both maternal or both paternal grandparents). The other two grandparents and the parents are unsampled and therefore have unknown genotypes. Although comprehensive sampling in the hatchery is straightforward, similar sampling of adults spawning naturally is often logistically prohibitive. The ability to infer

recent hatchery ancestry without sampling naturally spawning parents would overcome this issue.

DISCUSSION

Over the last year, we developed the mathematical framework necessary to confidently determine grandparent–grandchild trios by using only genotypes from two putative grandparents and one putative grandchild have been developed (Delomas and Campbell 2022). Specifically, estimators were developed to determine false-positive and false-negative error rates and show that genetic panels containing 500–700 single-nucleotide polymorphisms or 200–300 microhaplotypes are expected to allow application of this technique for monitoring segregated hatchery programs. An R package was developed which applies this method (https://rdrr.io/github/delomast/gRandma/). Efforts are now underway to develop new SNP marker panels for grandparentage analysis and genotype collections of broodstock for use in grandparentage analysis.

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TABLES

Table 1. Sample sizes and genotyping completion rate of SY2020 steelhead broodstock. Samples with ≥10% failed SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

Stock	Samples	Genotyped (%)	Tagging Rate (%)
Cottonwood Cr.	201	199 (99.0)	98.0
Dworshak	1120	1115 (99.6)	99.1
E.F. Salmon R.	34	34 (100.0)	100.0
Little Sheep Cr.	124	122 (98.4)	96.8
Oxbow	475	466 (98.1)	96.2
Pahsimeroi	934	930 (99.6)	99.1
S.F. Clearwater	210	208 (99.0)	98.1
Sawtooth	798	792 (99.2)	98.5
Tucannon R.	34	29 (85.3)	72.8
Touchet R.	17	15 (88.2)	77.9
Up. Sal. R. B-run	204	203 (99.5)	99.0
Wallowa	470	470 (100.0)	100.0
Total	4621	4583 (99.2)	98.4

Table 2. Sample sizes and genotyping completion rate of SY2020 spring-summer Chinook Salmon broodstock. Samples with ≥10% failed SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

Stock	Samples	Genotyped (%)	Tagging Rate (%)
Catherine Cr.	100	100 (100.0)	100.0
Dworshak	1477	1463 (99.1)	98.1
Grande Ronde	146	146 (100.0)	100.0
Imnaha	259	255 (98.5)	96.9
Johnson Cr.	92	90 (97.8)	95.7
Kooskia	1123	1120 (99.7)	99.5
Lookingglass Cr.	161	161 (100.0)	100.0
Lostine	131	124 (94.7)	89.6
Nez Perce FH	279	278 (99.6)	99.3
Pahsimeroi	304	302 (99.3)	98.7
Powell	147	147 (100.0)	100.0
Rapid River	2957	2908 (98.3)	96.7
S.F. Clearwater	706	704 (99.7)	99.4
S.F. Salmon	384	383 (99.7)	99.5
Sawtooth	757	753 (99.5)	98.9
Tucannon	43	43 (100.0)	100.0
Total	9066	8977 (99.0)	98.0

Table 3. Sample sizes and genotyping completion rate of SY2020 fall Chinook Salmon broodstock. Samples with ≥10% failed SNPs are not considered successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

Stock	Samples	Genotyped (%)	Tagging Rate (%)
Lyons Ferry	1767	1733 (98.1)	96.2
Nez Perce	709	708 (99.9)	99.7
Total	2476	2441 (98.6)	97.2

Table 4. List of loci that failed to genotype in >5% of the steelhead SY2020 samples.

Locus	Fail Rate (%)
OMS00154	5.4
Omy_RAD7606020	5.4
OMS00095	6.0
Omy_BAMBI4238	6.0
Omy_RAD3379824	6.1
Omy_RAD4667227	6.7
Omy_104569114	7.3
Omy_RAD5281228	9.9
Omy_114315438	10.4
Omy_RAD6595969	10.4
Omy_arp630	10.6
Omy_RAD1957859	11.2
OMY1011SNP	11.6
Omy25_61286316	12.3
Omy_RAD3667	12.9
Omy_RAD900413	12.9
OMS00039	13.0
Ocl_gshpx357	13.7
Omy_RAD9871553	13.8
OMGH1PROM1SNP1	14.1
Omy_RAD7814727	14.6
Omy_RAD6640236	14.8
Omy_GREB1_05	15.3
Omy_RAD5599710	15.9
Omy_RAD7850257	17.5
Omy_99300202	20.7
Omy_cd28130	23.1

Table 5. List of loci that failed to genotype in >5% of the spring-summer Chinook Salmon SY2020 samples.

Locus	Fail Rate (%)
Ots_12241456	5.1
Ots_101554407	5.6
Ots_U5049250	5.7
Ots_Myc366	6.9
Ots_GH2	7.0
Ots28_11071377	7.4
Ots_ARNT	7.6
Ots4_41638710	7.9
Ots28_11205423	8.5
Ots7_53291035	8.7
Ots28_11210919	8.8
Ots_12830257	8.9
Ots29_18791740	10.0
Ots_131906141	10.0
Ots_NOD1	11.6
Ots_u0757120	11.7
Ots_TNF	12.9
Ots_CCR7	13.2
Ots_105385421	13.5
Ots_MHC2	14.2
Ots3_57055518	14.8
Ots_105401325	16.8
Ots_CHI06027687_143477	18.9
Ots19_46172427	21.8
Ots_U512134	25.7
Ots28_11143508	26.3

Table 6. List of loci that failed to genotype >5% of the fall Chinook Salmon SY2020 samples.

Locus	Fail Rate (%)
Ots_108735302	5.1
Ots_u0725325	5.1
Ots_129170683	5.1
Ots_crRAD5547526	6.6
Ots_pigh105	7.9
Ots_GPDH338	9.2
Ots_u0757120	10.4
Ots_sept978	12.6
Ots_crRAD3607229	12.8
Ots_u202161	16.6
Ots_u0707161	25.2
Ots_MetA	45.4

Table 7. Results of comparisons between phenotypic sex and genetically determined sex using the sex-specific assay for SY2020 steelhead (Omy1_2SEXY).

Stock	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotype	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Cottonwood Cr.	199	0 (0.0%)	199 (100.0%)	199 (100.0%)	0 (0.0%)	0	0	98	101
Dworshak	1115	0 (0.0%)	1115 (100.0%)	1115 (100.0%)	0 (0.0%)	0	0	452	663
E.F. Salmon R.	34	0 (0.0%)	34 (100.0%)	34 (100.0%)	0 (0.0%)	0	0	18	16
Little Sheep Cr.	122	0 (0.0%)	122 (100.0%)	120 (98.4%)	2 (1.6%)	0	2	61	61
Oxbow	466	0 (0.0%)	466 (100.0%)	461 (98.9%)	5 (1.1%)	3	2	232	234
Pahsimeroi	930	0 (0.0%)	930 (100.0%)	928 (99.8%)	2 (0.2%)	2	0	463	467
S.F. Clearwater	208	0 (0.0%)	208 (100.0%)	208 (100.0%)	0 (0.0%)	0	0	66	142
Sawtooth	792	0 (0.0%)	792 (100.0%)	792 (100.0%)	0 (0.0%)	0	0	394	398
Tucannon R.	29	0 (0.0%)	29 (100.0%)	29 (100.0%)	0 (0.0%)	0	0	3	26
Touchet R.	15	0 (0.0%)	15 (100.0%)	15 (100.0%)	0 (0.0%)	0	0	5	10
Up. Sal. R. B-run	203	0 (0.0%)	203 (100.0%)	203 (100.0%)	0 (0.0%)	0	0	83	120
Wallowa	470	2 (0.4%)	468 (99.6%)	468 (100.0%)	0 (0.0%)	0	0	235	235

Table 8. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for spring-summer Chinook Salmon (Ots_SEXY3-1) from the SY2020 broodstocks.

Stock	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotype	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Catherine Cr.	100	1 (1.0%)	99 (99.0%)	99 (100.0%)	0 (0.0%)	0	0	45	55
Dworshak	1463	4 (0.3%)	1459 (99.7%)	1452 (99.5%)	7 (0.5%)	4	3	618	845
Grande Ronde	146	3 (2.1%)	143 (97.9%)	143 (100.0%)	0 (0.0%)	0	0	64	82
Imnaha	255	0 (0.0%)	255 (100.0%)	255 (100.0%)	0 (0.0%)	0	0	120	135
Johnson Cr.	90	1 (1.1%)	89 (98.9%)	89 (100.0%)	0 (0.0%)	0	0	46	44
Kooskia	1120	13 (1.2%)	1107 (98.8%)	1102 (99.5%)	5 (0.5%)	4	1	429	691
Lookingglass Cr.	161	3 (1.9%)	158 (98.1%)	157 (99.4%)	1 (0.6%)	0	1	82	79
Lostine	124	1 (0.8%)	123 (99.2%)	123 (100.0%)	0 (0.0%)	0	0	52	72
Nez Perce FH	278	2 (0.7%)	276 (99.3%)	276 (100.0%)	0 (0.0%)	0	0	108	170
Pahsimeroi	302	0 (0.0%)	302 (100.0%)	302 (100.0%)	0 (0.0%)	0	0	149	153
Powell	147	1 (0.7%)	146 (99.3%)	146 (100.0%)	0 (0.0%)	0	0	58	89
Rapid River	2908	0 (0.0%)	2908 (100.0%)	2908 (100.0%)	0 (0.0%)	0	0	1184	1724
S.F. Clearwater	704	5 (0.7%)	699 (99.3%)	699 (100.0%)	0 (0.0%)	0	0	253	451
S.F. Salmon	383	0 (0.0%)	383 (100.0%)	383 (100.0%)	0 (0.0%)	0	0	139	244
Sawtooth	753	0 (0.0%)	753 (100.0%)	753 (100.0%)	0 (0.0%)	0	0	350	403
Tucannon	43	0 (0.0%)	43 (100.0%)	43 (100.0%)	0 (0.0%)	0	0	21	22

Table 9. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for fall Chinook Salmon (Ots_SEXY3-1) from the SY2020 broodstocks.

Stock	Total Samples	Missing Sex Marker Genetic Data	Total Successful Sex Marker Genotype	Corresponding	Non-corresponding	Phenotypic Males Misidentified as Female	Phenotypic Females Misidentified as Male	Total Phenotypic Males	Total Phenotypic Females
Lyons Ferry	1733	4 (0.2%)	1729 (99.8%)	1724 (99.7%)	5 (0.3%)	2	3	632	1101
Nez Perce	708	23 (3.2%)	685 (96.8%)	685 (100.0%)	0 (0.0%)	0	0	263	422

Table 10. Average observed and expected heterozygosity with associated standard error of hatchery steelhead stocks for SY2020.

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

Table 11. Average observed and expected heterozygosity with associated standard error of hatchery spring-summer Chinook Salmon stocks in SY2020.

Stock	Avg. Het. (Obs)	SE	Avg. Het. (Exp)	SE
Catherine Cr.	0.21	0.01	0.21	0.01
Dworshak	0.21	0.01	0.21	0.01
Grande Ronde	0.20	0.01	0.21	0.01
Imnaha	0.24	0.01	0.24	0.01
Johnson Cr.	0.21	0.01	0.21	0.01
Kooskia	0.20	0.01	0.20	0.01
Lookingglass Cr.	0.22	0.01	0.22	0.01
Lostine	0.23	0.01	0.23	0.01
Nez Perce FH	0.21	0.01	0.21	0.01
Pahsimeroi	0.22	0.01	0.23	0.01
Powell	0.22	0.01	0.22	0.01
Rapid River	0.20	0.01	0.20	0.01
S.F. Clearwater	0.20	0.01	0.20	0.01
S.F. Salmon	0.22	0.01	0.21	0.01
Sawtooth	0.22	0.01	0.22	0.01
Tucannon	0.22	0.01	0.22	0.01

Table 12. Average observed and expected heterozygosity with associated standard error of hatchery fall Chinook Salmon stocks in SY2020.

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

Table 13. Population structure (F_{ST}) (below diagonal) among steelhead hatchery stocks sampled in SY2020. P-values are shown above the diagonal. Asterisks (*) indicate that the genotypic differentiation (exact G test) was highly significant and the combined p-value (Fisher's method) could not be calculated.

Stock	Cotton-wood Cr.	Dworshak	E.F. Salmon R.	Little Sheep Cr.	Oxbow	Pahsimeroi	S.F. Clearwater	Sawtooth	Tucannon R.	Touchet R.	Up. Sal. R. B-run
Cottonwood Cr.	---	*	*	*	*	*	*	*	*	*	*
Dworshak	0.060	---	*	*	*	*	*	*	*	*	*
E.F. Salmon R.	0.025	0.048	---	*	*	*	*	*	*	0.000	*
Little Sheep Cr.	0.032	0.068	0.037	---	*	*	*	*	*	*	*
Oxbow	0.028	0.064	0.021	0.027	---	*	*	*	*	*	*
Pahsimeroi	0.027	0.064	0.019	0.030	0.012	---	*	*	*	*	*
S.F. Clearwater	0.057	0.005	0.046	0.065	0.061	0.061	---	*	*	*	*
Sawtooth	0.028	0.063	0.020	0.031	0.014	0.009	0.061	---	*	*	*
Tucannon R.	0.018	0.052	0.022	0.023	0.021	0.022	0.048	0.025	---	1.000	*
Touchet R.	0.020	0.060	0.024	0.029	0.022	0.026	0.057	0.026	-0.001	---	*
Up. Sal. R. B-run	0.057	0.017	0.045	0.068	0.059	0.061	0.019	0.057	0.049	0.057	---

Table 14. Population structure (F_{ST}) (below diagonal) among spring-summer Chinook Salmon hatchery stocks sampled in SY2020. P-values are shown above the diagonal. Asterisks (*) indicate that the genotypic differentiation (exact G test) was highly significant and the combined p-value (Fisher's method) could not be calculated.

Stock	Catherine Cr.	Dworshak	Grande Ronde	Imnaha	Johnson Cr.	Kooskia	Looking-glass Cr.	Lostine	Nez Perce FH	Pahsimeroi	Powell	Rapid River	S.F. Clear-water	S.F. Salmon	Sawtooth	Tucan-non
Catherine Cr.	---	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Dworshak	0.009	---	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.013	0.012	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Imnaha	0.013	0.009	0.012	---	*	*	*	*	*	*	*	*	*	*	*	*
Johnson Cr.	0.025	0.021	0.025	0.024	---	*	*	*	*	*	*	*	*	*	*	*
Kooskia	0.011	0.001	0.014	0.010	0.024	---	*	*	*	*	*	*	*	*	*	*
Lookingglass Cr.	0.008	0.010	0.013	0.012	0.026	0.013	---	*	*	*	*	*	*	*	*	*
Lostine	0.025	0.023	0.028	0.023	0.039	0.027	0.030	---	*	*	*	*	*	*	*	*
Nez Perce FH	0.010	0.001	0.013	0.010	0.023	0.002	0.012	0.027	---	*	*	*	*	*	*	*
Pahsimeroi	0.032	0.029	0.036	0.031	0.035	0.032	0.032	0.036	0.032	---	*	*	*	*	*	*
Powell	0.021	0.017	0.023	0.019	0.013	0.021	0.025	0.025	0.019	0.026	---	*	*	*	*	*
Rapid River	0.018	0.012	0.013	0.012	0.028	0.014	0.015	0.030	0.014	0.037	0.025	---	*	*	*	*
S.F. Clearwater	0.013	0.005	0.014	0.012	0.027	0.004	0.013	0.026	0.006	0.031	0.022	0.014	---	*	*	*
S.F. Salmon	0.022	0.016	0.023	0.018	0.012	0.019	0.024	0.028	0.018	0.027	0.004	0.025	0.020	---	*	*
Sawtooth	0.032	0.026	0.033	0.032	0.031	0.030	0.032	0.041	0.030	0.023	0.027	0.031	0.028	0.024	---	*
Tucannon	0.020	0.020	0.029	0.027	0.040	0.023	0.026	0.033	0.020	0.046	0.035	0.038	0.026	0.034	0.053	---

Table 15. Estimates of effective population size (N_E) and 95% confidence intervals for steelhead hatchery stocks in SY2020.

Stock	Ne	CI (95%)
Cottonwood Cr.	69.8	57.0 - 86.5
Dworshak	204.3	189.7 - 220.1
E.F. Salmon R.	223.3	127.8 - 756.1
Little Sheep Cr.	89	69.4 - 118.2
Oxbow	88.9	79.4 - 99.5
Pahsimeroi	175.1	164.4 - 186.4
S.F. Clearwater	109.8	91.8 - 133.4
Sawtooth	208.9	193.4 - 226.0
Tucannon R.	762.8	178.8 - Infinite
Touchet R.	2364.2	182.2 - Infinite
Up. Sal. R. B-run	57.9	49.6 - 67.9
Wallowa	216.5	190.5 - 247.4

Table 16. Estimates of effective population size and 95% confidence intervals for SY2020 spring-summer Chinook Salmon hatchery stocks.

Stock	Ne	CI (95%)
Catherine Cr.	151.5	100.7 - 270.8
Dworshak	448.1	419.2 - 479.4
Grande Ronde	113.5	89.8 - 148.4
Imnaha	165.5	132.1 - 212.9
Johnson Cr.	423.7	255.0 - 1112.3
Kooskia	246.8	229.3 - 265.8
Lookingglass Cr.	74.1	59.1 - 94.5
Lostine	196.7	145.4 - 290.0
Nez Perce FH	148.7	123.6 - 181.5
Pahsimeroi	214.4	174.0 - 270.8
Powell	156.1	118.8 - 217.3
Rapid River	549.3	525.9 - 573.8
S.F. Clearwater	213.5	190.9 - 239.5
S.F. Salmon	276.7	226.7 - 345.7
Sawtooth	346.1	314.4 - 382.3
Tucannon	341.5	148.4 - Infinite

Table 17. Estimates of effective population size and 95% confidence intervals for fall Chinook Salmon SY2020 hatchery stocks.

Stock	Ne	CI (95%)
Lyons Ferry	1256.2	1144.4 - 1385.8
Nez Perce	1258.5	1047.2 - 1558.7

Table 18. Average observed and expected heterozygosity with associated standard error of hatchery steelhead stocks for SY2020 at 92 loci comparing the originally targeted SNPs with the loci treated as microhaplotypes.

	SNP				Microhaplotype			
Pop	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**
Cottonwood Cr.	0.260	0.018	0.252	0.018	0.340	0.019	0.340	0.020
Dworshak	0.230	0.019	0.224	0.019	0.294	0.020	0.292	0.020
E.F. Salmon R.	0.255	0.019	0.254	0.020	0.327	0.019	0.328	0.020
Little Sheep Cr.	0.242	0.019	0.244	0.020	0.315	0.020	0.321	0.021
Oxbow	0.263	0.018	0.260	0.018	0.337	0.019	0.340	0.020
Pahsimeroi	0.256	0.017	0.246	0.017	0.329	0.018	0.326	0.018
S.F. Clearwater	0.227	0.019	0.220	0.019	0.288	0.020	0.284	0.020
Sawtooth	0.252	0.018	0.247	0.018	0.329	0.019	0.332	0.020
Tucannon R.	0.259	0.018	0.258	0.018	0.331	0.019	0.334	0.020
Touchet R.	0.256	0.018	0.262	0.021	0.329	0.019	0.338	0.021
Up. Sal. R. B-run	0.229	0.019	0.228	0.019	0.295	0.020	0.297	0.021
Wallowa	0.261	0.017	0.250	0.017	0.339	0.019	0.333	0.019

Table 19. Average observed and expected heterozygosity with associated standard error of hatchery spring-summer Chinook Salmon stocks for SY2020 at 53 loci comparing the originally targeted SNPs with the loci treated as microhaplotypes.

	SNP				Microhaplotype			
Pop	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**
Catherine Cr.	0.255	0.025	0.258	0.026	0.342	0.027	0.351	0.029
Dworshak	0.249	0.024	0.247	0.024	0.337	0.026	0.336	0.026
Grande Ronde	0.255	0.024	0.263	0.025	0.341	0.026	0.348	0.027
Imnaha	0.257	0.025	0.251	0.024	0.346	0.026	0.344	0.026
Johnson Cr.	0.231	0.025	0.237	0.026	0.310	0.027	0.319	0.029
Kooskia	0.247	0.024	0.245	0.024	0.336	0.026	0.337	0.027
Lookingglass Cr.	0.264	0.024	0.270	0.025	0.352	0.026	0.357	0.026
Lostine	0.258	0.025	0.254	0.024	0.330	0.028	0.326	0.027
Nez Perce FH	0.252	0.024	0.254	0.024	0.340	0.026	0.348	0.027
Pahsimeroi	0.226	0.025	0.224	0.025	0.310	0.026	0.304	0.026
Powell	0.233	0.024	0.233	0.024	0.316	0.027	0.313	0.027
Rapid River	0.241	0.024	0.239	0.024	0.333	0.025	0.334	0.026
S.F. Clearwater	0.242	0.025	0.237	0.024	0.335	0.026	0.334	0.026
S.F. Salmon	0.234	0.024	0.231	0.023	0.315	0.026	0.315	0.026
Sawtooth	0.228	0.026	0.228	0.027	0.310	0.028	0.311	0.029
Tucannon	0.268	0.023	0.268	0.024	0.345	0.026	0.348	0.027

Table 20. Average observed and expected heterozygosity with associated standard error of hatchery fall Chinook Salmon stocks for SY2020 adults at Lower Granite Dam at 53 loci comparing the originally targeted SNPs with the loci treated as microhaplotypes.

	SNP				Microhaplotype			
Pop	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**	**Avg. Het. (Exp)**	**SE**	**Avg. Het. (Obs)**	**SE**
Lyons Ferry	0.256	0.021	0.250	0.021	0.336	0.021	0.335	0.021
Nez Perce	0.259	0.021	0.252	0.021	0.335	0.021	0.341	0.022

Table 21. Haplotypes and relative frequencies in SY2020 steelhead broodstocks for a candidate region on chromosome 25 associated with age-at-maturity.

Haplotype																						
1	2	3	4	5	6	7	8	9	10	Total	Cotton-wood Cr.	Dworshak	E.F. Salmon R.	Little Sheep Cr.	Oxbow	Pahsimeroi	S.F. Clear-water	Sawtooth	Tucannon R.	Touchet R.	Up. Sal. R. B-run	Wallowa
C	A	G	C	G	G	A	A	A	C	0.5331	0.7814	0.5417	0.7935	0.8195	0.3651	0.3828	0.4543	0.5840	0.6369	0.3990	0.4679	0.7340
A	C	T	T	A	T	G	G	G	G	0.3054	0.0879	0.4525	0.0877	0.1146	0.3327	0.3136	0.5433	0.1621	0.2748	0.4990	0.5123	0.0989
A	C	T	T	A	G	A	A	A	C	0.0605	0.0955	0.0027	0.0441	0.0205	0.0769	0.0882	0.0024	0.0998	0.0528	0.0343	0.0074	0.1276
C	A	G	C	G	G	G	G	G	G	0.0569	0.0327	-	-	0.0043	0.0839	0.1143	-	0.1243	-	0.0333	-	0.0255
C	A	G	C	G	T	G	G	G	G	0.0293	-	-	-	-	0.1004	0.0803	-	0.0222	0.0183	0.0343	0.0050	-
A	C	T	T	A	G	G	G	G	G	0.0039	<0.0001	-	0.0153	-	0.0173	0.0027	-	0.0037	-	<0.0001	0.0025	<0.0001
A	C	T	T	A	T	G	A	G	C	0.0038	-	-	-	-	-	0.0172	-	0.0025	-	-	-	-
A	C	T	T	A	T	A	A	A	C	0.0035	-	0.0004	-	0.0371	0.0216	-	-	-	0.0172	-	0.0025	-
C	A	T	T	A	G	A	A	A	C	0.0012	<0.0001	-	-	-	-	-	-	0.0006	-	-	-	0.0096
C	A	G	C	G	G	A	A	G	C	0.0009	-	0.0027	-	-	-	-	-	-	-	-	-	0.0021
C	A	G	C	G	T	A	A	A	C	0.0004	-	-	0.0594	-	-	-	-	-	<0.0001	-	-	-
A	C	G	T	A	T	G	G	G	G	0.0002	-	-	<0.0001	-	0.0012	0.0008	-	-	-	-	-	-
A	C	G	C	G	G	A	A	A	C	0.0002	-	-	-	-	-	-	-	-	-	-	-	0.0021
C	A	G	C	G	T	G	A	G	C	0.0002	-	-	-	-	-	<0.0001	-	-	-	-	-	-
A	C	G	T	A	G	G	G	G	G	0.0001	-	-	-	-	-	-	-	0.0009	-	-	-	-
A	A	T	T	A	T	G	G	G	G	0.0001	-	-	-	0.0041	-	-	-	-	-	-	-	-
C	A	T	T	A	G	A	G	A	C	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	T	T	A	G	A	G	G	C	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	T	T	A	G	A	G	G	G	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	T	T	A	G	A	G	A	G	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	T	C	G	T	A	G	A	G	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	T	C	G	T	A	G	A	C	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	T	C	G	T	A	G	G	G	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	T	C	G	T	A	G	G	C	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
C	A	G	C	G	G	G	A	G	C	<0.0001	-	-	-	-	-	-	-	-	-	-	0.0025	-
C	A	T	C	G	T	G	A	G	C	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-

Table 22. Haplotypes and relative frequencies in SY2020 steelhead broodstocks for a candidate region on chromosome 28 associated with migration timing.

Haplotype																								
1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Cotton-wood Cr.	Dworshak	E.F. Salmon R.	Little Sheep Cr.	Oxbow	Pahsi-meroi	S.F. Clear-water	Saw-tooth	Tucannon R.	Touchet R.	Up. Sal. R. B-run	Wallowa
G	A	G	G	A	G	C	C	G	T	G	T	A	0.6951	0.6784	0.8885	0.5000	0.7910	0.5948	0.5408	0.9277	0.6319	0.8276	0.8000	0.9187	0.5372
G	A	G	G	A	G	C	T	A	C	T	G	T	0.2103	0.2588	0.0271	0.1323	0.1967	0.3216	0.3302	0.0239	0.2317	0.0862	0.1333	0.0493	0.4095
G	A	G	G	A	G	C	T	A	C	T	G	A	0.0211	0.0427	0.0199	-	0.0123	0.0158	0.0257	0.0049	0.0290	-	-	-	0.0182
G	A	G	G	G	G	C	C	G	T	G	T	A	0.0135	-	-	0.0033	-	0.0070	0.0297	-	0.0343	-	0.0333	-	-
G	C	G	G	G	G	C	T	A	T	T	G	A	0.0114	-	0.0432	-	-	-	-	0.0120	-	-	-	0.0025	-
G	C	T	G	G	G	C	T	A	T	T	G	T	0.0109	-	0.0030	-	-	-	0.0315	0.0024	0.0195	0.0172	-	0.0025	0.0054
G	A	G	G	A	G	C	C	G	T	G	T	T	0.0064	-	0.0106	-	-	-	-	0.0171	0.0124	0.0345	0.0333	0.0148	-
G	C	T	G	G	G	C	T	A	C	T	G	T	0.0060	-	0.0004	0.2353	-	0.0086	0.0025	0.0047	0.0107	-	-	0.0049	-
G	A	G	G	G	G	C	C	G	T	G	T	T	0.0045	0.0201	-	-	-	-	0.0123	-	0.0062	-	-	0.0025	0.0011
G	C	T	G	G	G	C	T	A	C	T	G	A	0.0042	-	-	0.0100	-	0.0074	0.0158	0.0073	0.0013	-	-	-	-
G	A	G	G	A	G	C	C	A	C	T	G	T	0.0034	-	-	-	-	0.0287	0.0005	-	0.0023	-	-	-	-
G	C	T	G	G	G	C	T	A	T	T	G	A	0.0031	-	0.0048	-	-	-	-	<0.0001	0.0045	-	-	0.0049	0.0106
G	A	G	G	A	G	C	C	A	C	T	G	A	0.0027	-	-	-	-	0.0068	0.0006	-	0.0109	-	-	-	-
G	C	G	G	G	G	C	T	A	T	T	G	T	0.0010	-	0.0015	-	-	-	0.0019	-	-	-	-	-	-
G	A	G	G	G	G	C	C	A	T	G	T	T	0.0008	-	-	-	-	-	0.0039	-	-	-	-	-	-
A	C	T	A	G	T	A	T	A	C	T	G	T	0.0007	-	-	-	-	-	-	-	-	0.0172	-	-	0.0053
G	C	T	A	G	G	A	T	A	C	T	G	A	0.0007	-	-	-	-	-	-	-	-	-	-	-	0.0064
G	C	T	G	A	G	C	C	G	T	G	T	A	0.0006	-	-	0.0735	-	-	-	-	-	-	-	-	-
A	C	G	G	G	G	C	C	G	C	T	T	A	0.0005	-	-	-	-	-	0.0027	-	-	-	-	-	-
G	A	G	G	G	G	C	C	G	T	G	G	T	0.0004	-	-	0.0126	-	-	-	-	0.0019	-	-	-	-
G	A	G	G	G	G	C	C	A	T	G	G	A	0.0004	-	-	-	-	-	-	-	-	-	-	-	0.0038
G	A	T	G	A	G	C	C	G	T	G	T	A	0.0004	-	-	-	-	0.0023	-	-	0.0009	-	-	-	-
G	A	G	G	A	G	C	C	G	C	G	T	T	0.0003	-	-	-	-	-	-	-	0.0019	-	-	-	-
G	A	G	G	G	G	C	C	G	T	G	G	A	0.0003	-	-	-	-	-	-	-	-	-	-	-	0.0026
A	C	G	G	G	G	C	C	G	T	G	T	A	0.0002	-	-	-	-	-	0.0011	-	-	-	-	-	-
G	C	G	G	G	G	C	T	A	C	T	G	A	0.0002	-	0.0005	0.0047	-	-	<0.0001	-	-	-	-	-	-

Table 22. Continued

G	C	T	G	A	G	C	T	A	C	T	G	A	0.0002	-	-	-	-	0.0022	-	-	-	-	-	-	-
G	A	G	G	G	G	C	C	A	C	T	G	A	0.0002	-	-	-	-	-	0.0010	-	-	-	-	-	-
G	C	T	G	G	G	C	C	G	T	G	T	A	0.0001	-	-	-	-	<0.0001	-	-	-	0.0172	-	-	-
G	A	G	G	A	G	C	T	A	C	T	T	A	0.0001	-	0.0004	-	-	-	-	-	-	-	-	-	-
G	A	G	G	G	G	C	C	G	C	G	G	T	0.0001	-	-	-	-	-	-	-	0.0006	-	-	-	-
G	A	G	G	G	G	C	T	A	C	T	G	T	0.0001	-	-	-	-	0.0048	-	-	<0.0001	-	-	-	-
G	A	G	G	G	G	C	T	A	C	T	G	A	<0.0001	-	-	-	-	-	-	-	<0.0001	-	-	-	-

Table 23. Spring-summer Chinook Salmon SY2020 haplotype key for a candidate region on chromosome 28 associated with migration timing.

	Chromosome 28 Marker Number																											
Haplotype	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
I	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	T
II	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	C
III	G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C
IV	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	C	C	T	C
V	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	G	C	C	C	G	C	C	T	C
VI	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	A	G	T	C	G	T
VII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	G	T
VIII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C
IX	G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	T
X	A	A	G	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C
XI	A	A	G	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	G	T
XII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	C	C	G	C	C	T	C
XIII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	C	C	G	C	C	T	T
XIV	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	T	T	C	C	A	C	T	T	C
XV	G	C	A	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C
XVI	G	C	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C
XVII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	G	C	A	T	T	C	C	A	C	T	T	C
XVIII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	C	C	C	G	C	C	T	C
XIX	G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	C
XX	G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	G	C	C	C	G	C	C	T	C
XXI	A	A	A	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	C	C	G	C	C	T	T
XXII	A	A	G	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	A	G	T	C	G	T
XXIII	G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	T	A	T	G	C	T	A	G	T	C	G	T
XXIV	A	A	G	C	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C
XXV	A	A	G	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	C
XXVI	G	C	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	C	C	C	A	C	T	T	C
XXVII	G	C	A	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	C	G	T	C	T	T

Table 23. Continued

XXVIII	G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	T	G	C	T	C	G	T	C	T	T
XXIX	A	A	G	C	G	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	T
XXX	G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	C	C	G	C	C	T	T
XXXI	A	A	G	C	A	C	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	C
XXXII	A	A	A	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	G	T
XXXIII	A	A	G	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	C	C	G	C	C	G	T
XXXIV	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	T	T	C	C	A	C	T	G	C
XXXV	A	A	G	C	A	C	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	C	C	T	C
XXXVI	G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	T	A	T	G	C	T	C	G	T	C	T	T
XXXVII	A	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	G	C
XXXVIII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	C	T	C	G	T	C	T	T
XXXIX	A	A	A	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	C	A	G	C	C	G	T
XL	G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	G	C	A	T	T	C	C	A	C	T	T	C
XLI	G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	T	T	C	C	A	C	T	T	C
XLII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	G	C	C	C	G	C	C	T	T
XLIII	G	C	A	G	G	T	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C
XLIV	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	A	G	T	C	T	T
XLV	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	A	T	C	T	C
XLVI	A	A	G	G	G	C	T	A	A	A	T	T	A	G	A	A	T	A	T	G	C	T	C	G	T	C	T	T
XLVII	A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	C	G	T	C	T	C
XLVIII	A	A	A	G	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	C	C	T	C
XLIX	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	A	T	A	A	G	C	C	C	G	C	C	G	C
L	G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	C
LI	A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	A	G	T	C	G	T
LII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	A	T	A	T	G	C	T	C	G	T	C	T	T
LIII	A	A	G	G	A	T	C	T	T	G	C	C	G	A	T	A	G	C	A	T	T	C	C	A	C	T	T	C
LIV	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	G	C	T	C	G	T	C	T	T
LV	G	C	A	C	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C
LVI	A	A	G	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C
LVII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	G	C	T	C	G	T	C	T	C

Table 23. Continued

LVIII	A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	A	G	T	T	G	T
LIX	G	C	A	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	A	G	C	C	C	G	C	C	T	C
LX	A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	A	G	T	C	T	T
LXI	A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	A	G	T	T	T	T
LXII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	A	G	T	C	T	C

Table 24. Haplotypes and relative frequencies in SY2020 spring-summer Chinook Salmon broodstocks for a candidate region on chromosome 28 associated with migration timing. See Table 23 for alleles in each haplotype.

Haplotype	Total	Cather-ine Cr.	Dwor-shak	Grande Ronde	Imnaha	John-son Cr.	Koos-kia	Looking -glass Cr.	Lostine	Nez Perce FH	Pahsi-meroi	Powell	Rapid River	S.F. Clear-water	S.F. Salmon	Saw-tooth	Tuca-nnon
I	0.4008	0.4031	0.4026	0.4178	0.2348	0.2704	0.3496	0.3667	0.1721	0.4204	0.3236	0.3010	0.4755	0.4927	0.2946	0.3440	0.5000
II	0.2080	0.2588	0.2267	0.2773	0.1352	0.1184	0.2299	0.2978	0.0287	0.2265	0.1662	0.1004	0.2108	0.2511	0.1298	0.1605	0.2093
III	0.0723	0.0250	0.0055	-	0.3045	0.1110	0.0020	0.0341	0.2092	0.0072	0.3460	0.0971	0.0314	-	0.1613	0.3165	0.0116
IV	0.0617	0.0800	0.0882	0.0616	0.0394	0.0111	0.0854	0.0590	0.0040	0.0788	0.0232	0.0442	0.0612	0.0243	0.0094	0.0667	-
V	0.0514	0.0380	0.0763	0.1164	0.0098	0.0611	0.0661	0.0621	0.0161	0.0881	0.0149	0.0189	0.0474	0.0532	0.0292	0.0200	0.0233
VI	0.0509	0.0817	0.0693	0.0445	0.0390	0.0222	0.0826	0.0466	0.0323	0.0337	0.0057	-	0.0525	0.0321	0.0039	0.0359	0.0581
VII	0.0410	0.0163	0.0373	0.0171	0.0285	<0.0001	0.0425	0.0311	0.0089	0.0466	0.0470	-	0.0534	0.0569	0.0131	0.0095	0.1628
VIII	0.0254	-	0.0010	0.0034	0.0610	0.3222	0.0009	0.0155	0.0801	-	0.0216	0.2257	0.0281	0.0007	0.1232	0.0087	-
IX	0.0154	-	0.0250	-	-	0.0057	0.0464	0.0032	-	0.0181	-	0.0379	0.0018	0.0143	0.0633	-	-
X	0.0141	0.0070	0.0003	0.0034	0.0560	0.0168	-	0.0218	0.2465	-	0.0404	0.1125	0.0075	-	0.0659	-	-
XI	0.0130	-	0.0202	0.0034	0.0020	-	0.0266	0.0062	-	0.0133	-	-	0.0087	0.0257	0.0097	-	-
XII	0.0112	0.0112	0.0207	<0.0001	-	-	0.0207	0.0280	-	0.0240	-	-	0.0041	0.0256	-	-	-
XIII	0.0089	0.0108	0.0115	0.0342	-	0.0389	0.0240	-	-	0.0111	-	-	0.0072	0.0022	-	-	-
XIV	0.0034	0.0330	0.0053	-	-	-	0.0071	0.0155	-	0.0090	-	-	0.0001	0.0052	-	-	-
XV	0.0033	-	0.0007	-	0.0269	<0.0001	0.0018	-	0.0045	0.0036	-	<0.0001	0.0026	-	0.0109	-	-
XVI	0.0028	-	-	-	0.0078	-	-	-	0.1694	-	-	-	0.0001	-	0.0040	-	-
XVII	0.0023	0.0150	0.0017	0.0069	-	-	0.0046	-	-	0.0054	-	-	0.0012	0.0043	-	-	0.0349
XVIII	0.0018	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0212	-
XIX	0.0014	0.0050	-	-	-	-	0.0006	-	-	-	-	0.0186	-	-	0.0213	-	-
XX	0.0014	-	0.0006	-	-	-	<0.0001	-	-	-	-	0.0185	0.0001	-	0.0204	-	-
XXI	0.0012	-	0.0025	-	-	-	-	0.0031	-	-	-	-	0.0023	-	-	-	-
XXII	0.0010	-	0.0017	-	-	-	0.0036	-	-	-	-	-	0.0007	-	-	-	-
XXIII	0.0008	-	-	-	0.0255	-	-	-	-	-	-	-	0.0002	-	-	-	-
XXIV	0.0008	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0146	0.0013	-
XXV	0.0007	0.0050	0.0004	0.0035	-	-	0.0019	0.0031	-	-	-	-	-	0.0015	-	-	-
XXVI	0.0006	-	-	-	0.0078	-	-	-	0.0282	-	-	-	<0.0001	-	-	-	-

Table 24. Continued

XXVII	0.0006	-	-	-	-	-	-	-	-	-	<0.0001	0.0046	-	-	0.0074	-	-
XXVIII	0.0006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0067	-
XXIX	0.0003	-	-	-	-	-	-	0.0031	-	-	-	-	-	-	-	0.0034	-
XXX	0.0003	-	-	-	-	-	-	-	-	0.0031	-	-	0.0007	0.0028	-	-	-
XXXI	0.0003	-	-	-	-	-	-	-	-	-	0.0072	-	0.0002	-	-	-	-
XXXII	0.0003	-	-	-	0.0131	-	-	-	-	-	-	-	-	-	-	-	-
XXXIII	0.0003	-	0.0006	-	-	-	-	-	-	-	-	-	0.0006	-	-	-	-
XXXIV	0.0002	0.0037	0.0005	-	-	-	0.0018	-	-	-	-	-	-	-	-	-	-
XXXV	0.0002	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0047	-	-
XXXVI	0.0002	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0039	-	-
XXXVII	0.0001	-	-	-	0.0026	-	-	-	-	-	-	-	-	-	-	-	-
XXXVIII	0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0013	0.0013	-
XXXIX	0.0001	-	0.0003	-	-	-	-	-	-	-	-	-	0.0003	-	-	-	-
XL	0.0001	-	-	-	-	-	0.0012	-	-	-	-	-	-	-	-	-	-
XLI	0.0001	-	0.0007	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XLII	0.0001	-	-	-	-	-	-	-	-	-	-	-	0.0003	-	-	-	<0.0001
XLIII	0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0008	-
XLIV	0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XLV	0.0001	-	-	-	-	-	-	-	-	-	-	-	0.0002	-	-	-	-
XLVI	0.0001	-	-	-	0.0021	-	-	-	-	-	-	-	-	-	-	-	-
XLVII	0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0015	-	-
XLVIII	0.0001	-	-	-	-	-	-	0.0031	-	-	-	-	-	-	-	-	-
XLIX	0.0001	-	-	-	-	0.0056	-	-	-	-	-	-	-	-	-	-	-
L	0.0001	-	-	-	-	-	-	-	-	0.0018	-	-	-	-	-	-	-
LI	0.0001	-	-	-	0.0021	-	-	-	-	-	-	-	-	-	-	-	-
LII	0.0001	-	-	-	0.0018	-	-	-	-	-	-	-	-	-	-	-	-
LIII	<0.0001	-	-	0.0033	-	-	-	-	-	-	-	-	-	-	-	-	-
LIV	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0003	-
LV	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0007	-
LVI	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 24. Continued

LVII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0003	-
LVIII	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
LIX	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LX	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
LXI	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
LXII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	<0.0001	-	-

Table 25. Haplotypes and relative frequencies in SY2020 fall Chinook Salmon for a candidate region on chromosome 28 associated with migration timing. Note that frequencies were only calculated for the Nez Perce broodstock because the panel used to genotype Lyons Ferry broodstock does not include markers from chromosome 28.

Haplotype																											
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**Nez Perce**
G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.72974
G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.04597
A	A	G	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.04352
G	C	A	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.02420
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	C	0.01778
A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.01549
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	G	T	0.01473
G	C	A	G	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00934
G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	C	C	T	C	0.00900
A	A	G	C	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00799
G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	T	0.00763
G	C	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00658
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	G	C	T	A	G	T	C	G	T	0.00424
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	A	G	T	C	G	T	0.00414
G	C	A	C	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00392
A	A	G	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00380
G	C	A	C	G	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00370
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	T	T	0.00325
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	A	G	C	T	C	G	T	C	G	T	0.00282
G	C	A	C	A	C	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00282
G	C	A	G	A	T	C	T	T	G	C	C	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00282
A	A	G	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.00246
G	C	A	G	G	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00227
A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00219
G	C	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	C	T	C	G	T	C	G	T	0.00212

Table 25. Continued

A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	C	C	C	G	C	C	T	T	0.00209
A	A	G	G	G	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00207
G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	A	G	T	C	G	T	0.00197
G	C	A	G	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	G	C	T	C	G	T	C	T	T	0.00148
A	A	G	G	G	C	T	A	A	A	T	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00141
G	C	A	G	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.00125
G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	G	T	0.00105
A	A	G	C	A	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	C	G	T	C	T	T	0.00085
A	A	G	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	C	C	C	A	C	T	T	C	0.00082
G	C	A	C	A	T	C	T	T	G	C	C	G	A	T	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00075
G	A	G	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00074
A	A	G	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	T	G	C	C	C	G	C	C	T	T	0.00073
A	A	G	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	A	C	T	T	C	0.00072
G	C	A	G	G	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	C	T	C	G	T	C	T	C	0.00072
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	C	C	G	C	C	T	T	0.00071
A	C	A	C	A	T	C	T	T	G	C	C	G	A	T	A	T	A	T	G	C	T	C	G	T	C	G	T	0.00071
G	C	A	C	A	T	C	T	T	G	C	C	G	A	A	G	G	A	T	G	C	T	C	G	T	C	T	T	0.00071
G	C	A	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	C	C	C	G	C	C	T	C	0.00071
G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	C	T	C	0.00071
A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	C	T	C	G	T	C	G	T	0.00070
G	A	G	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	T	0.00070
G	C	A	C	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.00070
G	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00068
G	C	A	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.00066
A	A	G	G	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	G	C	T	C	G	T	C	T	T	0.00064
A	A	G	G	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.00063
A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00059
A	A	G	C	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.00049
G	C	A	C	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.00048
G	C	A	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	C	C	C	G	C	C	T	C	0.00041

Table 25. Continued

G	C	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	T	C	C	C	G	T	C	T	C	0.00029
G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	T	C	C	C	G	T	C	T	T	0.00029

Table 26. Summary of SY2021 adult and MY2021 juvenile steelhead and Chinook Salmon samples from Lower Granite Dam (LGR). Summary includes the number of samples that arrived from LGR (inventoried) and the number inventoried that were queued for genotyping. Of queued samples, we show the number that genotyped successfully and the number that failed genotyping. For samples that genotyped successfully, we show the number of individuals with a parentage based tag (PBT; i.e., of hatchery origin) and the number that were assigned a genetic stock based on individual assignment (IA) using SNP baselines v3.1.

Sample Group	**Total Samples Queued for Genotyping**	**Failed Genotyping (NG)**	**Successfully Genotyped**	**PBT Assignments**	**GSI Assignments**
Steelhead					
SY2021 Adults (Natural-origin Phenotype)	3,504	37 (1.1%)	3,467 (98.9%)	362 (10.4%)	3,105 (89.6%)
SY2021 Adults (Hatchery Phenotype)	611	4 (0.7%)	607 (99.3%)	565 (93.1%)	42 (6.9%)
MY2021 Juveniles	1,346	8 (0.6%)	1,338 (99.4%)	19 (1.4%)	1,319 (98.6%)
Chinook Salmon					
SY2021 Adults	2,676	3 (0.1%)	2,673 (99.9%)	666 (24.9%)	2,007 (75.1%)
MY2021 Juveniles	844	23 (2.7%)	821 (97.3%)	245 (29.8%)	576 (70.2%)
Total:	*8,981*	*75 (0.8%)*	*8,906 (99.2%)*	*1,857 (20.9%)*	*7,049 (89.1%)*

Table 27. Summary of Lower Granite Dam (LGR) natural-origin adult steelhead samples from SY2021 assigned to a genetic stock using individual assignment based on the Snake River steelhead SNP baseline v3.1. Summaries of life history diversity information (sex, length, and ocean age) for each genetic stock are shown. The 'Other' saltwater age category includes fish that were not queued to be aged, fish that could not be aged, and fish with spawn checks.

			Sex					Length							Ocean (Saltwater) Age						
			Frequency			Percentage		Mean Length (cm FL) by Ocean Age			Frequency		Percentage		Frequency				Percentage		
Genetic Stock	**Total Assignments**	**% Stock Composition**	**F**	**M**	**U**	**F**	**M**	**1**	**2**	**3**	**A-Run**	**B-Run**	**A-Run**	**B-Run**	**1**	**2**	**3**	**Other**	**1**	**2**	**3**
GRROND	1,049	34%	805	244	-	77%	23%	58.4	69.7	-	1,005	44	96%	4%	82	920	-	9	8%	92%	-
IMNAHA	188	6%	154	34	-	82%	18%	56.4	69.2	-	183	5	97%	3%	24	158	-	2	13%	87%	-
LOCWLR	182	6%	144	38	-	79%	21%	58.0	72.6	-	162	20	89%	11%	11	165	-	1	6%	94%	-
LOSALM	75	2%	55	20	-	73%	27%	59.7	71.0	-	72	3	96%	4%	14	58	-	-	19%	81%	-
LSNAKE	345	11%	267	78	-	77%	23%	56.8	69.9	-	331	14	96%	4%	38	287	-	1	12%	88%	-
MFSALM	222	7%	183	39	-	82%	18%	62.2	74.9	82.0	171	51	77%	23%	28	179	1	2	13%	86%	1%
SFCLWR	233	8%	160	72	1	69%	31%	62.3	79.5	-	81	152	35%	65%	6	200	-	1	3%	97%	-
SFSALM	95	3%	79	16	-	83%	17%	61.4	78.4	81.0	47	48	49%	51%	5	81	1	-	6%	93%	1%
UPCLWR	331	11%	266	65	-	80%	20%	62.4	79.7	86.5	135	196	41%	59%	10	304	2	1	3%	96%	1%
UPSALM	385	12%	293	92	-	76%	24%	58.1	69.6	79.0	374	11	97%	3%	50	315	1	4	14%	86%	1%
Total:	*3,105*		*2,406*	*698*	*-*	*78%*	*22%*	*58.7*	*72.4*	*82.4*	*2,561*	*544*	*82%*	*18%*	*268*	*2,667*	*5*	*75*	*9%*	*90%*	*<1%*

Table 28. Summary of Lower Granite Dam (LGR) juvenile steelhead samples from MY2021 assigned to a genetic stock using individual assignment based on the Snake River steelhead SNP baseline v3.1. Summaries of life history diversity information (sex, length, and freshwater age) for each genetic stock are shown. The 'Other' freshwater age category includes fish that were not queued to be aged or could not be aged.

			Sex					Length	Freshwater Age							
			Frequency			Percentage			Frequency				Percentage			
Genetic Stock	**Total Assignments**	**% Stock Composition**	**F**	**M**	**U**	**F**	**M**	**Mean Length (mm FL)**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
GRROND	442	34%	264	178	-	60%	40%	198	45	267	105	4	11%	63%	25%	1%
IMNAHA	86	7%	61	25	-	71%	29%	190	4	60	19	-	5%	72%	23%	-
LOCWLR	82	6%	55	27	-	67%	33%	201	16	52	10	1	20%	66%	13%	1%
LOSALM	11	1%	9	2	-	82%	18%	177	2	8	1	-	18%	73%	9%	-
LSNAKE	165	13%	96	68	1	58%	41%	195	26	96	28	4	17%	62%	18%	3%
MFSALM	47	4%	36	11	-	77%	23%	189	-	15	20	12	-	32%	43%	26%
SFCLWR	238	18%	136	102	-	57%	43%	187	22	174	26	2	10%	78%	12%	1%
SFSALM	19	1%	13	6	-	68%	32%	186	1	10	6	-	6%	59%	35%	-
UPCLWR	61	5%	52	9	-	85%	15%	182	4	31	16	8	7%	53%	27%	14%
UPSALM	168	13%	110	58	-	65%	35%	193	16	106	40	2	10%	65%	24%	1%
Total:	*1,319*		*832*	*486*	*1*	*63%*	*37%*	*194*	*136*	*819*	*271*	*33*	*11%*	*65%*	*22%*	*3%*

Table 29. Summary of Lower Granite Dam (LGR) adult Chinook Salmon samples sampled from SY2021 assigned to a genetic stock using individual assignments based on the Snake River Chinook Salmon SNP baseline v3.1. Summaries of life history diversity information (sex, length, and ocean age) for each genetic stock are shown. MJ = minijack.

			Sex					Ocean (Saltwater) Age											Length Mean Length (cm FL) by Ocean Age			
			Frequency		Percentage			Frequency						Percentage								
Genetic Stock	Total Assignments	% Stock Composition	F	M	F	M	MJ	1	2	3	4	Other	MJ	1	2	3	4	Other	1	2	3	4
CHMBLN	35	2%	10	25	29%	71%	-	15	19	1	-	-	-	43%	54%	3%	-	-	51.8	73.1	93.0	-
FALL	135	7%	55	80	41%	59%	7	20	78	19	1	10	6%	16%	62%	15%	1%	7%	51.2	75.5	83.4	85.0
HELLSC	668	33%	276	392	41%	59%	-	157	440	52	-	19	-	24%	68%	8%	-	3%	52.0	71.6	84.0	-
MFSALM	243	12%	92	151	38%	62%	-	52	140	44	-	7	-	22%	59%	19%	-	3%	49.4	72.7	86.7	-
SFSALM	528	26%	186	342	35%	65%	-	152	320	36	-	20	-	30%	63%	7%	-	4%	52.7	72.7	86.6	-
TUCANO	4	<1%	4	-	100%	-	-	-	4	-	-	-	-	-	100%	-	-	-	-	67.8	-	-
UPSALM	394	20%	159	235	40%	60%	-	76	246	58	-	14	-	20%	65%	15%	-	4%	51.6	73.8	86.1	-
Total:	2,007		782	1,225	39	61	7	472	1,247	210	1	70	<1%	24%	64%	11%	<1%	1%	52.0	72.9	85.5	85.0

Table 30. Summary of Lower Granite Dam (LGR) juvenile Chinook Salmon samples from MY2021 assigned to a genetic stock using individual assignment based on the Snake River Chinook Salmon SNP baseline v3.1. Summaries of life history diversity information (sex and length) by genetic stock are shown. Freshwater age is not summarized because scales were not collected from juvenile Chinook Salmon at Lower Granite Dam.

			Sex					Length
			Frequency			Percentage		
Genetic Stock	**Total Assignments**	**% Stock Composition**	**F**	**M**	**U**	**F**	**M**	**Mean Length (mm FL)**
CHMBLN	4	1%	2	2	-	50%	50%	126
FALL	21	4%	8	12	1	38%	57%	107
HELLSC	392	68%	215	177	-	55%	45%	118
MFSALM	38	7%	18	20	-	47%	53%	116
SFSALM	84	15%	47	37	-	56%	44%	116
TUCANO	-	0%	-	-	-	-	-	-
UPSALM	37	6%	21	16	-	57%	43%	119
Total:	*576*		*311*	*264*	1	*54%*	*46%*	*118*

Table 31. Proportionate natural influence (PNI) estimates for Chinook Salmon populations supplemented with integrated broodstocks. PNI is calculated for the portion of the populations spawning upstream of hatchery weirs based on origin determination based on visual identifiers (i.e., marks and tags) and verified through parentage-based tagging (PBT) analyses. Composite PNI estimates are also calculated to include portions of the population spawning up- and downstream of the Sawtooth and McCall weirs. Only mark data are used in the composite estimate.

		PNI		
		Upstream		Composite
Hatchery	**Year**	**Mark**	**PBT**	**Mark**
Sawtooth	2020	0.97	0.83	0.17
Sawtooth	2019	1.00	0.72	0.14
Sawtooth	2018	0.38	0.25	0.07
Sawtooth	2017	0.27	0.18	0.07
Sawtooth	2016	0.49	0.40	0.18
Sawtooth	2015	0.52	0.49	0.13
Sawtooth	2014	0.43	0.40	0.26
Pahsimeroi	2020	0.80	0.75	N/A
Pahsimeroi	2019	0.58	0.53	N/A
Pahsimeroi	2018	0.40	0.39	N/A
Pahsimeroi	2017	0.48	0.36	N/A
Pahsimeroi	2016	0.85	0.65	N/A
Pahsimeroi	2015	0.84	0.67	N/A
Pahsimeroi	2014	0.86	0.75	N/A
McCall	2020	0.90	0.83	0.53
McCall	2019	0.32	0.09	0.13
McCall	2018	0.46	0.32	0.13
McCall	2017	0.30	0.18	0.28
McCall	2016	0.47	0.39	0.44
McCall	2015	0.46	0.32	0.44
McCall	2014	0.66	0.62	0.66

FIGURES

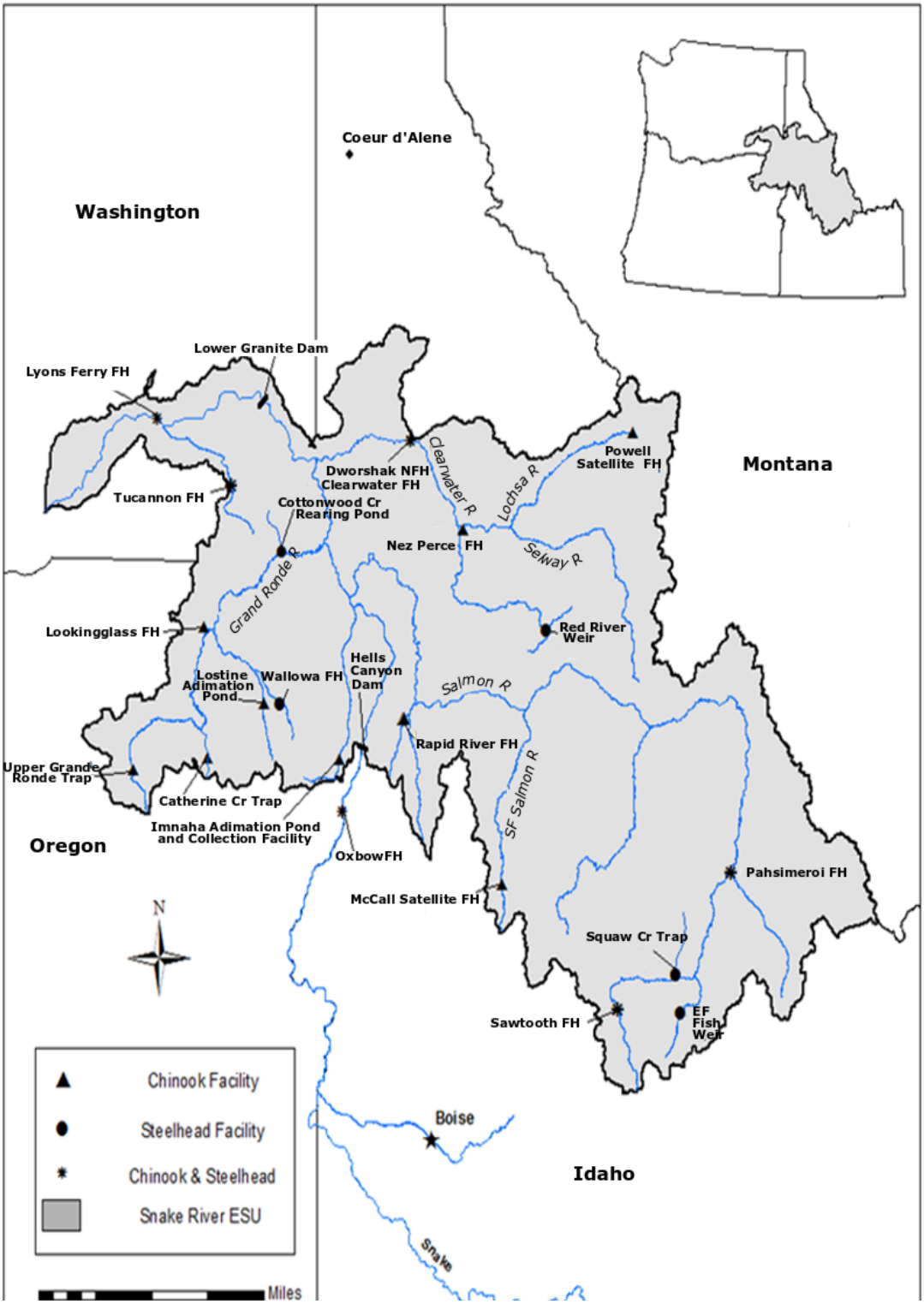

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

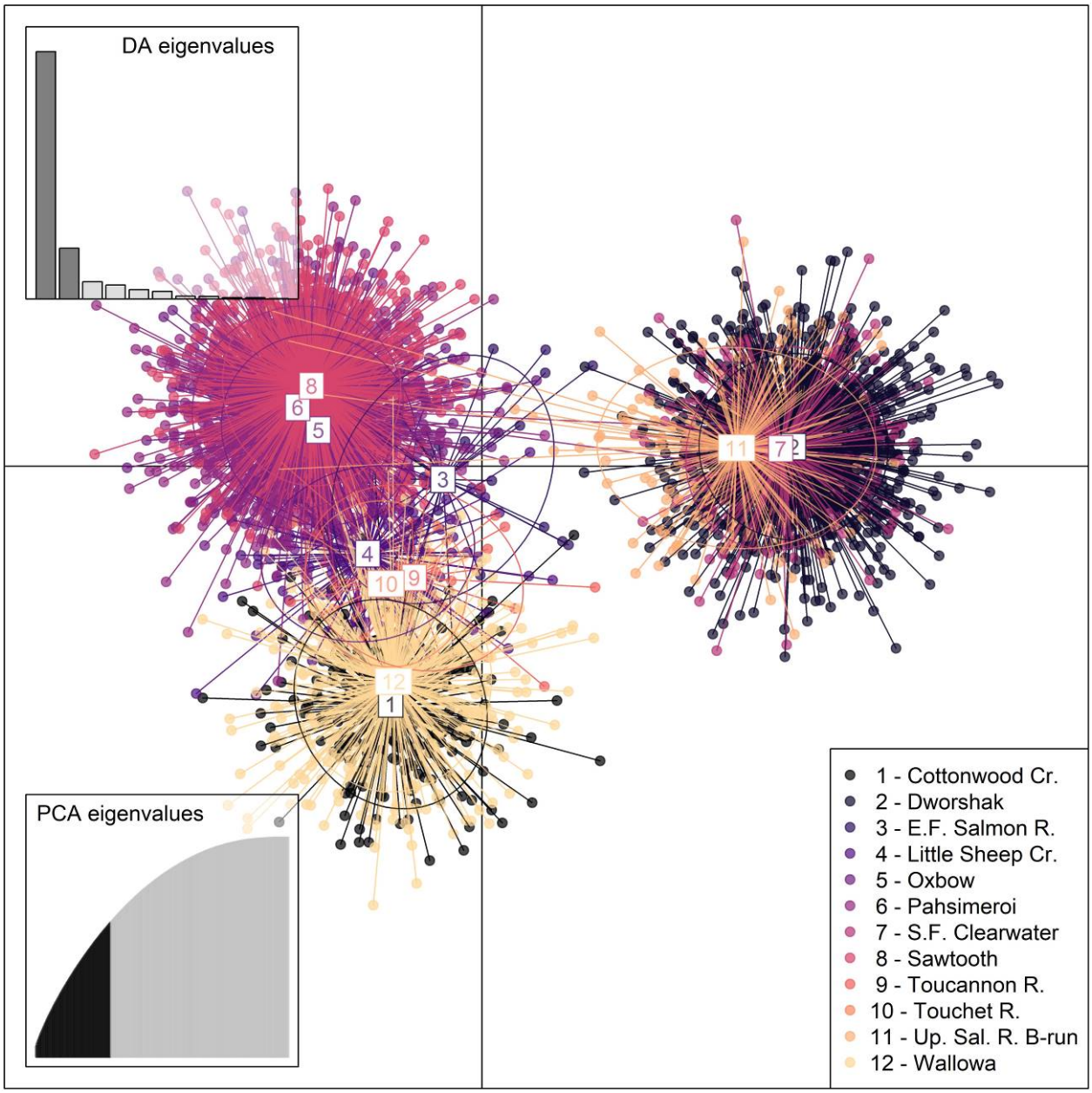

Figure 2. Discriminant analysis of principle components (DAPC) of SY2020 steelhead broodstocks. Each point represents an individual, and the colors of points and inertia ellipses correspond to each steelhead broodstock.

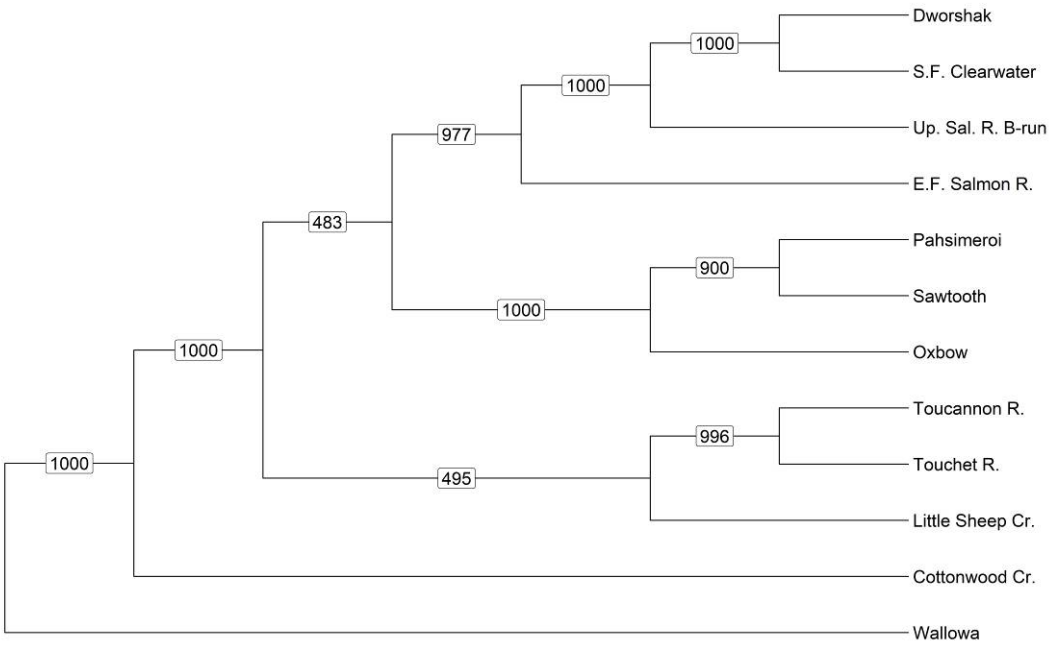

Figure 3. Unrooted neighbor-joining dendrogram of SY2020 steelhead broodstocks, generated using Cavalli-Sforza genetic chord distances and 1,000 bootstrap replicates. Values on the dendrogram represent the number of times a clade was represented within 1,000 bootstrap replicates.

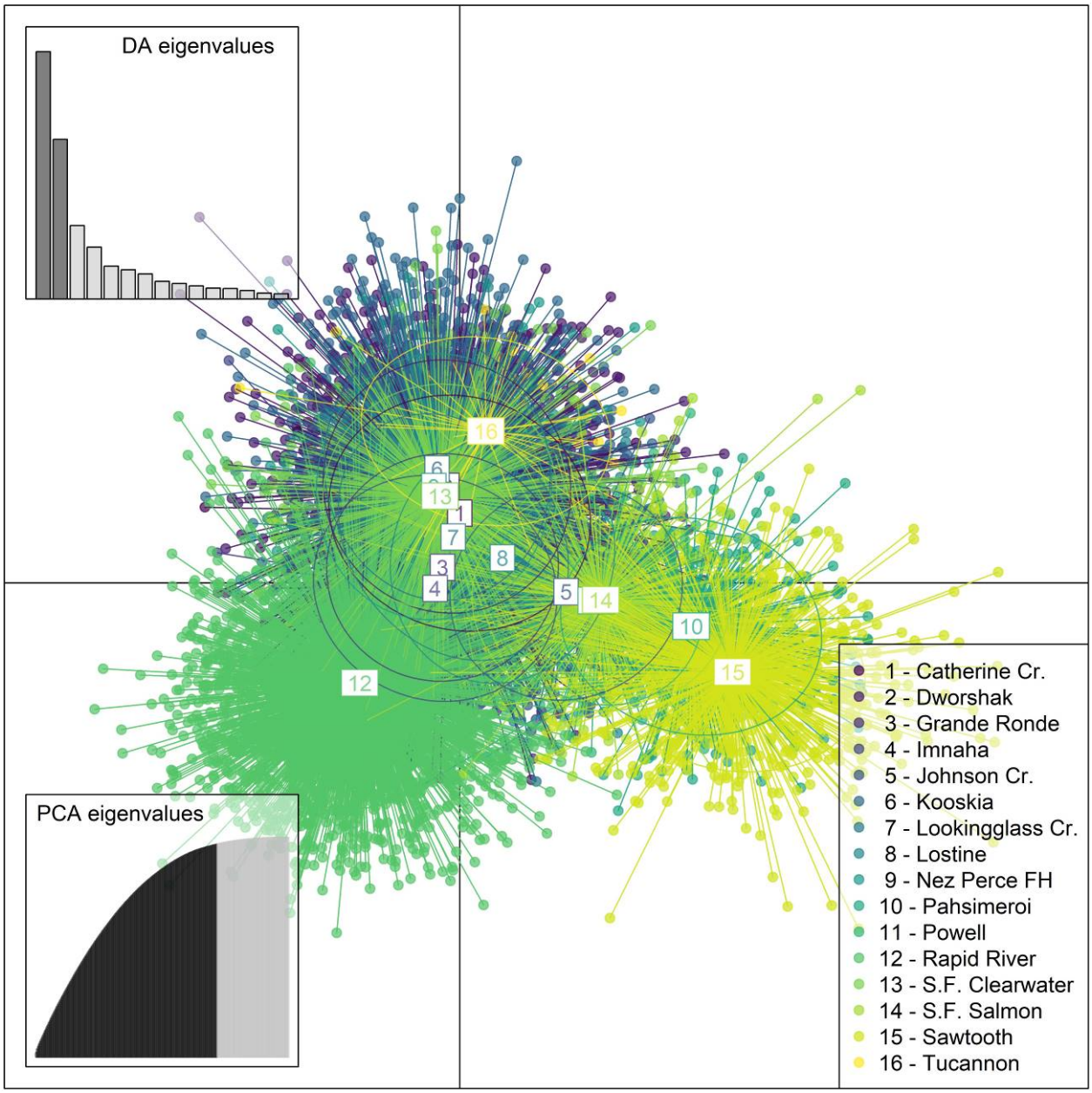

Figure 4. Discriminant analysis of principle components (DAPC) of SY2020 spring-summer Chinook Salmon broodstocks. Each point represents an individual, and the colors of points and inertia ellipses correspond to each Chinook Salmon broodstock.

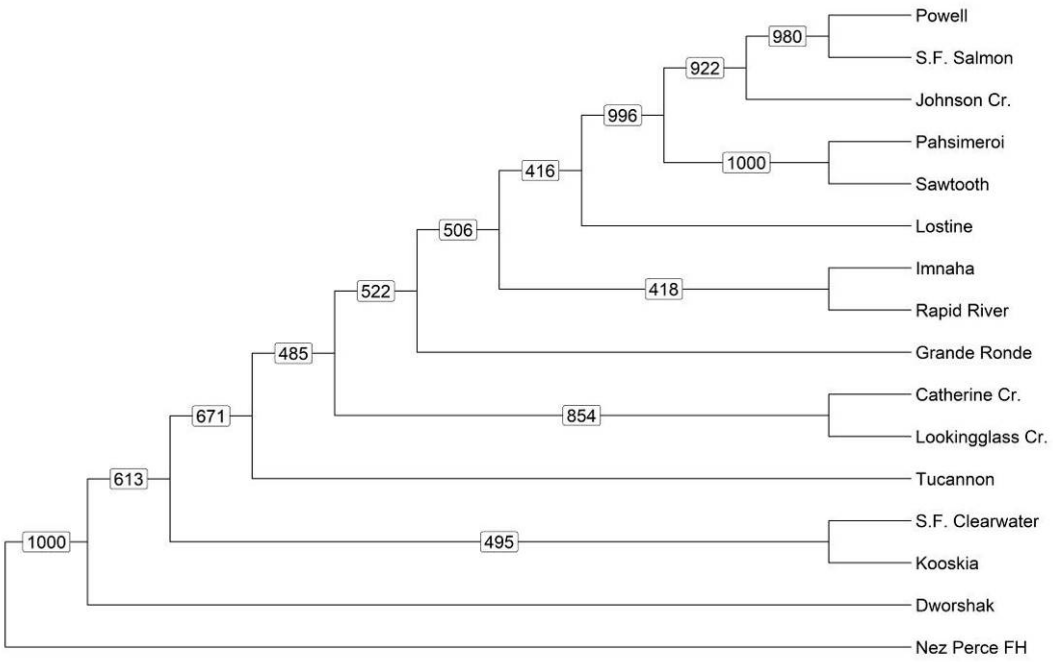

Figure 5. Unrooted neighbor-joining dendrogram of spring-summer SY2020 Chinook Salmon broodstocks, generated using Cavalli-Sforza genetic chord distances and 1,000 bootstrap replicates. Values on the dendrogram represent the number of times a clade was represented within 1,000 bootstrap replicates.

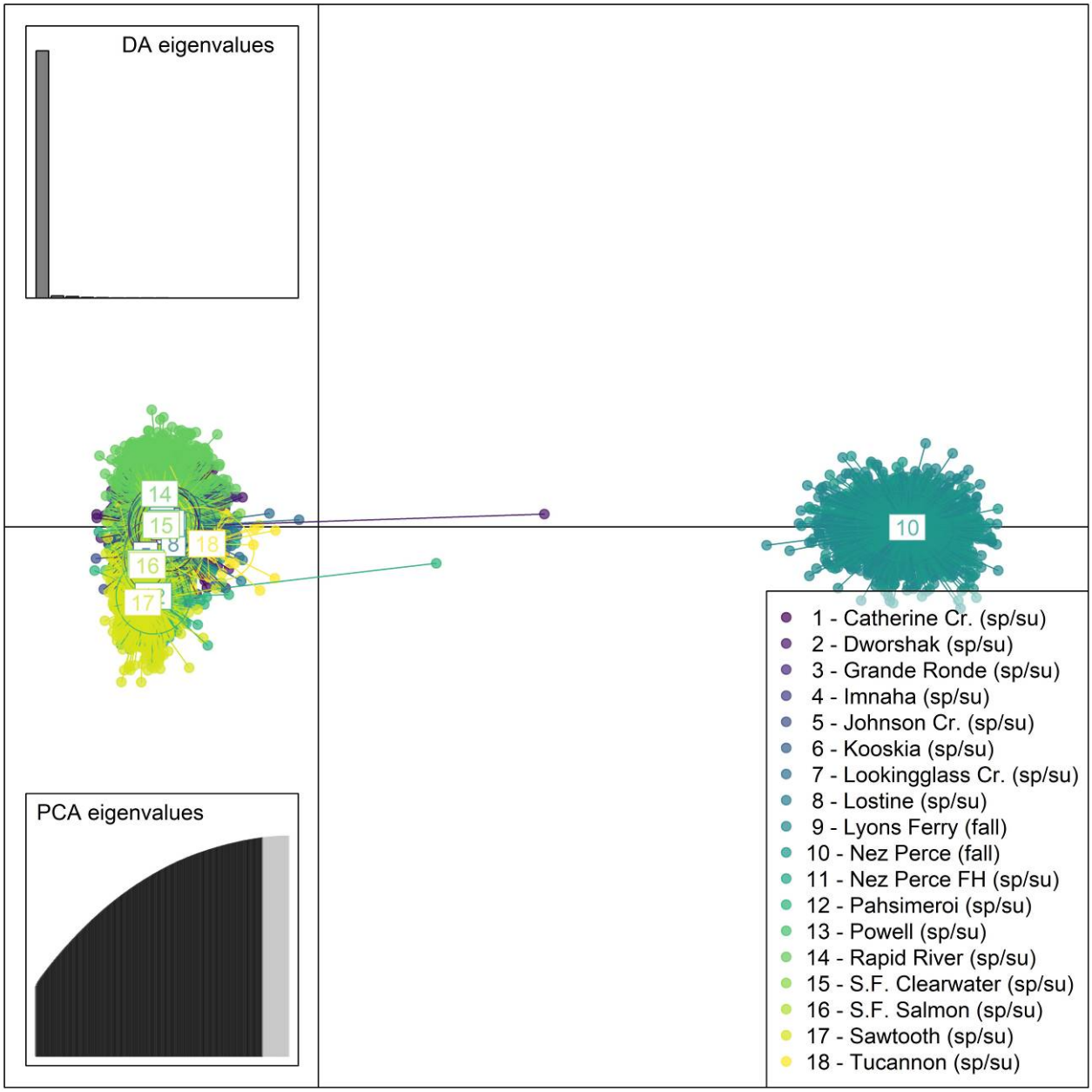

Figure 6. Discriminant analysis of principle components (DAPC) of all SY2020 Chinook Salmon broodstocks (both spring-summer and fall). Each point represents an individual, and the colors of points and inertia ellipses correspond to each Chinook Salmon broodstock. Spring-summer Chinook Salmon broodstocks are designated with (sp/su) and fall Chinook Salmon broodstocks are designated with (fall) in the legend.

APPENDIX A.

A DESCRIPTION OF GENETIC DIVERSITY FOR SPRING-SUMMER CHINOOK SALMON IN IDAHO

INTRODUCTION

A goal of salmon and steelhead conservation is to maintain natural patterns of genotypic expression (ICTRT 2003). Here we produce metrics related to genetic variation within TRT populations (expected heterozygosity, H_e) and levels of differentiation between populations (fixation index, F_{ST}) for spring-summer Chinook Salmon in the Snake River basin.

METHODS

We provided a summary of the major genetic diversity and structure patterns observed within the Snake River Chinook Salmon (hereafter: Chinook Salmon) ESU using a genetic baseline developed by Vu et al. (2015) and modified by Powell et al. (2018). These collections include tissues from juveniles and adults collected from across the Snake River basin which are amplified using a panel of 173 single nucleotide polymorphism (SNP) loci. Individual data and genotypes for all samples included in the Snake River Chinook Salmon genetic baseline are publically available at www.fishgen.net. Powell et al. (2018) provide an overview of different baselines that have been used for annual reports, which have varied in the number of markers and sample collections used for reference. The primary difference between the Chinook Salmon Snake River basin Baseline v3.1 and v3 was that the latter contained adults that were PIT tagged at LGR and subsequently detected at PIT tag instream arrays throughout the Snake River basin. Adults with PIT tags present in baseline v3 were removed from baseline v3.1. For more details on analyses and baseline summaries, we encourage seeing Vu et al. (2015) and Powell et al. (2018).

We calculated estimates of genetic diversity including expected heterozygosity (H_e) and genetic differentiation (F_{ST}) using GenAlEx v 6.503 (Peakall and Smouse 2006, 2012) by TRT population (ICTRT 2003). As part of this, collections of samples from multiple locations and years within a TRT boundary were pooled and a mean value was generated for these pooled collections. To visualize the genetic relationships among collections, a neighbor-joining (NJ) tree was constructed using the entire Snake River Chinook Salmon baseline collection including populations of the Fall Chinook Salmon ESU. The NJ tree was generated using Nei's genetic distance (Nei 1972) as calculated in GENDIST (PHYLIP; Felsenstein 1993), with trees constructed in NEIGHBOR (PHYLIP) and topology consistency was estimated via 1,000 bootstrap replicates (across loci) in SEQBOOT (PHYLIP). We show bootstrap support for major nodes that occurred in 700 (70%) or more bootstrap replicates. The final NJ trees was constructed and edited using FigTree v1.31 (Rambaut 2012).

RESULTS

A total of 4,356 samples collected from 43 locations are present in the Snake River basin spring-summer Chinook Salmon baseline V3.1 (Table A1, Figure A1). Here, we report on diversity and differentiation among populations from Idaho (3,155 individuals from 32 locations; Table A2), which includes functionally extirpated populations in the Clearwater River system. Metrics of genetic differentiation (F_{ST}) reflect values generated using the entire Snake River Chinook Salmon ESU collection.

At the MPG level, we observed the highest mean levels of expected heterozygosity in the Wet Clearwater (23.9%) and Dry Clearwater (23.7%), both of which are considered functionally extirpated (Table A2). Next, levels of heterozygosity were equal in the South Fork Salmon River (22.4%) and Upper Salmon River (22.4%) followed by the Middle Fork Salmon River (21.1%). Within the Wet Clearwater River MPG, the Lolo Creek population had slightly higher levels of heterozygosity (23.9%) than the Lochsa River (23.8%). Within the South Fork Salmon River, heterozygosity was highest in the Little Salmon River population (SRLSR, H_e = 22.8%), which has been heavily influenced by Rapid River Hatchery stock. The lowest levels of heterozygosity in the South Fork Salmon River MPG was observed on the Secesh River (21.8%). Within the Middle Fork Salmon River MPG, the highest and lowest levels of heterozygosity were observed in Big Creek (21.6%) and Sulphur Creek (20.5%), respectively. In the Upper Salmon River MPG, the highest levels of heterozygosity were observed in Valley Creek (23.0%) and the lowest in the Lemhi River (21.6%).

Pairwise genetic differentiation among MPGs was highest for the Middle Fork Salmon River (F_{ST} = 0.016), followed closely by the Upper Salmon River (0.015) and South Fork Salmon River (0.014; Table A3). Both the Wet Clearwater and Dry Clearwater River MPG had lower levels of F_{ST} at 0.012 and 0.011, respectively. Within the Middle Fork Salmon River MPG, the most differentiated population was Chamberlain Creek (F_{ST} = 0.021) and the least was Big Creek (F_{ST} = 0.012). We observed lower variation in values of genetic differentiation among populations of the Upper Salmon River; the lowest value was 0.012 for the Upper Salmon main stem and highest was 0.018 for the Yankee Fork population. Values of differentiation in the South Fork Salmon River MPG ranged from a low of 0.011 in the South Fork main stem to a high of 0.015 for both the Little Salmon River and Secesh River populations. We observed no variation in estimates of F_{ST} within the Wet Clearwater MPG.

Patterns of genetic differentiation identified via F_{ST} metrics mirrored those displayed in our neighbor-joining tree (Figure A2). The Upper Salmon River MPG formed a well-supported clade, although samples from the NF Salmon River fell out as sister to this clade. Likewise, the Middle Fork Salmon River consisted of a single well-supported clade, with the exception of samples from Chamberlain Creek. With only minor exceptions (e.g., GRLOS), genetic relationships among collections of samples conformed to delineations as defined by TRT populations (Figure A2).

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Table A1. Summary of sample collections included in the current Snake River spring-summer Chinook Salmon SNP baseline v3.1 (Powell et al. 2018). Summaries include the TRT population that the collection was taken in, the genetic stock (reporting group) used for GSI at Lower Granite Dam, Major Population Group (MPG), sample size (n), the years(s) that samples were collected, the lab where samples were genotyped, latitude/longitude, expected heterozygosity (HE), and fixation indices (F_{ST}).

Map#[A]	Collection Name	TRT Population	Genetic Stock[A]	MPG	n	Year Collected	Genotype Agency[C]	Latitude[D]	Longitude	He[E]	F_{ST}[F]
1	Decker Flat	SRUMA	UPSALM	Upper Salmon River	95	10, 11	NWFSC	44.06537	-114.85580	22.8%	0.013
2	Sawtooth Weir	SRUMA	UPSALM	Upper Salmon River	91	09, 10	IDFG	44.15066	-114.88545	22.0%	0.013
3	Valley Cr	SRVAL	UPSALM	Upper Salmon River	101	07, 08, 09, 10, 11	IDFG	44.24084	-115.00155	23.0%	0.014
4	WF Yankee Fork	SRYFS	UPSALM	Upper Salmon River	75	05	CRITFC	44.34484	-114.72517	22.4%	0.019
5	upper Salmon mainstem	SRLMA	UPSALM	Upper Salmon River	83	05, 06, 07, 08, 09, 10	IDFG	44.25568	-114.56477	22.4%	0.014
6	Herd Cr	SREFS	UPSALM	Upper Salmon River	99	10, 11	NWFSC	44.12319	-114.26642	21.6%	0.016
7	East Fork SR	SREFS	UPSALM	Upper Salmon River	187	04, 05, 11	IDFG/CRITFC	44.20019	-114.28613	22.4%	0.013
8	Pahsimeroi R	SRPAH	UPSALM	Upper Salmon River	92	07, 08, 09, 10	IDFG	44.56296	-113.91237	22.7%	0.016
10	upper Lemhi R	SRLEM	UPSALM	Upper Salmon River	96	09, 10	IDFG	44.82673	-113.60684	21.6%	0.017
12	NF Salmon R	SRNFS	UPSALM	Upper Salmon River	55	05, 06, 07, 08, 09, 10	IDFG	45.50104	-113.96306	22.5%	0.016
14	Marsh Cr	MFMAR	MFSALM	Middle Fork Salmon River	116	07, 08, 09, 10, 11	IDFG	44.41532	-115.18423	21.4%	0.014
15	Capehorn Cr	MFMAR	MFSALM	Middle Fork Salmon River	112	05, 06, 07, 09, 10	IDFG/CRITFC	44.35864	-115.22362	21.3%	0.018
16	Elk Cr (MF Salmon R)	MFBEA	MFSALM	Middle Fork Salmon River	135	07, 08, 09, 10, 11	IDFG/NWFSC	44.43041	-115.47107	20.9%	0.016
17	Bear Valley Cr	MFBEA	MFSALM	Middle Fork Salmon River	81	07, 08, 09, 10	IDFG	44.37347	-115.39544	21.3%	0.015
18	Sulphur Cr	MFSUL	MFSALM	Middle Fork Salmon River	135	08, 09, 10, 11	IDFG/NWFSC	44.54330	-115.39615	20.5%	0.019
19	Loon Cr	MFLOO	MFSALM	Middle Fork Salmon River	94	10, 11	IDFG	44.59815	-114.81104	21.4%	0.016
20	Camas Cr	MFCAM	MFSALM	Middle Fork Salmon River	107	06, 09, 10	IDFG/CRITFC	44.82550	-114.49964	20.8%	0.017
21	upper Big Cr	MFBIG	MFSALM	Middle Fork Salmon River	55	10, 11	IDFG/CRITFC	45.15304	-115.29609	21.5%	0.017
22	lower Big Cr	MFBIG	MFSALM	Middle Fork Salmon River	139	01, 11	CRITFC/NWFSC	45.10717	-114.80611	21.6%	0.012
23	Chamberlain Cr (pre-2008)	SRCHA	CHMBLN	Middle Fork Salmon River	70	03, 04, 06, 07	IDFG	45.39364	-115.19440	20.9%	0.020
24	Chamberlain Cr (post-2008)	SRCHA	CHMBLN	Middle Fork Salmon River	149	09, 10	IDFG/CRITFC	45.37078	-115.19671	20.9%	0.023
25	Summit and Lake Cr	SFSEC	SFSALM	South Fork Salmon River	122	07, 08, 09, 10, 11	IDFG	45.27121	-115.91413	21.6%	0.016
26	Sesech R	SFSEC	SFSALM	South Fork Salmon River	131	01, 07, 08, 09, 10	IDFG/CRITFC	45.21723	-115.80862	21.9%	0.015
27	Johnson Cr	SFMAI	SFSALM	South Fork Salmon River	137	02, 11	CRITFC/NWFSC	44.90585	-115.48672	22.2%	0.015
28	SF Salmon R mainstem	SFMAI	SFSALM	South Fork Salmon River	139	09, 10	IDFG	44.66661	-115.70292	22.9%	0.011
29	Rapid R	SRLSR	HELLSC	South Fork Salmon River	91	06	IDFG	45.31630	-116.41804	22.8%	0.015
30	Crooked F (Lochsa R)	CRLOC	HELLSC	Wet Clearwater[G]	28	07, 08, 09, 10	IDFG	46.61875	-114.66711	24.2%	0.016
31	Powell Weir	CRLOC	HELLSC	Wet Clearwater[G]	30	09	IDFG	46.50701	-114.68739	23.3%	0.014
32	Red R	SCUMA	HELLSC	Dry Clearwater[G]	72	07, 08, 09, 10	IDFG	45.70942	-115.33989	24.1%	0.013

Table A1. Continued

Map#[A]	Collection Name	TRT Population	Genetic Stock[A]	MPG	n	Year Collected	Genotype Agency[C]	Latitude[D]	Longitude	He[E]	F_{ST}[F]
33	Crooked R Weir	SCUMA	HELLSC	Dry Clearwater[G]	67	09, 10	IDFG	45.76553	-115.54375	24.1%	0.013
34	Newsome Cr	SCUMA	HELLSC	Dry Clearwater[G]	82	01	CRITFC	45.83376	-115.61115	22.9%	0.015
35	Lolo Cr	CRLOL	HELLSC	Wet Clearwater[G]	89	01, 02	IDFG/CRITFC	46.28015	-115.77274	23.9%	0.012
36	Imnaha R (08' & 10')	IRMAI	HELLSC	Grande Ronde / Imnaha	96	08, 10	IDFG/NOAA	45.49004	-116.80393	23.4%	0.012
37	upper Grande Ronde R	GRUMA	HELLSC	Grande Ronde / Imnaha	43	08	IDFG/NOAA	45.19318	-118.39441	24.6%	0.016
28	Catherine Cr	GRCAT	HELLSC	Grande Ronde / Imnaha	140	04, 06, 11	IDFG/CRITFC/NWFSC	45.15485	-117.77926	24.8%	0.013
39	Minam R	GRMIN	HELLSC	Grande Ronde / Imnaha	131	94, 02, 10	IDFG/CRITFC/NWFSC	45.34755	-117.65335	25.0%	0.013
40	Wallowa R & Hurricane Cr	GRLOS	HELLSC	Grande Ronde / Imnaha	37	11	IDFG	45.42408	-117.29267	25.5%	0.019
41	Lostine R	GRLOS	HELLSC	Grande Ronde / Imnaha	175	03, 05, 09	IDFG//NOAA	45.47359	-117.42573	23.0%	0.015
42	Wenaha R	GRWEN	HELLSC	Grande Ronde / Imnaha	179	02, 06, 09, 10	CRITFC	45.96890	-117.69559	26.4%	0.014
43	Tucannon R	SNTUC	TUCANO	Tucannon river	81	03	CRITFC	46.50530	-118.01440	25.9%	0.026
N/A	Clearwater R	FALL ESU	FALL	Snake River	144	08	IDFG/CRITFC	46.52285	-116.61520	N/A	N/A
N/A	Nez Perce Tribal H.	FALL ESU	FALL	Snake River	85	03	CRITFC	46.51910	-116.66460	N/A	N/A
N/A	Lyons Ferry H.	FALL ESU	FALL	Snake River	90	00	CRITFC	46.58940	-118.21950	N/A	N/A

Footnotes:

A. Only a subset of collections in the map are presented here. Updates to the previous GSI baseline involved removing selected populations

B. This is the genetic stock (reporting group) used for genetic individual assignment tests for Chinook Salmon adults captured at the Lower Granite Dam (LGR) adult trapping facility and smolts captured at the LGR juvenile fish facility (Vu et al. 2015).

C. Denotes the agency that genotyped the samples. IDFG = Idaho Department of Fish and Game's Eagle Fish Genetics Lab in Eagle, ID (Matthew Campbell: matthew.campbell@idfg.idaho.gov; John Hargrove: john.hargrove@idfg.idaho.gov). CRITFC = Columbia River Inter-Tribal Fish Commission's genetics lab in Hagerman, ID (Sean Narum: nars@critfc.org). NWFSC = NOAA Northwest Fisheries Science Center.

D. WGS84 Datum

E. The mean expected heterozygosity across all SNPs for each populations. Calculated per SNP as 1 minus the sum of the squared allele frequencies, p_i^2 using GENALEX 6.5 (Peakall and Smouse 2006, 2012).

F. The average of pairwise F_{ST} when compared to all other populations in the Snake River baseline. Calculated in GENALEX 6.5 (Peakall and Smouse 2006, 2012)

G. Denotes functionally extirpated populations of Chinook Salmon

Table A2. A summary of genetic differentiation (fixation index, F_{ST}) and diversity (expected heterozygosity, H_E) for sample collections of spring-summer Chinook Salmon populations in the state of Idaho. Listed are the TRT populations for each suite of collections as well as the number of sites sampled, number of years in which sampling occurred, and number of individuals analyzed at a panel of 173 SNP markers.

			Samples Collected				
MPG	**TRT**	**Description**	**Sites**	**Years**	**Individuals**	H_e	F_{ST}
Middle Fork Salmon River	MFBIG	Big Cr	2	3	194	21.6%	0.012
Middle Fork Salmon River	MFMAR	Marsh Cr	2	7	228	21.5%	0.014
Middle Fork Salmon River	MFLOO	Loon Cr	1	2	94	21.4%	0.015
Middle Fork Salmon River	MFBEA	Bear Valley Cr/ Elk Cr	2	5	216	21.0%	0.014
Middle Fork Salmon River	SRCHA	Chamberlain Cr	2	6	219	21.0%	0.021
Middle Fork Salmon River	MFCAM	Camas Cr	1	3	107	20.8%	0.017
Middle Fork Salmon River	MFSUL	Sulphur Cr	1	4	135	20.5%	0.019
South Fork Salmon River	SRLSR	Little Salmon R	1	1	91	22.8%	0.015
South Fork Salmon River	SFMAI	South Fork Salmon R	2	4	276	22.7%	0.011
South Fork Salmon River	SFSEC	Secesh R	2	6	253	21.8%	0.015
Upper Salmon River	SRVAL	Valley Cr	1	5	101	23.0%	0.013
Upper Salmon River	SRPAH	Pahsimeroi R	1	4	92	22.7%	0.016
Upper Salmon River	SRNFS	NF Salmon R	1	6	55	22.5%	0.016
Upper Salmon River	SLRMA	Upper Salmon River lower main stem	1	6	83	22.4%	0.014
Upper Salmon River	SRYFS	Yankee Fork	1	1	75	22.4%	0.018
Upper Salmon River	SRUMA	Upper Salmon River main stem	2	3	186	22.4%	0.012
Upper Salmon River	SREFS	EF Salmon R	2	4	286	22.2%	0.013
Upper Salmon River	SRLEM	Lemhi R	1	2	96	21.6%	0.017
Dry Clearwater*	SCUMA	Upper South Fork Clearwater	3	5	221	23.7%	0.011
Wet Clearwater*	CRLOL	Lolo Creek	1	2	89	23.9%	0.012
Wet Clearwater*	CRLOC	Lochsa River	2	4	58	23.8%	0.012

* denotes a functionally extirpated TRT population

Table A3. A pairwise matrix of genetic differentiation among populations of Idaho Chinook Salmon as quantified by F_{ST} values (fixation index). Values closer to zero represent lower levels of genetic differentiation and larger values imply populations are more genetically distinct. Values are color coded with green and red implying lower and higher levels of differentiation, respectively. Numbers in correspond to the same populations labeled in the rows.

TRT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Avg
1) SRUMA	-	0.004	0.010	0.005	0.005	0.010	0.012	0.013	0.010	0.011	0.015	0.011	0.015	0.009	0.019	0.012	0.007	0.014	0.011	0.010	0.012	0.012
2) SRVAL	0.004	-	0.011	0.005	0.006	0.010	0.012	0.015	0.014	0.015	0.019	0.014	0.017	0.010	0.021	0.016	0.010	0.016	0.012	0.012	0.012	0.013
3) SRYFS	0.010	0.011	-	0.012	0.015	0.019	0.020	0.020	0.017	0.017	0.023	0.018	0.024	0.015	0.025	0.019	0.014	0.020	0.017	0.016	0.018	0.018
4) SLRMA	0.005	0.005	0.012	-	0.005	0.009	0.013	0.014	0.013	0.014	0.019	0.014	0.016	0.010	0.019	0.015	0.010	0.019	0.014	0.013	0.014	0.014
5) SREFS	0.005	0.006	0.015	0.005	-	0.009	0.011	0.014	0.012	0.013	0.018	0.011	0.014	0.009	0.020	0.014	0.009	0.017	0.012	0.012	0.013	0.013
6) SRPAH	0.010	0.010	0.019	0.009	0.009	-	0.013	0.017	0.016	0.017	0.020	0.016	0.017	0.014	0.024	0.016	0.012	0.019	0.015	0.014	0.014	0.016
7) SRLEM	0.012	0.012	0.020	0.013	0.011	0.013	-	0.019	0.017	0.017	0.024	0.018	0.019	0.013	0.024	0.018	0.014	0.016	0.015	0.013	0.014	0.017
8) SRNFS	0.013	0.015	0.020	0.014	0.014	0.017	0.019	-	0.016	0.015	0.021	0.017	0.018	0.013	0.019	0.016	0.011	0.014	0.013	0.011	0.013	0.016
9) MFMAR	0.010	0.014	0.017	0.013	0.012	0.016	0.017	0.016	-	0.007	0.010	0.010	0.013	0.008	0.020	0.011	0.007	0.018	0.013	0.014	0.013	0.014
10) MFBEA	0.011	0.015	0.017	0.014	0.013	0.017	0.017	0.015	0.007	-	0.006	0.010	0.014	0.007	0.019	0.011	0.009	0.018	0.014	0.015	0.015	0.014
11) MFSUL	0.015	0.019	0.023	0.019	0.018	0.020	0.024	0.021	0.010	0.006	-	0.012	0.017	0.011	0.023	0.015	0.012	0.024	0.020	0.020	0.020	0.019
12) MFLOO	0.011	0.014	0.018	0.014	0.011	0.016	0.018	0.017	0.010	0.010	0.012	-	0.012	0.008	0.022	0.014	0.010	0.019	0.015	0.015	0.016	0.015
13) MFCAM	0.015	0.017	0.024	0.016	0.014	0.017	0.019	0.018	0.013	0.014	0.017	0.012	-	0.009	0.023	0.015	0.011	0.019	0.014	0.015	0.015	0.017
14) MFBIG	0.009	0.010	0.015	0.010	0.009	0.014	0.013	0.013	0.008	0.007	0.011	0.008	0.009	-	0.016	0.012	0.008	0.015	0.011	0.011	0.012	0.012
15) SRCHA	0.019	0.021	0.025	0.019	0.020	0.024	0.024	0.019	0.020	0.019	0.023	0.022	0.023	0.016	-	0.017	0.013	0.019	0.020	0.018	0.019	0.021
16) SFSEC	0.012	0.016	0.019	0.015	0.014	0.016	0.018	0.016	0.011	0.011	0.015	0.014	0.015	0.012	0.017	-	0.005	0.017	0.014	0.012	0.013	0.015
17) SFMAI	0.007	0.010	0.014	0.010	0.009	0.012	0.014	0.011	0.007	0.009	0.012	0.010	0.011	0.008	0.013	0.005	-	0.013	0.010	0.009	0.009	0.011
18) SRLSR	0.014	0.016	0.020	0.019	0.017	0.019	0.016	0.014	0.018	0.018	0.024	0.019	0.019	0.015	0.019	0.017	0.013	-	0.008	0.004	0.006	0.015
19) CRLOC	0.011	0.012	0.017	0.014	0.012	0.015	0.015	0.013	0.013	0.014	0.020	0.015	0.014	0.011	0.020	0.014	0.010	0.008	-	0.004	0.005	0.012
20) SCUMA	0.010	0.012	0.016	0.013	0.012	0.014	0.013	0.011	0.014	0.015	0.020	0.015	0.015	0.011	0.018	0.012	0.009	0.004	0.004	-	0.003	0.011
21) CRLOL	0.012	0.012	0.018	0.014	0.013	0.014	0.014	0.013	0.013	0.015	0.020	0.016	0.015	0.012	0.019	0.013	0.009	0.006	0.005	0.003	-	0.012

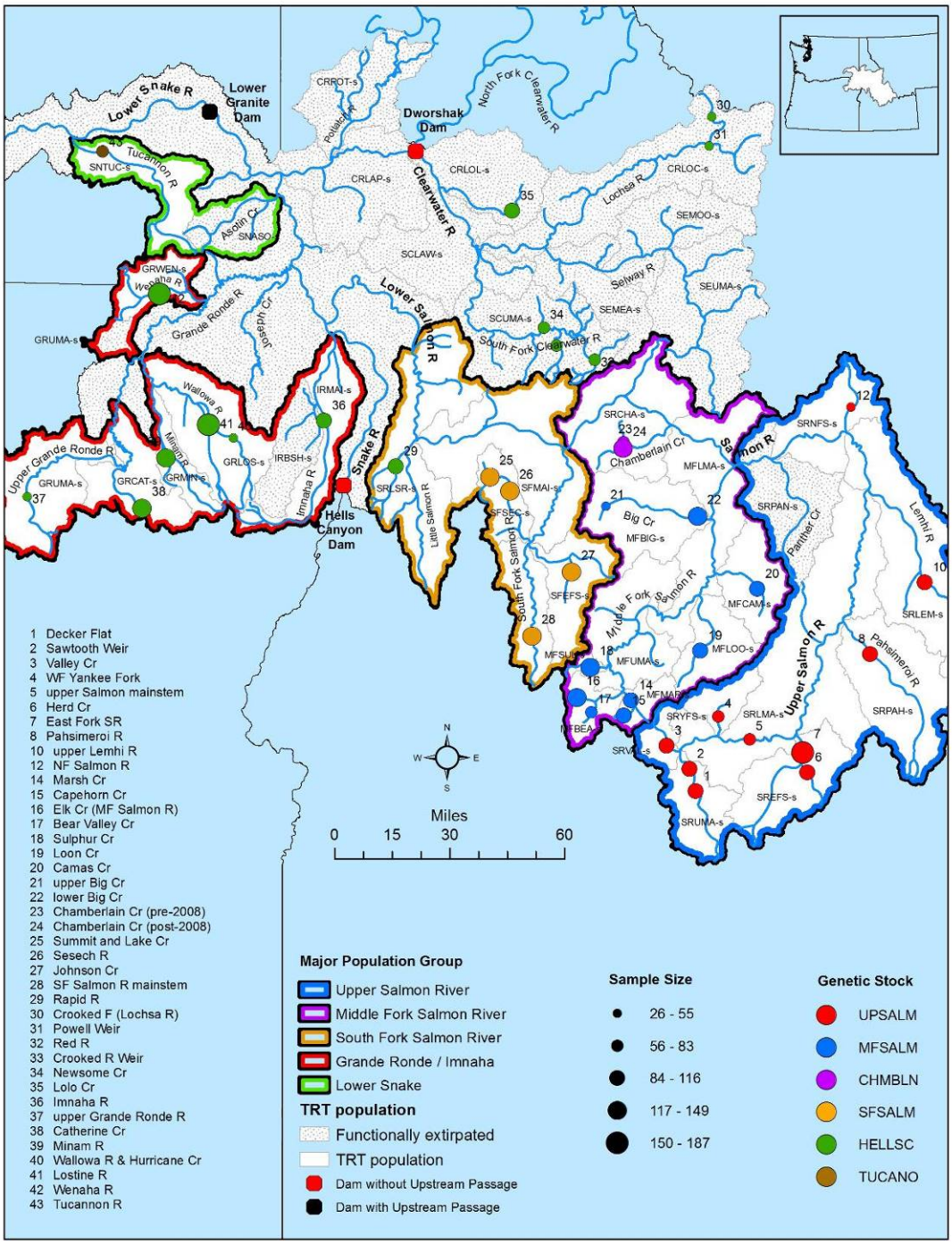

Figure A1. A map of steelhead collections used in the Chinook Salmon baseline v3.1 (Powell et al. 2018) and in the current summary of genetic diversity and genetic differentiation among Idaho Chinook Salmon populations. For a detailed description of collection characteristics see Table A1. The Hells Canyon Tributaries major population group (shaded gray) does not support independent populations and is considered extirpated.

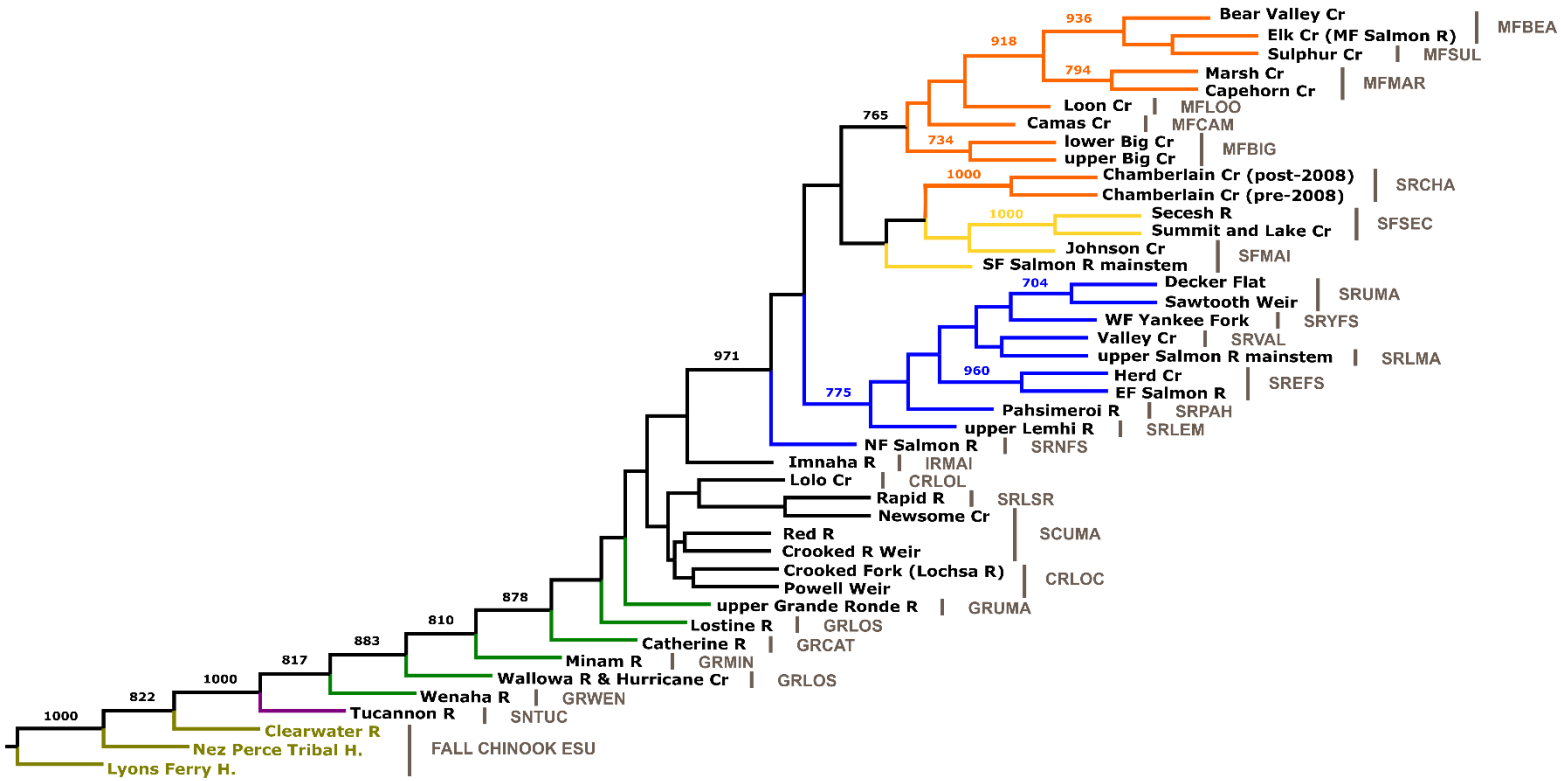

Figure A2. Genetic relationships among populations of spring-summer Chinook Salmon collected from the Snake River basin. Labels in black denote individual collections and labels in grey denote the TRT population for each collection. The tree is based on Nei's genetic distance and numbers along branches show display bootstraps values support the grouping greater than 70% (of 1,000 replicates).

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