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GENETIC MONITORING OF SNAKE RIVER SALMONIDS

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Genetic Monitoring of Snake River Salmonids

Project Progress Report

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

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 non-lethally 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 Northwest Power and Conservation 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 is 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 Northwest Power and Conservation 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 (LGR) 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 LGR (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 upstream 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 upstream 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 each 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 2021 (SY2022), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2021 (SY2022), 3) Origin of samples from various sport fisheries in Idaho in migration year 2021 (SY2022), 4) Age composition and origin of the SY2021 broodstocks, and 5) stock composition of returning adults during SY2022 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2021), 2) Age composition and origin of SY2021 broodstocks, 3) Stock composition of returning adults during SY2021 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. McCarrick and others will present results for SY2021 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 2022 marks our seventh 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 incorporate newly characterized microhaps into the Chinook Salmon GSI baseline. Importantly, we have modified existing bioinformatic pipelines to extract microhap 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., Baum et al. 2022). 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).

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 SY2021.

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). 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 twelfth 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 polymerase chain reaction (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 were 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 recorded length and cross information whenever possible. Tissue samples were 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 were shipped to the IDFG Eagle Fish Genetics Laboratory (EFGL) in Eagle, Idaho. Care was 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 was labeled with a field identification number, which was used to track the samples until they arrived at the lab, at which time they were given a standardized lab database code. The associated data was reviewed at the lab to ensure accurate information was recorded for every fish sampled. Any discrepancies that were discovered were 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 were extracted and genotyped, genetic data were 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. 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.

Standardized parental genotypes were stored on a Progeny database server housed at the 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 principal components (DAPC; Jombart et al. 2010). Unrooted neighbor-joining dendrograms were generated from Prevosti's genetic distance using the R package poppr (Kamvar et al. 2014) 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 SY2021; 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. 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).

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, SY2021 broodstocks 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 SY2021, we collected and inventoried approximately 4,487 genetic samples from steelhead broodstock (Table 1) and approximately 10,030 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 SY2021 (N = 2,533; 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

For steelhead SY2021, all samples were extracted and genotyped with the expanded panel of 368 SNPs which includes a sex-identification assay. Of the 4,487 total samples collected, 4,441 (99.0%) were genotyped with an acceptable level of missing data (Table 1).

For spring-summer Chinook Salmon SY2021, all samples were extracted and genotyped with the expanded panel of 343 PBT SNPs which includes a sex-identification assay. Of the 10,030 total samples collected, 9,853 (98.2%) were genotyped with an acceptable level of missing data (Table 2).

For fall Chinook Salmon SY2021, 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 2,533 total samples collected, 2,511 (99.1%) 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 SY2021 steelhead, 31 loci failed to genotype at >5% of samples (Table 4). For SY2021 spring-summer Chinook Salmon, 3 loci failed at >5% of the samples (Table 5). For SY2021 fall Chinook Salmon, 11 loci failed at >5% of the samples (Table 6).

Error Rate (Quality Control)

For steelhead SY2021, a subset of 190 samples were rerun and the resulting 64,849 genotypes (limited to non-missing genotypes in both runs) were checked for discrepancies. Of

these genotypes, there were 42 discrepancies, excluding any SNP failures in either the original or the rerun genotype, which resulted in a genotype error rate of 0.06%.

For spring-summer Chinook Salmon SY2021 genotyped at EFGL, a subset of 116 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 42,184 rerun genotypes being compared to the original genotypes. This resulted in 125 discrepancies, excluding any SNP failures in either the original or the rerun genotype, between the original and samples and a genotyping error rate of 0.3%.

Average Heterozygosity

Levels of observed heterozygosity within steelhead broodstocks were 0.23-0.27 for all hatchery broodstocks (Table 10). For Chinook Salmon, levels of observed heterozygosity were 0.20-0.23 in spring-summer stocks (Table 11) and 0.27-0.28 in fall stocks (Table 12).

Population Structure

Pairwise F_{ST} was calculated among the steelhead SY2021 hatchery broodstocks (Table 13). Values ranged from a low of 0.002 between the S.F. Clearwater and Dworshak stocks, and a high of 0.078 between the Little Sheep Creek and S.F. Clearwater 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 SY2021, pairwise F_{ST} values ranged from a low of 0.000 between the Dworshak and the S.F. Clearwater stocks and a high of 0.043 between the Tucannon and Sawtooth stocks and between the Johnson Creek and Lostine stocks (Table 14). All tests of genetic differentiation among spring-summer Chinook Salmon stocks were statistically significant ($p < 0.01$). Differentiation among the two fall Chinook Salmon stocks was very low ($F_{ST} = 0.005$), 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 SY2021 ranged from a low of 34.3 for the Tucannon R. broodstock to a high of 250.1 (for the Dworshak broodstock; Table 15). Previous research has indicated that when sample sizes are less than the true effective size, the accuracy and precision of N_E estimates decrease (Ackerman et al. 2017). Two stocks exhibited either wide or infinite confidence bounds (E.F. Salmon River, 39.1 – Infinite; Touchet R., 443.0 - Infinite). We suggest caution in interpreting N_E estimates for these two stocks.

Effective population size for spring-summer Chinook Salmon hatchery broodstock in SY2021 ranged from a low of 68.6 for Catherine Cr. to a high of 504.8 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 1,198.6 and the Lyons Ferry stock estimated at 1,244.4 (Table 17).

Sex Markers

The sex-specific assay for steelhead matched phenotypic sex in 99.9% of the samples (Table 7). In the instances (n = 6) 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 0.18% of the samples.

The sex-specific assay for spring-summer Chinook Salmon matched phenotypic sex in 99.8% of the samples (Table 8). In the instances (n = 17) 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 0.6% of the samples.

The sex-specific assay for fall Chinook Salmon matched phenotypic sex in 98.2% of the samples (Table 9). In the instances (n = 45) 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 2.1% 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). Stock-level tag rates met or exceeded 90% in all but three steelhead broodstocks. Stock-level tag rates were greater than 90% in all but five 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 SY2021.

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 were 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 release group level are generally over 90%.

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 0.30-0.34 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 0.23-0.26 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, 31 distinct haplotypes on chromosome 28 and 33 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 84 distinct haplotypes on chromosome 28 in spring-summer Chinook Salmon broodstocks (Table 23; Table 24) and 74 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 are likely lower than reported. 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 S.F. Clearwater stock) and other locations. The Dworshak, Upper Salmon B-run, and S.F. 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. Based on genetic theory, a population is at risk of inbreeding when N_E is <50 (Franklin 1980). The majority of steelhead and Chinook salmon broodstocks in the Snake River basin exceeded this threshold, but N_E for Tucannon R. steelhead broodstock was 34.3.

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 98.0% for steelhead, 96.5% for spring-summer Chinook Salmon, and 98.3% 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 (ICBTRT 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). 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 Baum et al. (2022) 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 10–25% and can be as high as 80%. 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 Ebel et al. (2022) 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 Baum et al. (2022) for adults and Ebel et al. (2022) 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. 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 MY2022 steelhead and Chinook Salmon juveniles and SY2022 adult steelhead and Chinook Salmon were processed at either IDFG's Eagle Fish Genetics Laboratory (EFGL) in Eagle, Idaho or the Columbia River Inter-Tribal Fish Commission Laboratory 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 MY2022 and adult fish sampled in SY2022 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 the R package rubias (Moran and Anderson 2019). 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). Both juvenile and adult steelhead were compared against the Snake River SNP baseline v3.1 (Powell et al. 2018; Hargrove et al. 2021b). 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:

- SY2022 steelhead adults

- MY2022 steelhead juveniles
- SY2022 Chinook Salmon adults
- MY2022 Chinook Salmon juveniles

RESULTS

We inventoried 9,015 samples (including both steelhead and Chinook Salmon) from SY2022 adults and MY2022 juveniles at LGR (Table 26). A total of 66 (0.7%) samples were not genotyped successfully (<90% complete). We assigned 1,942 (21.7%) samples to hatchery parents in our PBT baseline despite all samples being collected from fish with intact adipose fins. For the remaining 7,007 samples, we performed IA, which are summarized below and in Tables 27–30.

SY2022 Steelhead Adults

Of the 2,326 unclipped adult steelhead sampled in SY2022, 2,097 (90.2%) were phenotypically identified as natural-origin because they had no physical mark (e.g., ventral fin clip) or tag (e.g., CWT; Table 26). The remaining 229 (9.8%) fish were identified as hatchery origin via physical marks or tags. A total of 2,318 (99.7%) of the queued samples were successfully genotyped. We assigned 161 (7.7%) phenotypically natural-origin fish to hatchery parents. Of the 228 fish that were successfully genotyped and phenotypically identified as hatchery origin fish, 218 individuals (95.6%) were assigned via PBT to hatchery parents. All remaining successfully genotyped, phenotypically natural-origin (1,929) 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 1,929 unclipped, phenotypically wild adult steelhead sampled in SY2022 and assigned a genetic stock is summarized in Table 27. Steelhead were assigned to genetic stock in the following numbers, 452 fish (23.4%) assigned to GRROND, 358 (18.6%) to LSNAKE, 284 (14.7%) to UPSALM, 211 (10.9%) to UPCLWR, 202 (10.5%) to MFSALM, 181 (9.4%) to IMNAHA, 94 (4.9%) to SFCLWR, 64 (3.3%) to LOCLWR, 61 (3.2%) to SFSALM, and 22 (1.1%) to LOSALM.

MY2022 Steelhead Juveniles

A total of 1,241 samples were queued for genotyping of which 1,223 (98.5%) were successfully amplified. Twenty juveniles (1.6%) were assigned to hatchery parents. The remaining 1,203 (98.4%) were assigned a genetic stock via IA.

Life history diversity information for the 1,203 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, 379 fish (31.5%) assigned to GRROND, 192 (16.0%) to LSNAKE, 182 (15.1%) to UPSALM, 107 (8.9%) to SFCLWR, 100 (8.3%) to IMNAHA, 75 (6.2%) to UPCLWR, 60 (5.0%) to MFSALM, 50 (4.2%) to LOCLWR, 42 (3.5%) to SFSALM, and 16 (1.3%) to LOSALM.

SY2021 Chinook Salmon Adults

Of the 3,851 unclipped adult Chinook Salmon sampled in SY2022, all but 9 (0.2%) samples were genotyped successfully. We assigned 1,018 (26.5%) Chinook Salmon to hatchery parents. The remaining 2,824 (73.5%) Chinook Salmon were assigned to a genetic stock via IA.

Life history diversity information (sex, length, and ocean age) for the 2,824 adult Chinook Salmon sampled in SY2022 and assigned a genetic stock is summarized in Table 29. The largest number of Chinook Salmon were assigned to HELLSC 1,106 (39.2%), followed by 754 (26.7%) to SFSALM, 392 (13.9%) to UPSALM, 384 (13.6%) to MFSALM, 112 (4.0%) to FALL, and 76 (2.7%) to CHMBLN. No assignments were made to the TUCANO reporting unit.

MY2021 Chinook Salmon Juveniles

We queued 1,597 unclipped juvenile Chinook Salmon from MY2022 for genotyping, and 31 (1.9%) of samples failed to genotype (Table 26). Of the juveniles that were successfully genotyped, 525 (33.5%) were assigned back to hatchery parents and the remaining 1,041 (66.5%) were assigned a genetic stock via IA.

Life history diversity information for the 1,041 Chinook Salmon smolts assigned a genetic stock is summarized in Table 30. Of the smolts assigned a genetic stock, 538 fish (51.7%) assigned to HELLSC, 224 (21.5%) to SFSALM, 154 (14.8%) to MFSALM, 86 (8.3%) to UPSALM, 21 (2.0%) to FALL, 18 (1.7%) 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; Lawry et al. 2020; Baum et al. 2022; Ebel et al. 2022).

Trapped adult fish are also PIT tagged by the Integrated Status and Effectiveness Monitoring Project (ISEMP; BPA Project 2003-017-00). Detections 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). 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. 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 2021 (SY2022), 2) Origin of samples from tribal fisheries in Columbia River Zone 6 during migration year 2021 (SY2022), 3) Origin of samples from various sport fisheries in Idaho in migration year 2021 (SY2022), 4) Age composition and origin of the SY2021 broodstocks, and 5) stock composition of returning adults during SY2022 at Lower Granite Dam.

For Chinook Salmon, the PBT baselines were used to determine: 1) Origin of samples from various sport fisheries in Idaho (SY2021), 2) Age composition and origin of SY2021 broodstocks, 3) stock composition of returning adults during SY2021 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 2021 (SY2022). A total of 128 samples were processed for PBT assignment. An example of the methods used for this annual sampling and PBT assignment results can be found in Byrne et al. (*In prep*).

Steelhead Tribal Fisheries in Zone 6 of Columbia River

IDFG coordinated sampling of steelhead harvested in the tribal fishery between Bonneville Dam and McNary Dam (Zone 6) during collection year (CY) 2021 (i.e., spawn year 2022). A total of 462 steelhead samples were analyzed. Description of the methods used for this annual sampling can be found in Byrne et al. (2015) and Byrne et al. (*In prep*).

Steelhead Sport Fisheries in Idaho

IDFG collected samples of steelhead harvested in the SY2022 sport fishery from various river systems including the Clearwater and Salmon. A total of 2,428 samples were processed for PBT assignment. A more detailed description of this project is in McBaine et al. (*In prep*). Results from a previous year are available in Warren et al. (2018).

Age Composition of SY2021 Steelhead Broodstock

PBT was used to determine age composition of steelhead broodstocks in Idaho by assigning the SY2021 broodstocks back to all previously sampled broodstocks, thereby identifying the age of each fish. A total of 4,682 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 2021 and spring of 2022 (McBaine et al. *In prep*) and 2,309 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 SY2021. A total of 1,254 samples representative of the various time strata and river sections were analyzed with PBT. Complete methodology and results are presented in Noyes et al. (*In prep*). Results for a previous year are available in Belnap et al. (2022).

Age Composition of SY2021 Chinook Salmon Broodstock

PBT was used to determine age composition of Chinook Salmon broodstocks in Idaho by assigning the SY2021 broodstocks back to previously sampled broodstocks, thereby identifying the age of each fish. A total of 10,938 hatchery-origin broodstock samples were analyzed with PBT.

Stock Composition of Adult Chinook Salmon at Lower Granite Dam

Representative samples of the adult Chinook run across Lower Granite Dam were collected in 2021 (Noyes et al. *In prep*) and 3,709 samples were analyzed with PBT. Results for a previous year are available in Belnap et al. (2022).

RESULTS

Steelhead Sport Fisheries in Columbia River

Of the 128 samples analyzed, 57 (45%) assigned to the PBT baseline. A detailed breakdown of stock composition in these fisheries is presented in Byrne et al. (*In prep*).

Steelhead Tribal Fisheries in Zone 6 of Columbia River

Of the 452 samples analyzed from clipped and unclipped steelhead, 318 (70%) assigned to the PBT baseline. A detailed breakdown of stock composition in this fishery is presented in Byrne et al. (*In prep*).

Steelhead Sport Fisheries in Idaho

Of the 2,386 samples analyzed after removing duplicates and genotyping failures, 2,206 (92%) assigned to the baseline. A detailed breakdown of stock composition in this fishery is presented in McBaine et al. (*In prep*).

Age Composition of SY2021 Steelhead Broodstock

Of the samples collected, 4,441 were analyzed with PBT after excluding duplicate and un-genotyped samples. Of these, 4,060 (91%) 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, 2,303 were analyzed after removing duplicated samples or samples that failed to genotype. Of these, 2,156 (94%) assigned to the baseline. A summary of stock composition and age will be provided in an upcoming IDFG technical report (McBaine et al. *In prep*).

Chinook Salmon Sport Fishery in Idaho

Of the samples collected, 1,225 were analyzed after removal of samples that failed to genotype. Of these, 1,047 (85%) received a PBT assignment. A detailed breakdown of stock and age composition of the harvest in this fishery is presented in Noyes et al. (*In prep*).

Age Composition of SY2021 Chinook Salmon Broodstock

Of the samples collected, 9,921 were analyzed after removing samples that failed to genotype and duplicate samples. Of these, 9,489 assigned (96%) 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

Of the samples collected, 3,680 were analyzed after removing duplicated samples and those that failed to genotype. Of these, 3,312 (90%) assigned to the PBT baseline. A summary of stock composition and age will be provided in an upcoming IDFG technical report (Noyes et al. *In prep*).

DISCUSSION

The PBT baselines being developed and maintained are made available to fisheries managers to help address a variety of management questions for steelhead and Chinook Salmon. While specific implications and interpretations are presented in separate reports, the number and

diversity of projects that made use of the PBT baselines is noteworthy, especially since many of these projects would not have been possible without access to this technology.

Steelhead Sport Fisheries in Columbia River

This project represents some of the first comprehensive attempts to categorize the stock composition of the steelhead harvest in the Lower Columbia sport fishery. Results from this year's sampling (Byrne et al. *In prep*), as well as results from previous years (Byrne et al. 2015), will aid in monitoring needs for the *U.S. v Oregon* Management Agreement and in the management of ESA-listed B-run steelhead that return to the Dworshak Fish Hatchery.

Steelhead Tribal Fisheries in Zone 6 of Columbia River

This project also represents some of the first comprehensive attempts to categorize the stock composition of the steelhead harvest in the Zone 6 fishery. Implications of the results are more thoroughly explored in Byrne et al. (*In prep*).

Steelhead Sport Fisheries in Idaho

This project represents some of IDFG's first evaluations of stock composition of in-state fisheries using PBT. A complete evaluation can be found in McBaine 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 Noyes 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

McCarrick and others are presenting this information for SY2021 in a companion report titled *Integrated broodstock evaluation*.

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 fish 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 has 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.

METHODS

RADseq Data and Locus Selection

The Stacks v2.2 bioinformatic pipeline (Rochette et al. 2019) was used to identify SNPs and call genotypes from RADseq data from Hecht et al. (2015) informed by the most up-to-date *Oncorhynchus tshawytscha* reference genome (Otsh_v2.0; GenBank accession GCA_018296145.1). Read based phasing of the SNP genotypes was performed by WhatsHap (Patterson et al. 2015). Expected heterozygosity was calculated for microhaplotypes in this data set of 50bp in length (or less). A total of 500 of these loci were chosen for further development by applying the greedy algorithm described by Matukumalli et al. (2009) with the modification of using expected heterozygosity in the calculation instead of minor allele frequency. The greedy algorithm was applied to each chromosome independently and the goal was to identify loci distributed between chromosomes according to chromosome length in the reference genome.

Amplicon Sequencing Locus Selection

Since the primary purpose of this new genetic marker panel is to infer pedigree relationships, we wanted to maximize the genetic diversity (expected heterozygosity) of loci in the panel. Within each locus identified by Stacks, expected heterozygosity was calculated with a sliding window of 60 bp and the region with the highest expected heterozygosity was recorded as a candidate microhaplotype locus. A size of 60 bp was chosen because, in the most extreme scenario of SNPs being located at opposite ends, it allows sequencing of a 15 bp forward primer and the target region with a 75 bp read. We then eliminated any candidate loci that had more than six SNPs, loci with immediately adjacent SNPs, allelic richness greater than 1 + number of SNPs, or were significantly out of Hardy-Weinberg equilibrium ($p < 0.05$) as assessed by a permutation test (Graffelman and Weir 2018). The limit of six SNPs within a 60 bp region was a subjective choice intended to partially filter out errors from aligning paralogous regions to the same position. The maximum allelic richness given the number of SNPs represents the maximum theoretical number of alleles if each position can only be mutated once and there was historically no recombination within the locus. To select a candidate pool of loci, the locus with the highest expected heterozygosity that was at least 5 Mb away from all previously selected loci was chosen until no possible loci remained. The process was then repeated with a minimum distance of 2 Mb until ~700 total loci were chosen. Primers were attempted to be designed for all candidates using BatchPrimer3 (You et al. 2008) and default parameters except for a product size of 60-150 with optimum of 80, primer lengths of 10-25 with optimum of 20, and Tm of 55-60 with optimum of 57. Primers were then mapped against the reference Chinook Salmon genome with bowtie2. Any primer pair that aligned more than once within 1 kb of each other and with either primer having an edit distance of 1 or less was removed to filter out primers targeting duplicated regions. We successfully designed primers for 500 candidate loci.

DISCUSSION

During the performance period, we identified 500 candidate loci for Snake River Chinook Salmon from which to test and designed primers for loci. During the next performance period we will test and optimize a new GTseq panel for Grandparentage testing and genotype ~15,000–

20,000 Chinook Salmon samples for empirical testing of error rates. Once the panel is optimized and finalized, primer sequences for the panel will be available via the FishGen webpage (https://www.fishgen.net/Home.aspx). In the interim, primer sequences are available from the authors upon request.

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LITERATURE CITED

Ackerman, M. W., C. Habicht, and L. W. Seeb. 2011. Single-Nucleotide Polymorphisms (SNPs) under Diversifying Selection Provide Increased Accuracy and Precision in Mixed-Stock Analyses of Sockeye Salmon from the Copper River, Alaska. Transactions of the American Fisheries Society 140:865-881.

Ackerman, M. W., J. McCane, C. A. Steele, M. R. Campbell, A. P. Matala, J. E. Hess, and S. R. Narum. 2012. Chinook and Steelhead Genotyping for Genetic Stock Identification at Lower Granite Dam. Idaho Department of Fish and Game Report 12-15. Annual Report, BPA Project 2010-026-00.

Ackerman, M. W., B. K. Hand, R. K. Waples, G. Luikart, R. S. Waples, C. A. Steele, B. A. Garner, J. McCane, and M. R. Campbell. 2017. Effective number of breeders from sibship reconstruction: empirical evaluations using hatchery steelhead. Evolutionary Applications 10(2):146-160.

Anderson, E. C. 2010. Computational algorithms and user-friendly software for parentage-based tagging of Pacific salmonids. Final report submitted to the Pacific Salmon Commission's Chinook Technical Committee (US Section).

Anderson, E. C., and J. C. Garza. 2005. A description of full genotyping. Report submitted to the Pacific Salmon Commission, Vancouver, British Columbia. 11p. http://swfsc.noaa.gov/publications/FED/00675.pdf.

Anderson, E. C., and J. C. Garza. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics 172:2567–2582.

Anderson, E. C., R. S. Waples, and S. T. Kalinowski. 2008. An improved method for predicting the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 65(7):1475-1486.

Araki, H., W. R. Ardren, E. Olsen, B. Cooper, and M. S. Blouin. 2007. Reproductive success of captive-bred steelhead trout in the wild: evaluation of three hatchery programs in the Hood River. Conservation Biology 21:181–190.

Baetscher, D. S., A. J. Clemento, T. C. Ng, E. C. Anderson, and J. C. Garza. 2018. Microhaplotypes provide increased power from short-read DNA sequences for relationship inference. Molecular Ecology Resources. 18(2):296-305.

Baum, C. M., K. M. Lawry, C. L. Sullivan, J. S. Hargrove, T. Delomas, M. Davison, M. E. Dobos, N. R. Smith, W. C. Schrader, T. Copeland, and M. R. Campbell. 2022. Wild adult steelhead and Chinook Salmon abundance and composition at Lower Granite Dam, spawn year 2020. Annual Report 2020. Idaho Department of Fish and Game Report 22-11.

Beacham, T. D., S. Pollard, and K. D. Le. 2000. Microsatellite DNA population structure and stock identification of steelhead trout (*Oncorhynchus mykiss*) in the Nass and Skeena Rivers in northern British Columbia. Marine Biotechnology 2(6):587-600.

Beacham, T. D., J. R. Candy, K. J. Supernault, T. Ming, B. Deagle, A. Schulze, D. Tuck, K. H. Kaukinen, J. R. Irvine, K. M. Miller, and R. E. Withler. 2001. Evaluation and application of

microsatellite and major histocompatibility complex variation for stock identification of Coho Salmon in British Columbia. Transactions of the American Fisheries Society 130(6):1116-1149.

Beacham, T. D., C. Wallace, K. Jonsen, B. McIntosh, J. R. Candy, D. Willis, C. Lynch, J.-S. S. Moore, L. Bernatchez, and R. E. Withler. 2019. Comparison of coded-wire tagging with parentage-based tagging and genetic stock identification in a large-scale Coho Salmon fisheries application in British Columbia, Canada. Evolutionary Applications 12:230–254.

Belnap, M., F. Bohlen, C. Dondero, and R. Brown. 2022. IPC and LSRCP Monitoring and Evaluation Programs in the State of Idaho: Calendar Year 2019 and Brood Year 2013 Hatchery Chinook Salmon Reports. Idaho Department of Fish and Game, Report 22-09, Boise.

Bingham, D. M., P. C. Gerrity, and S. Painter. 2018. Genetic tagging is an effective way to monitor survival of released hatchery saugers: conservation efforts in the Wind River, Wyoming. Environmental Practice 20:92–103.

Busby, P. J., T. C. Wainwright, G. J. Bryant, L. J. Lierheimer, R. S. Waples, F. W. Waknitz, and I. L. Lagomarsino. 1996. Status review of (West Coast) steelhead from Washington, Idaho, Oregon, and California. National Marine Fisheries Technical Memorandum NMFS-NWFSC-27. Seattle.

Byrne, A., J. Hymer, S. Ellis, R. Dick II, K. Keller, T. A. Delomas, J. E. Hess, M. Begay, and T. Miller. In prep. A Genetic Analysis of the Summer Steelhead Stock Composition in the Columbia River and Snake River Tribal and Sport Fisheries from June 2021 to March 31, 2022.

Byrne, A., J. Hymer, S. Ellis, R. Dick, K. Keller, C. A. Steele, M. E. Hess, M. Begay, and T. Miller. 2015. A Genetic Analysis of the Summer Steelhead Stock Composition in the Columbia River and Snake River Tribal and Sport Fisheries. Idaho Department of Fish and Game, Report 15-06, Boise.

Camacho, C. A., K. K. Wright, J. Powell, W. C. Schrader, T. Copeland, M. W. Ackerman, M. Dobos, M. P. Corsi, and M. R. Campbell. 2017. Wild adult steelhead and Chinook Salmon abundance and composition at Lower Granite Dam, spawn years 2009-2016. Cumulative Report 2009 through 2016. Idaho Department of Fish and Game Report 17-06.

Camacho, C. A., J. Powell, M. Davison, M. E. Dobos, W. C. Schrader, T. Copeland, and M. R. Campbell. 2018a. Wild adult steelhead and Chinook Salmon abundance and composition at Lower Granite Dam, spawn year 2017. Annual Report. Idaho Department of Fish and Game Report 18-06.

Camacho, C. A., K. K. Wright, J. Powell, W. C. Schrader, T. Copeland, M. W. Ackerman, M. E. Dobos, M. Davison, M. P. Corsi, and M. R. Campbell. 2018b. Wild juvenile steelhead and Chinook Salmon abundance and composition at Lower Granite Dam, migratory years 2010 and 2017. Cumulative Report 2010 through 2017. Idaho Department of Fish and Game Report 18-05.

Campbell, M. R., C. C. Kozfkay, T. Copeland, W. C. Schrader, M. W. Ackerman, and S. R. Narum. 2012. Estimating abundance and life history characteristics of threatened wild Snake River

steelhead using genetic stock identification. Transactions of the American Fisheries Society. 141(5):1310-1327.

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 15:855-867.

Campbell, M. R., N. V. Vu, A. P. LaGrange, R. S. Hardy, T. J. Ross, and S. R. Narum. 2019. Development and application of single nucleotide polymorphism (SNP) genetic markers for conservation monitoring of Burbot populations. Transactions of the American Fisheries Society 148:661–670.

Christie, M. R., M. J. Ford, and M. S. Blouin. 2014. On the reproductive success of early-generation hatchery fish in the wild. Evolutionary Applications 7:883–896.

Collins, E. E., J. S. Hargrove, T. A. Delomas, and S. R. Narum. 2020. Distribution of genetic variation underlying adult migration timing in steelhead of the Columbia River basin. Ecology and Evolution 10(17):9486–9502.

DeHaan, P. W., G. R. Jordan, and W. R. Ardren. 2008. Use of genetic tags to identify captive-bred Pallid Sturgeon (*Scaphirhynchus albus*) in the wild: improving abundance estimates for an endangered species. Conservation Genetics 9:691–697.

Delomas, T. A., J. McCane, J. Hargrove, and M. Campbell. 2021. Parentage-based tagging of Snake River hatchery steelhead and Chinook Salmon. Annual Report. Idaho Department of Fish and Game Report 21-04.

Denson, M. R., K. Brenkert, W. E. Jenkins, and T. L. Darden. 2012. Assessing Red Drum juvenile stocking in a South Carolina estuary using genetic identification. North American Journal of Fisheries Management 32:32–43.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14(1):209–214.

Ebel, J. D., T. Copeland, J. S. Hargrove, M. Davison, M. E. Dobos, W. C. Schrader, and M. R. Campbell. 2022. Wild juvenile steelhead and Chinook Salmon abundance and composition at Lower Granite Dam, migratory year 2019. Annual Report 2019. Idaho Department of Fish and Game Report 22-08.

Evans, H. K., K. B. Carlson, R. Wisser, M. E. Raley, K. M. Potoka, and K. J. Dockendorf. 2018. Genetics and hatchery management: a parentage-based tagging approach to Blueback Herring conservation. Journal of Fish and Wildlife Management 9:1–13.

Ford, M. J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology 16:815–825.

Fountain, E. D., J. N. Pauli, B. N. Reid, P. J. Palsbøll, and M. Z. Peery. 2016. Finding the right coverage: the impact of coverage and sequence quality on single nucleotide polymorphism genotyping error rates. Molecular Ecology Resources 16:966-978.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135–140 in: M.E. Soule and B.A. Wilcox (eds.), Conservation Biology: An Evolutionary Ecological Perspective. Sunderland, Mass. Sinauer Associates.

Good, T. P., R. S. Waples, and P. B. Adams. 2005. Updated status of federally listed ESUs of West Coast Salmon and steelhead. U.S. Department of Commerce, NOAA Technical Memorandum, NMFS-NWFSC-66. 598 pp.

Goodman, D. 2005. Selection equilibrium for hatchery and wild spawning fitness in integrated breeding programs. Canadian Journal of Fisheries and Aquatic Sciences 62:374–389.

Graffelman, J., and B. S. Weir. 2018. Multi-allelic exact tests for Hardy-Weinberg equilibrium that account for gender. Molecular Ecology Resources 18(3):461-473.

Habicht, C., L. W. Seeb, K. W. Myers, E. V. Farley, and J. E. Seeb. 2010. Summer-fall distribution of stocks of immature sockeye Salmon in the Bering Sea as revealed by single-nucleotide polymorphisms. Transactions of the American Fisheries Society 139(4):1171-1191.

Hankin, D. G., J. Fitzgibbons, and T. Chen. 2009. Unnatural random mating policies select for younger age at maturity in hatchery Chinook Salmon (*Oncorhynchus tshawytscha*) populations. Canadian Journal of Fisheries and Aquatic Sciences 66:1505–1521.

Hargrove, J. S., M. Davison, and M. R. Campbell. 2019. Natural-origin Chinook Salmon and steelhead life history and genetic diversity at PIT tag detection locations throughout the Snake River basin. Idaho Department of Fish and Game Report 19-13. Annual Report, BPA Project 2010-026-00.

Hargrove, J. S., C. A. Camacho, W. C. Schrader, J. H. Powell, T. A. Delomas, J. E. Hess, S. R. Narum, and M. R. Campbell. 2021a. Parentage-based tagging improves escapement estimates for ESA-listed adult Chinook Salmon and Steelhead in the Snake River basin. Canadian Journal of Fisheries and Aquatic Sciences. 78(4):349-360.

Hargrove, J. S., T. A. Delomas, J. McCane, M. Davison, M. R. Campbell, R. L. Horn, and S. R. Narum. 2021b. Chinook and Steelhead Genotyping for Genetic Stock Identification at Lower Granite Dam. Idaho Department of Fish and Game Report 21-03. Annual Report, BPA Project 2010-026-00.

HSRG (Hatchery Scientific Review Group). 2009. Columbia River hatchery reform system-wide report. Bonneville Power Administration, Portland, Oregon.

Hecht, B. C., A. P. Matala, J. E. Hess, and S. R. Narum. 2015. Environmental adaptation in Chinook salmon (*Oncorhynchus tshawytscha*) throughout their North American range. Molecular Ecology 24(22):5573–5595.

Hess, J. E., J. S. Zendt, A. R. Matala, and S. R. Narum. 2016. Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. Proceedings of the Royal Society B: Biological Sciences 283(1830):20153064.

ICBTRT (Interior Columbia Basin Technical Recovery Team). 2003. Independent Populations of Chinook, steelhead, and sockeye for listed Interior Columbia Basin ESUs. Interior Columbia Basin Technical Recovery Team Report. July 2003.

IDFG (Idaho Department of Fish and Game). 2018. Fisheries management plan 2019-2024. IDFG, Boise.

ISRP/ISAB (Independent Scientific Review Panel and Independent Scientific Advisory Board). 2009. Tagging Report. A comprehensive review of Columbia River Basin fish tagging technologies and programs. ISRP/ISAB 2009-1. Northwest Power and Conservation Council. Portland, Oregon.

Johnson, K. J. 2004. Regional overview of coded wire tagging of anadromous salmon and steelhead in Northwest America. Regional Mark Processing Center, Pacific States Marine Fisheries Commission. Portland, Oregon.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11(1):94.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1106.

Kamvar, Z. N., J. F. Tabima, and N. J. Grünwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

LaHood, E. S., J. J. Miller, C. Apland, and M. J. Ford. 2008. A Rapid, Ethanol-Free Fish Tissue Collection Method for Molecular Genetic Analyses. Transactions of the American Fisheries Society 137:1104-1107.

Lawry, K. M., C. A. Camacho, J. S. Hargrove, T. Delomas, M. Davison, M. E. Dobos, W. C. Schrader, T. Copeland, and M. R. Campbell. 2020. Wild adult steelhead and Chinook Salmon abundance and composition at Lower Granite Dam, spawn year 2019. Annual Report. Idaho Department of Fish and Game Report 20-12.

Lehnert, S. J., S. M. Baillie, J. MacMillan, I. G. Paterson, C. F. Buhariwalla, I. R. Bradbury, and P. Bentzen. 2020. Multiple decades of stocking has resulted in limited hatchery introgression in wild Brook Trout (*Salvelinus fontinalis*) populations of Nova Scotia. Evolutionary Applications 13:1069–1089.

Leitwein, M., M. Duranton, Q. Rougemont, P. A. Gagnaire, and L. Bernatchez. 2020. Using haplotype information for conservation genomics. Trends in Ecology & Evolution 35:245-58.

Matala, A. P., J. E. Hess, and S. R. Narum. 2011. Resolving adaptive and demographic divergence among Chinook Salmon populations in the Columbia River basin. Transactions of the American Fisheries Society 140:783–807.

Matala, A. P., M. W. Ackerman, M. R. Campbell, and S. R. Narum. 2014. Relative contributions of neutral and non-neutral genetic differentiation to inform conservation of steelhead trout across highly variable landscapes. Evolutionary Applications 7:682-701.

Matukumalli, L. K., C. T. Lawley, R. D. Schnabel, J. F. Taylor, M. F. Allan, M. P. Heaton, J. O'Connell, S. S. Moore, T. P. L. Smith, T. S. Sonstegard, and C. P. Van Tassell. 2009.

Development and characterization of a high density SNP genotyping assay for cattle. PLoS One, 4(4):e5350.

May, S. A., G. J. McKinney, R. Hilborn, L. Hauser, and K. A. Naish. 2020. Power of a dual-use SNP panel for pedigree reconstruction and population assignment. Ecology and Evolution 10:9522-31.

McBaine, K., F. Bohlen, and R. Brown. In prep. IPC and LSRCP Monitoring and Evaluation Programs in the State of Idaho: Calendar Year 2021 and Brood Year 2016 Hatchery Steelhead Reports.

McElhany, P., M. H. Ruckelshaus, M. J. Ford, T. C. Wainwright, and E. P. Bjorkstedt. 2000. Viable Salmonid populations and the recovery of evolutionary significant units. U.S. Department of Commerce, NOAA Technical Memo. NMFS-NWFSC-42, 156 p.

McKinney G. J., J. E. Seeb, and L. W. Seeb. 2017. Managing mixed-stock fisheries: genotyping multi-SNP haplotypes increases power for genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 74:429-34.

McKinney G. J., C. E. Pascal, W. D. Templin, S. E. Gilk-Baumer, T. H. Dann, L. W. Seeb, and J. E. Seeb. 2020. Dense SNP panels resolve closely related Chinook Salmon populations. Canadian Journal of Fisheries and Aquatic Sciences 77:451-61.

Micheletti, S. J., J. E. Hess, J. S. Zendt, and S. R. Narum. 2018. Selection at a genomic region of major effect is responsible for evolution of complex life histories in anadromous steelhead. BMC Evolutionary Biology 18(1):140.

Miller, L. M., T. Close, and A. R. Kapuscinski. 2004. Lower fitness of hatchery and hybrid Rainbow Trout compared to naturalized populations in Lake Superior tributaries. Molecular Ecology 13:3379–3388.

Mobrand, L. E., J. Barr, L. Blankenship, D. E. Campton, T. T. P. Evelyn, T. A. Flagg, C. V. W. Mahnken, L. W. Seeb, P. R. Seidel, and W. W. Smoker. 2005. Hatchery reform in Washington State. Fisheries 30(6):11–23.

Moran, P. 2003. Genetic structure of *Oncorhynchus mykiss* populations in the Grande Ronde River, Imnaha River, and adjacent regions of the Snake River basin. Final report submitted to the U.S. Fish and Wildlife Service, Lower Snake River Compensation Plan Office, Boise, Idaho, in partial fulfillment of Contract No. 14110-1-H070. 28p. + Appendices.

Moran, B. M., and E. C. Anderson. 2019. Bayesian inference from the conditional genetic stock identification model. Canadian Journal of Fisheries and Aquatic Sciences 76(4):551-560.

Narum, S. R., J. J. Stephenson, and M. R. Campbell. 2007. Genetic variation and structure of Chinook Salmon life history types in the Snake River. Transactions of the American Fisheries Society 136(5):1252-1262.

Narum, S. R., D. Hatch, A. J. Talbot, P. Moran, and M. S. Powell. 2008. Iteroparity in complex mating systems of steelhead *Oncorhynchus mykiss* (Walbaum). Journal of Fish Biology 72(1):45-60.

Narum, S. R., A. Di Genova, S. J. Micheletti, and A. Maass. 2018. Genomic variation underlying complex life-history traits revealed by genome sequencing in Chinook Salmon. Proceedings of the Royal Society B: Biological Sciences 285(1883):20180935.

NMFS (National Marine Fisheries Service). 2017. ESA recovery plan for Snake River spring-summer Chinook Salmon (*Oncorhynchus tshawytscha*) & Snake River basin steelhead (*Oncorhynchus mykiss*) [online]. Retrieved from https://www.fisheries.noaa.gov/resource/document/recovery-plan-snake-river-spring-summer-chinook-salmon-and-snake-river-basin

Nielsen, J. L., A. Byrne, S. L. Graziano, and C. C. Kozfkay. 2009. Steelhead Genetic Diversity at Multiple Spatial Scales in a Managed Basin: Snake River, Idaho. North American Journal of Fisheries Management 29(3):680-701.

Noyes, C., F. Bohlen, C. Dondero, and R. Brown. In prep. IPC and LSRCP Monitoring and Evaluation Programs in the State of Idaho: Calendar Year 2022 and Brood Year 2016 Hatchery Chinook Salmon Reports.

Oldoni, F., K. K. Kidd, and D. Podini. 2019. Microhaplotypes in forensic genetics. Forensic Science International: Genetics 38:54-69.

Patterson, M., T. Marschall, N. Pisanti, L. Van Iersel, L. Stougie, G. W. Klau, and A. Schönhuth. 2015. WhatsHap: weighted haplotype assembly for future-generation sequencing reads. Journal of Computational Biology 22(6):498-509.

Powell, J. H., N. Vu, J. McCane, M. W. Ackerman, M. R. Campbell, D. J. Hasselman, and S. R. Narum. 2018. Chinook and Steelhead Genotyping for Genetic Stock Identification at Lower Granite Dam. Idaho Department of Fish and Game Report 18-03. Annual Report, BPA Project 2010-026-00.

QCI (Quantitative Consultants, Inc.). 2013. Integrated status and effectiveness monitoring project: Salmon subbasin cumulative analysis report. Quantitative Consultants, Inc. Annual report 2012, BPA Project 2003-017-00.

Rochette, N. C., A. G. Rivera-Colón, and J. M. Catchen. 2019. Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology 28(21):4737–4754.

Rousset, F. 2008. GENEPOP'007: a complete reimplementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103–106.

See, K., R. Orme, R. Kinzer, and M. W. Ackerman. 2016. PIT tag based escapement estimates to Snake Basin population. Prepared by ISEMP for the Bonneville Power Administration. Published by QCI. Available: https://www.researchgate.net/publication/303289911_PIT_Tag_Based_Escapement_Estimates_to_Snake_Basin_Populations. (December 2016).

Shaklee, J. B., T. D. Beacham, L. Seeb, and B. A. White. 1999. Managing fisheries using genetic data: case studies from four species of Pacific Salmon. Fisheries Research 43:45-78.

Sinnwell, J. P., and D. J. Schaid. 2021. haplo.stats: Statistical Analysis of Haplotypes with Traits and Covariates when Linkage Phase is Ambiguous. Mayo Clinic, Rochester, Minnesota.

Smith, C. T., W. D. Templin, J. E. Seeb, and L. W. Seeb. 2005. Single nucleotide polymorphisms provide rapid and accurate estimates of the proportions of US and Canadian Chinook Salmon caught in Yukon River fisheries. North American Journal of Fisheries Management 25(3):944-953.

Smith, C. T. 2007. Feasibility of genetic stock ID of Chinook Salmon sampled at Lower Granite Dam. U. S. Fish and Wildlife Service, Abernathy Fish Technology Center Report.

Stark, E. J., C. Bretz, A. Byrne, P. Cleary, T. Copeland, L. Denny, R. Engle, T. Miller, S. Rosenberg, E. R. Sedell, G. E. Shippentower, and C. Warren. 2016. Snake River Basin steelhead 2013/2014 Run Reconstruction. Report to Bonneville Power Administration, Portland, Oregon.

Steele, C. A., E. C. Anderson, M. W. Ackerman, M. A. Hess, N. R. Campbell, S. R. Narum, and M. R. Campbell. 2013a. A validation of parentage-based tagging using hatchery steelhead in the Snake River basin. Canadian Journal of Fisheries and Aquatic Sciences, 70:1046-1054.

Steele, C., J. McCane, M. Ackerman, M. Campbell, M. Hess, and S. Narum. 2013b. Parentage based tagging of Snake River hatchery steelhead and Chinook Salmon. Idaho Department of Fish and Game Report 13-23. Annual report 2013. BPA Project 2010-031-00.

Steele, C. A., M. Hess, S. Narum, and M. Campbell. 2019. Parentage-based tagging: Reviewing the implementation of a new tool for an old problem. Fisheries, 44:412-422.

Steele, C. A., M. Ackerman, J. McCane, M. Hess, N. Campbell, S. R. Narum, M. R. Campbell. 2011. Parentage Based Tagging Snake River hatchery steelhead and Chinook Salmon. Annual Report. BPA (Project No. 2010-031-00 Contract Number 48348). United States Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, P.O. Box 3621, Portland, Oregon 97208.

Tattam, I. A., and J. R. Ruzycki. 2020. Smolt transportation influences straying of wild and hatchery Snake River steelhead into the John Day River. Transactions of the American Fisheries Society 149:284–297.

Thurow, R. F. 1985. Middle Fork Salmon River Fisheries Investigations. Job Completion Report, Project F-73-R-6.

Thurow, R. F. 2000. Dynamics of Chinook Salmon populations within Idaho's Frank Church Wilderness-implications for persistence *in* McCool, S.F., and others, v3. Wilderness as a place for scientific inquiry: Proceedings of the wilderness science in a time of change conference, Missoula, Montana, May 23-27, 1999, U.S. Forest Service RMRS-P-15-VOL-3, p. 143-151.

Tiffan, K. F., D. W. Rondorf, R. D. Garland, and P. A. Verhey. 2000. Identification of fall versus spring Chinook Salmon migrating through the lower Snake River based on body morphology. Transactions of the American Fisheries Society 129:1389-1395.

Vähä, J. P., J. Erkinaro, M. Falkegård, P. Orell, and E. Niemelä. 2016. Genetic stock identification of Atlantic salmon and its evaluation in a large population complex. Canadian Journal of Fisheries and Aquatic Sciences 74:327-38.

Vandeputte, M., A. Bestin, L. Fauchet, J. M. Allamellou, S. Bosc, O. Menchi, P. Haffray, and C. Saavedra. 2021. Can we identify wild-born salmon from parentage assignment data? A case study in the Garonne-Dordogne rivers salmon restoration programme in France. Aquatic Living Resources 34: article 7.

van Doornik, D. M., D. L. Eddy, R. S. Waples, S. J. Boe, T. L. Hoffnagle, E. A. Berntson, and P. Moran. 2013. Genetic monitoring of threatened Chinook Salmon populations: estimating introgression of nonnative hatchery stocks and temporal genetic changes. North American Journal of Fisheries Management 33:693–706.

Vu, N., M. W. Ackerman, K. K. Wright, J. McCane, M. R. Campbell, J. E. Hess, and S. R. Narum. 2015. Chinook and steelhead genotyping for genetic stock identification at Lower Granite Dam. Idaho Department of Fish and Game Report 15-02. Annual Report, BPA Project 2010-026-00.

Waples, R. S., M. J. Ford, and D. Schmitt. 2007. Empirical results of salmon supplementation in the northeast Pacific: a preliminary assessment. Pages 383–403 in T. M. Bert, editor. Ecological and genetic implications of aquaculture activities. Springer, Dordrecht, The Netherlands.

Warren, C., S. Rosenberger, F. Bohlen. 2018. 2016 Calendar Year Hatchery Steelhead Report: IPC and LSRCP Monitoring and Evaluation Programs in the State of Idaho. Idaho Department of Fish and Game, Report 18-21, Boise.

Willis, S. C., J. E. Hess, J. K. Fryer, J. M. Whiteaker, C. Brun, R. Gerstenberger, and S. R. Narum. 2020. Steelhead (*Oncorhynchus mykiss*) lineages and sexes show variable patterns of association of adult migration timing and age-at-maturity traits with two genomic regions. Evolutionary Applications 13(10):2836–2856.

Willis, S. C., J. E. Hess, J. K. Fryer, J. M. Whiteaker, and S. R. Narum. 2021. Genomic region associated with run timing has similar haplotypes and phenotypic effects across three lineages of Chinook Salmon. Evolutionary Applications 14(9):2273–2285.

Wright, S. 1931. Evolution in Mendelian Populations. Genetics. 16(2):97-159.

Wright, K., W. Schrader, L. Reinhardt, K. Hernandez, C. Hohman, and T. Copeland. 2015. Process and methods for assigning ages to anadromous salmonids from scale samples. Idaho Department of Fish and Game Report 15-03.

You, F. M., N. Huo, Y. Q. Gu, M. Luo, Y. Ma, D. Hane, G. R. Lazo, J. Dvorak, and O. D. Anderson. 2008. BatchPrimer3: A high throughput web application for PCR and sequencing primer design. BMC Bioinformatics 9(1):253.

TABLES

Table 1. Sample sizes and genotyping completion rate of SY2021 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.	138	137 (99.3)	98.6
Dworshak	928	923 (99.5)	98.9
E.F. Salmon R.	27	14 (51.9)	26.9
Little Sheep Cr.	112	112 (100.0)	100.0
Oxbow	386	383 (99.2)	98.5
Pahsimeroi	806	799 (99.1)	98.3
S.F. Clearwater	366	366 (100.0)	100.0
Sawtooth	885	885 (100.0)	100.0
Tucannon R.	44	35 (79.5)	63.3
Touchet R.	27	23 (85.2)	72.6
Up. Sal. R. B-run	357	354 (99.2)	98.3
Wallowa	411	410 (99.8)	99.5
Total	4487	4441 (99.0)	98.0

Table 2. Sample sizes and genotyping completion rate of SY2021 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.	111	109 (98.2)	96.4
Dworshak	1631	1624 (99.6)	99.1
Grande Ronde	142	140 (98.6)	97.2
Imnaha	249	219 (88.0)	77.4
Johnson Cr.	75	72 (96.0)	92.2
Kooskia	557	554 (99.5)	98.9
Lookingglass Cr.	168	157 (93.5)	87.3
Lostine	134	125 (93.3)	87.0
Nez Perce FH	284	283 (99.6)	99.3
Pahsimeroi	151	150 (99.3)	98.7
Powell	125	110 (88.0)	77.4
Rapid River	2553	2515 (98.5)	97.0
S.F. Clearwater	1980	1966 (99.3)	98.6
S.F. Salmon	803	795 (99.0)	98.0
Sawtooth	974	972 (99.8)	99.6
Tucannon	93	62 (66.7)	44.4
Total	10030	9853 (98.2)	96.5

Table 3. Sample sizes and genotyping completion rate of SY2021 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	1838	1826 (99.3)	98.7
Nez Perce	695	685 (98.6)	97.1
Total	2533	2511 (99.1)	98.3

Table 4. List of loci that failed to genotype in >5% of the steelhead SY2021 samples.

Locus	Fail rate (%)
Omy_RAD3379824	5.1
Omy_RAD3840619	5.1
OMS00154	5.3
OMGH1PROM1SNP1	5.5
Omy_RAD7606020	5.6
Omy_hsp4786	5.8
Omy_RAD1307316	6.0
Omy_GH1P1_2	6.5
Omy_BAMBI4238	8.5
Omy_114315438	8.6
Omy_RAD5281228	8.6
Omy_RAD1784916	9.0
Omy_104569114	9.9
Omy_cd59b112	10.8
Omy_RAD7814727	13.4
Omy_RAD1957859	13.9
Omy_RAD900413	14.6
Omy_RAD5599710	14.7
Omy_GREB1_05	15.3
Omy_RAD4667227	15.7
OMY1011SNP	16.5
Omy_RAD6595969	17.0
Omy_RAD6640236	20.8
Omy_arp630	21.8
Omy_RAD9871553	22.7
OMS00039	23.8
Omy_99300202	24.1
Omy25_61286316	25.6
OMS00180	36.4
Omy_RAD7850257	37.3
Ocl_gshpx357	39.5

Table 5. List of loci that failed to genotype in >5% of the spring-summer Chinook Salmon SY2021 samples.

Locus	Fail rate (%)
Ots_CHI06105101_16717	12.3
Ots_CHI06027687_143477	17.1
Ots_105401325	18.0

Table 6. List of loci that failed to genotype >5% of the fall Chinook Salmon SY2021 samples.

Locus	Fail rate (%)
Ots_110689218	6.1
Ots_crRAD7651228	6.1
Ots_crRAD5547526	7.6
Ots_GPDH338	7.6
Ots_u0725325	9.2
Ots_sept978	10.5
Ots_crRAD3607229	13.8
Ots_u202161	14.6
Ots_pigh105	16.1
Ots_u0707161	30.5
Ots_MetA	42.3

Table 7. Results of comparisons between phenotypic sex and genetically determined sex using the sex-specific assay for SY2021 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.	137	0 (0.0%)	137 (100.0%)	137 (100.0%)	0 (0.0%)	0	0	42	95
Dworshak	923	1 (0.1%)	922 (99.9%)	919 (99.7%)	3 (0.3%)	2	1	426	497
E.F. Salmon R.	14	0 (0.0%)	14 (100.0%)	14 (100.0%)	0 (0.0%)	0	0	7	7
Little Sheep Cr.	112	4 (3.6%)	108 (96.4%)	107 (99.1%)	1 (0.9%)	1	0	57	55
Oxbow	383	0 (0.0%)	383 (100.0%)	382 (99.7%)	1 (0.3%)	1	0	189	194
Pahsimeroi	799	2 (0.3%)	797 (99.7%)	797 (100.0%)	0 (0.0%)	0	0	396	403
S.F. Clearwater	366	1 (0.3%)	365 (99.7%)	365 (100.0%)	0 (0.0%)	0	0	115	251
Sawtooth	885	0 (0.0%)	885 (100.0%)	885 (100.0%)	0 (0.0%)	0	0	444	441
Tucannon R.	35	0 (0.0%)	35 (100.0%)	35 (100.0%)	0 (0.0%)	0	0	5	30
Touchet R.	23	0 (0.0%)	23 (100.0%)	23 (100.0%)	0 (0.0%)	0	0	7	16
Up. Sal. R. B-run	354	0 (0.0%)	354 (100.0%)	354 (100.0%)	0 (0.0%)	0	0	156	198
Wallowa	410	0 (0.0%)	410 (100.0%)	409 (99.8%)	1 (0.2%)	0	1	201	209

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 SY2021 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.	109	1 (0.9%)	108 (99.1%)	108 (100.0%)	0 (0.0%)	0	0	53	56
Dworshak	1624	6 (0.4%)	1618 (99.6%)	1616 (99.9%)	2 (0.1%)	0	2	725	899
Grande Ronde	140	0 (0.0%)	140 (100.0%)	140 (100.0%)	0 (0.0%)	0	0	57	83
Imnaha	219	0 (0.0%)	219 (100.0%)	218 (99.5%)	1 (0.5%)	1	0	99	120
Johnson Cr.	72	1 (1.4%)	71 (98.6%)	70 (98.6%)	1 (1.4%)	0	1	33	39
Kooskia	554	20 (3.6%)	534 (96.4%)	531 (99.4%)	3 (0.6%)	0	3	261	293
Lookingglass Cr.	157	3 (1.9%)	154 (98.1%)	152 (98.7%)	2 (1.3%)	2	0	77	80
Lostine	125	0 (0.0%)	125 (100.0%)	125 (100.0%)	0 (0.0%)	0	0	52	73
Nez Perce FH	283	1 (0.4%)	282 (99.6%)	282 (100.0%)	0 (0.0%)	0	0	131	152
Pahsimeroi	150	0 (0.0%)	150 (100.0%)	150 (100.0%)	0 (0.0%)	0	0	73	77
Powell	110	0 (0.0%)	110 (100.0%)	110 (100.0%)	0 (0.0%)	0	0	42	68
Rapid River	2515	12 (0.5%)	2503 (99.5%)	2501 (99.9%)	2 (0.1%)	0	2	1124	1391
S.F. Clearwater	1966	17 (0.9%)	1949 (99.1%)	1943 (99.7%)	6 (0.3%)	2	4	737	1229
S.F. Salmon	795	0 (0.0%)	795 (100.0%)	795 (100.0%)	0 (0.0%)	0	0	354	441
Sawtooth	972	0 (0.0%)	972 (100.0%)	972 (100.0%)	0 (0.0%)	0	0	487	485
Tucannon	62	0 (0.0%)	62 (100.0%)	62 (100.0%)	0 (0.0%)	0	0	25	37

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 SY2021 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	1826	9 (0.5%)	1817 (99.5%)	1815 (99.9%)	2 (0.1%)	1	1	601	1225
Nez Perce	685	44 (6.4%)	641 (93.6%)	598 (93.3%)	43 (6.7%)	2	41	263	422

Table 10. Average observed (obs) and expected (exp) heterozygosity with associated standard error of hatchery steelhead stocks for SY2021.

Stock	Avg. het. (obs)	SE	Avg. het. (exp)	SE
Cottonwood Cr.	0.25	0.01	0.25	0.01
Dworshak	0.23	0.01	0.23	0.01
E.F. Salmon R.	0.25	0.01	0.25	0.01
Little Sheep Cr.	0.24	0.01	0.24	0.01
Oxbow	0.26	0.01	0.25	0.01
Pahsimeroi	0.27	0.01	0.26	0.01
S.F. Clearwater	0.23	0.01	0.23	0.01
Sawtooth	0.26	0.01	0.25	0.01
Tucannon R.	0.26	0.01	0.25	0.01
Touchet R.	0.25	0.01	0.24	0.01
Up. Sal. R. B-run	0.23	0.01	0.23	0.01
Wallowa	0.26	0.01	0.25	0.01

Table 11. Average observed (obs) and expected (exp) heterozygosity with associated standard error of hatchery spring-summer Chinook Salmon stocks in SY2021.

Stock	Avg. het. (obs)	SE	Avg. het. (exp)	SE
Catherine Cr.	0.21	0.01	0.22	0.01
Dworshak	0.21	0.01	0.21	0.01
Grande Ronde	0.21	0.01	0.21	0.01
Imnaha	0.23	0.01	0.24	0.01
Johnson Cr.	0.21	0.01	0.21	0.01
Kooskia	0.21	0.01	0.21	0.01
Lookingglass Cr.	0.22	0.01	0.23	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	0.21	0.01
Powell	0.21	0.01	0.21	0.01
Rapid River	0.20	0.01	0.19	0.01
S.F. Clearwater	0.21	0.01	0.21	0.01
S.F. Salmon	0.20	0.01	0.19	0.01
Sawtooth	0.21	0.01	0.21	0.01
Tucannon	0.22	0.01	0.22	0.01

Table 12. Average observed (obs) and expected (exp) heterozygosity with associated standard error of hatchery fall Chinook Salmon stocks in SY2021.

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 SY2021. 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.	0.027	0.045	---	*	*	*	*	*	<0.001	<0.001	*
Little Sheep Cr.	0.032	0.077	0.035	---	*	*	*	*	*	*	*
Oxbow	0.028	0.065	0.023	0.024	---	*	*	*	*	*	*
Pahsimeroi	0.028	0.070	0.024	0.027	0.014	---	*	*	*	*	*
S.F. Clearwater	0.060	0.002	0.046	0.078	0.064	0.070	---	*	*	*	*
Sawtooth	0.028	0.064	0.021	0.029	0.014	0.011	0.064	---	*	*	*
Tucannon R.	0.022	0.062	0.027	0.028	0.024	0.025	0.061	0.027	---	0.108	*
Touchet R.	0.018	0.059	0.026	0.026	0.025	0.026	0.060	0.028	0.009	---	*
Up. Sal. R. B-run	0.056	0.006	0.041	0.072	0.058	0.063	0.005	0.058	0.055	0.055	---

Table 14. Population structure (F_{ST}) (below diagonal) among spring-summer Chinook Salmon hatchery stocks sampled in SY2021. 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	0.013	---	*	*	*	*	*	*	*	*	*	*	<0.001	*	*	*
Grande Ronde	0.020	0.013	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Imnaha	0.018	0.010	0.019	---	*	*	*	*	*	*	*	*	*	*	*	*
Johnson Cr.	0.030	0.023	0.032	0.026	---	*	*	*	*	*	*	*	*	*	*	*
Kooskia	0.013	0.002	0.013	0.012	0.024	---	*	*	*	*	*	*	*	*	*	*
Lookingglass Cr.	0.004	0.008	0.012	0.013	0.025	0.007	---	*	*	*	*	*	*	*	*	*
Lostine	0.031	0.027	0.039	0.023	0.043	0.028	0.025	---	*	*	*	*	*	*	*	*
Nez Perce FH	0.013	0.001	0.012	0.012	0.024	0.002	0.007	0.027	---	*	*	*	*	*	*	*
Pahsimeroi	0.032	0.027	0.036	0.031	0.040	0.029	0.027	0.036	0.029	---	*	*	*	*	*	*
Powell	0.025	0.016	0.024	0.018	0.016	0.017	0.021	0.031	0.017	0.025	---	*	*	*	*	*
Rapid River	0.026	0.014	0.020	0.017	0.034	0.019	0.019	0.037	0.014	0.040	0.027	---	*	*	*	*
S.F. Clearwater	0.013	0.000	0.012	0.010	0.023	0.001	0.007	0.026	0.001	0.028	0.016	0.013	---	*	*	*
S.F. Salmon	0.026	0.017	0.027	0.019	0.016	0.018	0.022	0.031	0.019	0.029	0.004	0.030	0.017	---	*	*
Sawtooth	0.029	0.021	0.032	0.027	0.032	0.025	0.028	0.040	0.024	0.025	0.023	0.032	0.022	0.025	---	*
Tucannon	0.022	0.017	0.029	0.022	0.032	0.016	0.018	0.035	0.016	0.042	0.028	0.039	0.017	0.029	0.043	---

Table 15. Estimates of effective population size (N_E) and 95% confidence intervals for steelhead hatchery stocks in SY2021.

Stock	Ne	CI (95%)
Cottonwood Cr.	88.4	70.6 – 113.8
Dworshak	250.1	230.8 – 271.2
E.F. Salmon R.	98.4	39.1 – Infinite
Little Sheep Cr.	97.0	74.8 – 131.8
Oxbow	89.6	81.2 – 99.0
Pahsimeroi	111.5	103.3 – 120.2
S.F. Clearwater	126.9	111.3 – 145.3
Sawtooth	208.2	193.1 – 224.6
Tucannon R.	34.3	19.2 – 81.8
Touchet R.	Infinite	443.0 – Infinite
Up. Sal. R. B-run	61.4	53.8 – 69.9
Wallowa	180.0	160.4 – 203.0

Table 16. Estimates of effective population size and 95% confidence intervals for SY2021 spring-summer Chinook Salmon hatchery stocks.

Stock	Ne	CI (95%)
Catherine Cr.	68.6	54.0 – 89.6
Dworshak	394.9	362.1 – 431.2
Grande Ronde	99.5	83.4 – 120.8
Imnaha	226.2	182.5 – 289.9
Johnson Cr.	134.4	84.7 – 276.9
Kooskia	203.7	179.5 – 232.2
Lookingglass Cr.	105.0	87.2 – 128.8
Lostine	77.9	60.1 – 104.5
Nez Perce FH	114.7	96.4 – 137.9
Pahsimeroi	243.8	176.8 – 372.1
Powell	94.4	71.0 – 132.5
Rapid River	504.8	485.8 – 524.6
S.F. Clearwater	494.4	468.0 – 522.4
S.F. Salmon	150.6	139.0 – 163.3
Sawtooth	222.1	206.3 – 239.4
Tucannon	185.4	125.9 – 330.7

Table 17. Estimates of effective population size and 95% confidence intervals for fall Chinook Salmon SY2021 hatchery stocks.

Stock	Ne	CI (95%)
Lyons Ferry	1244.4	1155.3 – 1344.3
Nez Perce	1198.6	998.1 – 1482.9

Table 18. Average observed (obs) and expected (exp) heterozygosity with associated standard error of hatchery steelhead stocks for SY2021 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.	0.253	0.018	0.244	0.018	0.331	0.019	0.332	0.019
Dworshak	0.229	0.019	0.226	0.019	0.296	0.020	0.296	0.020
E.F. Salmon R.	0.250	0.019	0.251	0.021	0.315	0.019	0.326	0.022
Little Sheep Cr.	0.243	0.019	0.247	0.020	0.319	0.020	0.329	0.020
Oxbow	0.259	0.018	0.251	0.018	0.336	0.019	0.337	0.019
Pahsimeroi	0.255	0.018	0.249	0.018	0.326	0.019	0.326	0.019
S.F. Clearwater	0.233	0.019	0.228	0.019	0.299	0.020	0.298	0.020
Sawtooth	0.252	0.018	0.244	0.017	0.327	0.019	0.328	0.019
Tucannon R.	0.251	0.018	0.253	0.019	0.326	0.020	0.336	0.021
Touchet R.	0.247	0.018	0.244	0.018	0.322	0.019	0.323	0.020
Up. Sal. R. B-run	0.228	0.019	0.221	0.019	0.296	0.020	0.292	0.021
Wallowa	0.258	0.018	0.250	0.018	0.339	0.019	0.336	0.019

Table 19. Average observed (obs) and expected (exp) heterozygosity with associated standard error of hatchery spring-summer Chinook Salmon stocks for SY2021 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.	0.250	0.026	0.251	0.027	0.345	0.026	0.347	0.028
Dworshak	0.250	0.024	0.250	0.024	0.341	0.026	0.340	0.026
Grande Ronde	0.263	0.024	0.268	0.024	0.350	0.025	0.359	0.026
Imnaha	0.256	0.025	0.259	0.025	0.347	0.026	0.350	0.026
Johnson Cr.	0.233	0.024	0.218	0.023	0.309	0.027	0.299	0.026
Kooskia	0.247	0.025	0.244	0.025	0.333	0.026	0.334	0.027
Lookingglass Cr.	0.258	0.024	0.269	0.025	0.348	0.025	0.362	0.026
Lostine	0.246	0.025	0.253	0.025	0.320	0.028	0.331	0.029
Nez Perce FH	0.249	0.024	0.251	0.025	0.335	0.026	0.340	0.027
Pahsimeroi	0.225	0.025	0.226	0.025	0.306	0.027	0.307	0.027
Powell	0.243	0.025	0.246	0.026	0.319	0.028	0.322	0.028
Rapid River	0.238	0.025	0.238	0.025	0.330	0.025	0.332	0.026
S.F. Clearwater	0.249	0.024	0.246	0.024	0.337	0.026	0.336	0.026
S.F. Salmon	0.243	0.024	0.243	0.024	0.319	0.027	0.321	0.027
Sawtooth	0.233	0.025	0.237	0.026	0.315	0.028	0.319	0.028
Tucannon	0.256	0.024	0.255	0.025	0.328	0.026	0.331	0.028

Table 20. Average observed (obs) and expected (exp) heterozygosity with associated standard error of hatchery fall Chinook Salmon stocks for SY2021 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	0.258	0.021	0.251	0.021	0.336	0.022	0.335	0.022
Nez Perce	0.257	0.022	0.253	0.021	0.336	0.021	0.337	0.021

Table 21. Haplotypes and relative frequencies in SY2021 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
A	C	T	T	A	T	G	G	G	G	0.4412	0.1277	0.5975	0.3862	0.1473	0.5394	0.4266	0.6052	0.3295	0.2423	0.3251	0.59	0.1975
C	A	G	C	G	G	A	A	A	C	0.3971	0.7482	0.3907	0.4538	0.7044	0.1949	0.3213	0.3907	0.3833	0.6423	0.5643	0.368	0.5622
A	C	T	T	A	G	A	A	A	C	0.074	0.0949	0.0098	<0.0001	0.088	0.0696	0.0724	0.0041	0.1481	0.0434	0.0227	0.026	0.1793
C	A	G	C	G	G	G	G	G	G	0.0537	0.0292	-	0.0398	-	0.0761	0.1151	-	0.1073	0.0286	-	0.003	0.0461
C	A	G	C	G	T	G	G	G	G	0.0192	-	-	0.0289	-	0.0566	0.0436	-	0.0278	0.0145	0.0443	0.0117	-
A	C	T	T	A	G	G	G	G	G	0.0057	-	-	0.0424	-	0.0488	0.001	-	0.0017	-	-	0.0012	<0.0001
A	C	T	T	A	T	G	A	G	C	0.0037	-	-	-	-	-	0.0194	-	0.0006	0.0143	-	-	-
A	C	T	T	A	T	A	A	A	C	0.0022	-	-	-	0.037	0.0145	-	-	-	-	<0.0001	-	-
C	A	T	T	A	G	A	A	A	C	0.0012	-	-	-	0.0026	-	-	-	-	-	0.0217	-	0.0107
C	A	T	C	G	G	A	A	A	C	0.0006	-	0.0009	-	0.0054	<0.0001	-	<0.0001	0.0008	-	-	-	0.0014
C	A	G	C	G	G	A	A	G	C	0.0003	-	0.0012	-	-	-	-	-	-	0.0143	-	-	-
A	C	G	T	A	T	G	G	G	G	0.0003	<0.0001	<0.0001	-	<0.0001	-	0.0007	<0.0001	0.0009	-	-	-	-
A	C	G	C	G	G	A	A	A	C	0.0002	-	-	-	-	-	-	-	-	-	-	-	0.0025
C	A	G	C	G	T	A	A	A	C	0.0002	-	-	0.0462	-	-	-	-	-	-	0.0217	-	-
A	A	T	T	A	T	G	G	G	G	0.0002	-	-	-	0.0056	-	-	-	-	-	-	-	-
A	A	G	T	A	T	G	G	G	G	0.0001	-	-	-	0.0078	-	-	-	-	-	-	-	-
A	A	T	T	A	T	A	A	A	C	0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	T	A	T	A	A	A	C	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	T	T	A	T	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	T	A	T	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	T	A	G	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	T	T	A	G	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	T	T	A	G	A	A	A	C	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	T	A	G	A	A	A	C	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	T	C	A	T	A	A	A	C	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	T	C	A	T	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-

Table 21. Continued

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
A	A	T	C	A	G	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	C	A	T	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	T	C	A	G	A	A	A	C	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	C	A	G	A	A	A	C	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	C	A	T	A	A	A	C	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
A	A	G	C	A	G	A	A	A	G	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-
C	A	G	C	G	G	G	A	G	C	<0.0001	-	-	-	-	-	<0.0001	-	-	-	-	-	-

Table 22. Haplotypes and relative frequencies in SY2021 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	0.6905	0.708	0.9078	0.75	0.8294	0.565	0.4686	0.9078	0.5748	0.7857	0.8257	0.9152	0.5317
G	A	G	G	A	G	C	T	A	C	T	G	T	0.2148	0.2591	0.0313	0.0357	0.1429	0.2934	0.3894	0.0245	0.2849	0.1286	0.1083	0.0546	0.4146
G	A	G	G	A	G	C	T	A	C	T	G	A	0.0267	0.0219	0.0185	-	0.0179	0.0147	0.0505	0.0212	0.0405	-	0.0221	0.009	0.0098
G	A	G	G	G	G	C	C	G	T	G	T	A	0.015	-	-	-	-	0.042	0.0327	-	0.0365	-	0.0217	-	-
G	A	G	G	A	G	C	C	G	T	G	T	T	0.0128	-	0.0315	-	-	0.0014	-	0.0315	0.0118	-	0.0221	0.0071	0.0025
G	C	T	G	G	G	C	T	A	C	T	G	A	0.0086	-	-	0.0357	-	0.0282	0.0279	<0.0001	0.0028	-	-	0.0038	-
G	C	T	G	G	G	C	T	A	T	T	G	T	0.0072	-	-	-	-	0.0065	0.0143	-	0.0175	0.0286	-	-	0.0049
G	C	T	G	G	G	C	T	A	C	T	G	T	0.0055	-	0.0038	0.1071	-	0.0149	0.0071	0.0055	0.0057	0.0143	-	0.0047	-
G	C	T	G	G	G	C	T	A	T	T	G	A	0.0037	-	<0.0001	-	-	-	0.0069	0.0031	0.0012	-	-	0.0014	0.0122
G	A	G	G	A	G	C	C	A	C	T	G	A	0.0032	-	-	-	-	0.0011	-	-	0.0155	-	-	-	-
G	A	G	G	A	G	C	C	A	C	T	G	T	0.0029	-	-	-	-	0.0302	-	-	0.002	-	-	-	-
G	A	G	G	G	G	C	T	A	C	T	G	T	0.002	-	-	-	-	<0.0001	-	-	<0.0001	-	-	-	-
G	A	G	G	G	G	C	C	G	T	G	T	T	0.0015	0.0109	-	-	-	-	-	-	0.0035	0.0143	-	-	0.0049
G	C	G	G	G	G	C	T	A	T	T	G	A	0.0009	-	0.006	-	-	-	-	0.0037	-	-	-	<0.0001	-
G	C	T	A	G	G	A	T	A	C	T	G	A	0.0006	-	-	-	-	-	-	-	-	-	-	-	0.0052
A	C	G	G	G	G	C	C	G	T	G	T	A	0.0006	-	-	-	-	-	0.0025	-	-	-	-	-	-
G	A	T	A	G	G	A	T	A	C	T	G	A	0.0005	-	-	-	-	-	-	-	-	-	-	-	0.007
G	A	G	G	A	G	C	C	G	C	G	T	T	0.0005	-	-	-	-	-	-	<0.0001	0.0023	-	-	-	-
A	C	T	A	G	T	A	T	A	C	T	G	T	0.0005	-	0.0011	-	-	-	-	-	-	0.0286	-	-	-
G	C	T	G	G	G	C	C	G	T	G	T	A	0.0004	-	-	<0.0001	-	-	<0.0001	-	-	-	-	-	0.0037
G	A	G	G	G	G	C	C	G	T	G	G	A	0.0003	-	-	-	-	-	-	-	-	-	-	-	0.0037
G	A	G	G	A	G	C	C	G	C	G	T	A	0.0003	-	-	-	-	-	-	0.0027	-	-	-	0.0014	-
G	A	T	G	A	G	C	C	G	T	G	T	A	0.0003	-	-	-	0.0099	-	-	-	-	-	-	-	-
G	A	G	G	G	G	C	C	G	T	G	G	T	0.0002	-	-	-	-	-	-	-	0.0011	-	-	-	-
G	C	T	G	A	G	C	C	G	T	G	T	A	0.0002	-	-	0.0714	-	-	-	-	-	-	-	-	-
G	C	T	G	A	G	C	T	A	C	T	G	A	0.0001	-	-	-	-	0.0014	-	-	-	-	-	-	-

Table 22. Continued

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	C	T	G	G	G	C	T	A	T	G	G	T	0.0001	-	-	-	-	0.0013	-	-	-	-	-	-	-
G	C	T	A	G	G	C	T	A	T	G	G	T	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
G	A	G	G	G	G	C	C	A	C	T	G	T	<0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-
G	C	T	A	G	G	C	T	A	C	G	G	A	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
G	C	T	G	G	G	C	T	A	C	G	G	A	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-

Table 23. Spring-summer Chinook Salmon SY2021 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	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
IV	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
V	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
VI	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
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	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
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	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
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	C
XIV	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
XV	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
XVI	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
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	T	A	T	A	A	T	T	C	C	A	C	T	T	C
XIX	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
XX	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
XXI	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
XXII	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
XXIII	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
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	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
XXVI	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
XXVII	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

Table 23. Continued.

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

Table 23. Continued.

	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**
LVI	G	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	T	C
LVII	G	A	A	G	A	T	C	T	T	G	C	C	A	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C
LVIII	G	A	A	G	A	T	C	T	T	G	C	C	A	A	A	G	T	C	A	T	T	C	C	A	C	T	T	C
LIX	A	A	G	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
LX	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
LXI	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	G	T	A	T	G	C	T	C	G	T	C	T	C
LXII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	C	T	A	G	T	C	G	T
LXIII	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	T	C	A	T	T	C	C	A	C	T	T	C
LXIV	G	C	A	G	G	C	T	A	A	A	T	T	A	A	A	G	G	C	A	T	T	C	C	G	C	T	T	C
LXV	G	C	A	G	G	C	T	A	A	A	T	T	A	A	A	G	T	C	A	T	T	C	C	A	C	T	T	C
LXVI	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	T	T	C	G	C	C	T	C
LXVII	A	A	G	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	T	T	C	G	C	T	T	C
LXVIII	G	C	A	G	G	C	T	A	A	A	T	T	A	A	A	G	T	C	A	T	T	C	C	G	C	T	T	C
LXIX	A	A	G	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	T	T	C	G	C	C	T	C
LXX	A	A	G	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	T	T	C	G	C	T	T	C
LXXI	A	A	G	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	T	T	C	G	C	C	T	C
LXXII	A	A	G	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	T	T	C	G	C	T	T	C
LXXIII	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	T	T	C	G	C	T	T	C
LXXIV	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	T	T	C	G	C	T	T	C
LXXV	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	T	T	C	G	C	C	T	C
LXXVI	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	G	C	A	G	T	T	C	G	C	C	T	C
LXXVII	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	T	C	T	G	T	T	C	G	C	C	T	C
LXXVIII	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	T	C	A	G	T	T	C	G	C	C	T	C
LXXIX	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	C
LXXX	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	T	C	T	G	T	T	C	G	C	T	T	C
LXXXI	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	T	T	C
LXXXII	G	A	A	G	A	T	C	T	T	G	C	C	G	A	A	G	T	C	A	G	T	T	C	G	C	T	T	C
LXXXIII	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	T	T	T

Table 23. Continued.

	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**
LXXXIV	A	A	G	C	A	T	C	T	T	G	C	C	G	A	T	A	G	C	A	T	T	C	A	A	C	T	G	C

Table 24. Haplotypes and relative frequencies in SY2021 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	0.4248	0.4015	0.3933	0.3405	0.1316	0.1984	0.4052	0.2712	0.2194	0.4254	0.2524	0.4988	0.5164	0.3776	0.5175	0.4702	0.3733
II	0.2019	0.36	0.2461	0.2857	0.0947	0.1597	0.1818	0.3546	0.0234	0.2192	0.1403	0.0773	0.2181	0.2161	0.1294	0.1285	0.3065
III	0.0669	<0.0001	0.0734	0.0407	0.0365	0.0347	0.0862	0.0725	-	0.0624	0.0167	0.0158	0.0824	0.0887	0.0203	0.0304	0.0595
IV	0.0631	0.0734	0.0787	0.15	0.0255	0.0556	0.1074	0.0701	0.016	0.1081	-	0.0687	0.0314	0.0921	0.0433	0.0458	0.0192
V	0.0565	0.0275	0.0246	-	0.3729	0.1107	0.0101	0.0827	0.1505	0.0025	0.4131	0.063	0.0197	0.0242	0.0104	0.2051	0.0563
VI	0.0524	0.0826	0.0632	0.0607	0.0487	-	0.0587	0.0559	0.02	0.053	0.0367	0.0091	0.0457	0.0638	0.0225	0.0617	0.0242
VII	0.0445	0.0183	0.039	0.0688	0.0133	0.0296	0.0777	0.0132	<0.0001	0.0363	0.0405	0.0156	0.0585	0.0489	0.0375	0.0081	0.0912
VIII	0.018	-	0.01	0.009	0.0744	0.3056	0.0009	0.012	0.054	0.0039	0.0299	0.0939	0.0055	0.0113	0.0619	0.0088	0.0161
IX	0.0117	-	0.0056	-	0.0031	0.029	0.0092	-	-	0.0088	<0.0001	0.0289	-	0.01	0.0811	-	0.0082
X	0.0107	0.0092	0.0208	0.0134	-	0.0278	0.0329	0.01	-	0.0229	-	-	-	0.0215	-	-	0.0161
XI	0.0105	<0.0001	0.0126	-	0.0068	0.0069	0.0162	0.0095	0.0041	0.03	-	-	0.0086	0.0163	0.0088	-	-
XII	0.008	0.0046	0.0009	0.0122	0.088	-	-	<0.0001	0.231	-	0.0569	0.0684	0.0014	0.0005	0.0094	0.0015	0.0108
XIII	0.0077	-	0.0158	0.0071	-	<0.0001	0.005	-	0.032	0.0069	-	-	0.0004	0.0169	-	-	-
XIV	0.0039	-	0.003	-	0.019	-	-	-	0.0045	-	0.0034	0.0379	0.0065	0.0032	0.001	-	-
XV	0.0037	-	-	-	0.0599	-	-	0.0032	0.2115	-	-	0.0046	-	-	0.0019	-	-
XVI	0.0023	-	0.004	-	-	-	-	-	-	-	-	-	0.004	0.0025	-	-	-
XVII	0.0017	0.0046	0.0034	-	-	-	0.0045	0.0096	-	0.0018	-	-	-	0.0018	-	-	0.0081
XVIII	0.0016	-	0.0003	-	-	-	0.0009	-	-	0.0018	0.0031	-	-	0.0013	0.0108	0.0016	-
XIX	0.0015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0157	-
XX	0.0009	-	0.0003	-	-	-	-	0.0066	-	-	-	-	-	0.0003	0.0076	-	-
XXI	0.0008	-	0.0004	-	-	-	-	-	-	0.0013	-	0.004	-	-	0.0115	-	-
XXII	0.0007	-	-	-	0.0042	-	-	-	-	-	-	-	-	-	-	-	-
XXIII	0.0007	-	-	-	-	-	<0.0001	-	0.0051	-	0.0035	-	-	-	0.0145	-	-
XXIV	0.0007	-	-	-	-	-	-	-	-	-	-	0.0046	-	-	0.0006	0.004	-
XXV	0.0006	-	-	-	-	0.0081	-	-	-	-	0.0014	-	-	-	-	0.0046	-
XXVI	0.0006	-	0.0007	-	-	-	0.0009	-	-	-	-	-	0.0008	0.0012	-	-	-

Table 24. Continued.

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
XXVII	0.0005	-	0.0011	-	-	-	-	-	-	0.0009	-	-	0.0002	0.001	-	-	-
XXVIII	0.0005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0037	-
XXIX	0.0004	-	0.0012	-	-	-	-	-	-	-	-	-	-	0.0003	-	0.0015	-
XXX	0.0003	-	-	-	0.006	-	-	-	0.0104	-	-	-	-	-	-	-	-
XXXI	0.0003	-	-	-	0.0023	-	-	-	-	-	-	-	-	-	0.0022	-	-
XXXII	0.0002	-	0.0003	-	-	-	-	-	-	-	-	-	-	0.0005	<0.0001	-	-
XXXIII	0.0002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0015	-
XXXIV	0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0015	-	-
XXXV	0.0001	-	-	-	-	<0.0001	-	-	-	-	-	-	-	-	0.0014	-	-
XXXVI	0.0001	-	-	-	0.0046	-	-	-	-	-	-	-	-	-	-	-	-
XXXVII	0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0012	-	-
XXXVIII	0.0001	-	-	-	0.0023	-	-	-	-	-	-	-	-	-	-	-	-
XXXIX	0.0001	-	-	-	-	-	-	-	0.0045	-	-	-	-	-	-	-	-
XL	0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XLI	0.0001	-	-	-	0.0026	-	-	-	-	-	-	-	-	-	-	-	-
XLII	0.0001	-	-	-	-	0.0074	-	-	-	-	-	-	-	-	-	-	-
XLIII	0.0001	-	-	-	-	-	-	0.0032	-	-	-	-	-	-	-	-	-
XLIV	0.0001	-	-	-	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-
XLV	<0.0001	-	-	-	-	-	0.0005	-	-	-	-	-	-	-	-	-	-
XLVI	<0.0001	-	-	-	0.0022	-	-	-	-	-	-	-	-	-	-	-	-
XLVII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0004	-
XLVIII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0013	-
XLIX	<0.0001	-	-	-	-	0.0069	-	-	-	-	-	-	-	-	-	-	-
L	<0.0001	-	-	-	-	0.0058	-	-	-	-	-	-	-	-	-	-	-
LI	<0.0001	-	-	-	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-
LII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0003	-
LIII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0002	-
LIV	<0.0001	-	-	-	-	-	-	-	-	-	-	0.0045	-	-	-	-	-

Table 24. Continued.

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
LV	<0.0001	-	-	-	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-
LVI	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LVII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LVIII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LIX	<0.0001	-	-	-	0.0009	-	-	-	-	-	-	-	-	-	-	-	-
LX	<0.0001	-	0.0003	-	-	-	-	-	-	-	-	-	-	-	0.0006	-	-
LXI	<0.0001	-	-	-	-	-	0.0004	-	-	-	-	-	-	-	-	-	-
LXII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0008	-
LXIII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXIV	<0.0001	-	-	-	0.0001	-	-	-	-	-	-	-	-	-	-	-	-
LXV	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
LXVI	<0.0001	-	-	-	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-
LXVII	<0.0001	-	-	-	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-
LXVIII	<0.0001	-	-	-	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
LXIX	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXX	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXI	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXIII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXIV	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXV	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXVI	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXVII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXVIII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXIX	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXX	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXXI	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXXII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 24. Continued.

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
LXXXIII	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LXXXIV	<0.0001	-	0.0009	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 25. Haplotypes and relative frequencies in SY2021 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	0.6876
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	0.0568
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	0.044
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	0.0321
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	0.0271
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	0.023
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	0.0144
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	0.0102
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	0.0102
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	0.0089
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	0.0084
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	0.0077
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	0.0064
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	0.0045
A	A	G	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	0.0042
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	0.0036
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	0.0034
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	0.003
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	0.0028
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	0.0028
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	0.0023
G	C	A	G	G	C	T	A	A	A	T	T	A	G	A	G	G	C	A	G	C	C	C	G	C	C	T	C	0.0022
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	0.0019
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	0.0018
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	0.0015

Table 25. Continued

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	A	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	0.0015
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	0.0015
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	0.0015
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	0.0014
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	0.0014
A	A	G	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	0.0012
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	0.0012
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	0.0009
A	A	G	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	0.0009
G	C	A	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	0.0008
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	0.0008
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	0.0008
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	0.0008
G	C	A	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	0.0008
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	0.0008
A	A	G	C	A	T	C	T	T	G	C	C	A	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.0008
G	C	A	G	G	C	T	A	A	G	T	T	A	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.0008
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	0.0007
G	C	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	0.0007
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	0.0007
G	C	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	0.0007
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	T	0.0007
G	C	G	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	0.0007
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	0.0007
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	T	T	0.0006
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	T	0.0006
A	A	G	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	0.0006
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	0.0006

Table 25. Continued

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	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	0.0006
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	0.0005
A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	G	G	C	T	T	T	C	C	A	C	T	T	C	0.0005
G	C	A	G	G	C	T	A	A	A	T	T	G	G	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.0004
G	C	A	C	A	T	C	T	T	G	C	C	G	A	A	G	G	C	T	G	C	T	C	G	C	C	G	T	0.0004
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	0.0003
G	C	A	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	0.0003
A	A	G	G	G	C	T	A	A	A	T	T	A	G	T	G	G	C	T	T	C	C	C	A	C	T	T	C	0.0003
G	C	A	G	G	C	T	A	A	A	T	T	G	A	A	G	G	C	A	T	T	C	C	A	C	T	T	C	0.0003
A	A	G	C	A	C	T	A	A	A	T	T	A	G	T	A	T	A	T	G	T	T	C	G	T	C	T	T	0.0002
A	A	G	G	A	C	T	A	A	A	T	T	A	G	A	G	G	C	A	G	T	T	C	G	T	C	T	T	0.0001
G	C	A	G	G	C	T	A	A	A	T	T	G	G	A	G	G	C	A	T	C	C	C	A	C	T	T	C	<0.0001
G	C	A	G	G	C	T	A	A	A	T	T	G	A	A	G	G	C	A	T	C	C	C	A	C	C	T	C	<0.0001
G	C	A	G	G	C	T	A	A	A	T	T	G	A	A	G	G	C	A	T	C	C	C	A	C	T	T	C	<0.0001
G	C	A	G	G	C	T	A	A	A	T	T	G	A	A	G	G	C	A	T	T	C	C	A	C	C	T	C	<0.0001
G	C	A	G	G	C	T	A	A	A	T	T	G	G	A	G	G	C	A	T	C	C	C	A	C	C	T	C	<0.0001
G	C	A	G	G	C	T	A	A	A	T	T	G	G	A	G	G	C	A	T	T	C	C	A	C	C	T	C	<0.0001
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	G	T	<0.0001
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	T	<0.0001
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	T	T	C	<0.0001
G	C	A	G	G	C	T	A	A	A	T	T	A	G	T	G	G	C	A	T	C	C	C	A	C	T	T	C	<0.0001

Table 26. Summary of SY2022 adult and MY2022 juvenile steelhead and Chinook Salmon samples from Lower Granite Dam. Summary includes the number of samples inventoried and queued for genotyping. For samples that genotyped successfully, we show the number of individuals with a parentage-based tag (PBT assignment, 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 to genotype (NG)**	**Successfully genotyped**	**PBT assignments**	**GSI assignments**
Steelhead					
SY2022 Adults (Natural-origin Phenotype)	2,097	7 (0.3%)	2,090 (99.7%)	161 (7.7%)	1,929 (92.3%)
SY2022 Adults (Hatchery Phenotype)	229	1 (0.4%)	228 (99.6%)	218 (95.6%)	10 (4.4%)
MY2022 Juveniles	1,241	18 (1.5%)	1,223 (98.5%)	20 (1.6%)	1,203 (98.4%)
Chinook Salmon					
SY2022 Adults	3,851	9 (0.2%)	3,842 (99.8%)	1,018 (26.5%)	2,824 (73.5%)
MY2022 Juveniles	1,597	31 (1.9%)	1,566 (98.1%)	525 (33.5%)	1,041 (66.5%)
Total:	*9,015*	*66 (0.7%)*	*8,949 (99.3%)*	*1,942 (21.7%)*	*7,007 (78.3%)*

Table 27. Summary of Lower Granite Dam natural-origin adult steelhead samples from SY2022 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.

			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**	**1**	**2**	**3**
GRROND	452	23%	299	153	-	66%	34%	57.0	70.0	-	445	7	98%	2%	273	171	-	61%	39%	-
IMNAHA	181	9%	113	68	-	62%	38%	56.7	70.2	-	179	2	99%	1%	118	61	-	66%	34%	-
LOCLWR	64	3%	48	16	-	75%	25%	59.1	72.5	-	57	7	89%	11%	24	37	-	39%	61%	-
LOSALM	22	1%	13	9	-	59%	41%	57.3	71.6	-	21	1	95%	5%	15	7	-	68%	32%	-
LSNAKE	358	19%	211	147	-	59%	41%	56.5	71.5	-	347	11	97%	3%	225	120	-	65%	35%	-
MFSALM	202	11%	146	56	-	72%	28%	59.9	74.9	-	169	33	54%	16%	95	106	-	47%	53%	-
SFCLWR	94	5%	57	36	1	61%	38%	62.7	79.3	91.0	54	40	57%	43%	19	71	2	21%	77%	2%
SFSALM	61	3%	49	12	-	80%	20%	61.4	78.0	-	38	23	62%	38%	14	47	-	23%	77%	-
UPCLWR	211	11%	133	78	-	63%	37%	63.1	78.5	86.0	138	73	65%	35%	73	131	1	36%	64%	<1%
UPSALM	284	15%	168	116	-	59%	41%	57.3	70.3	-	278	6	98%	2%	193	89	-	68%	32%	-
Total:	*1,929*		*1,237*	*691*	1	*64%*	*36%*	*57.8*	*73.6*	*89.9*	*1,726*	*203*	*82%*	*18%*	*1,049*	*840*	*3*	*55%*	*44%*	*<1%*

Table 28. Summary of Lower Granite Dam juvenile steelhead samples from MY2022 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.

			Sex				Length	Freshwater age											
			Frequency		Percentage			Frequency						Percentage					
Genetic stock	Total assignments	% Stock composition	F	M	F	M	Mean length (mm FL)	1	2	3	4	5	6	1	2	3	4	5	6
GRROND	379	32%	231	148	61%	39%	179	74	231	66	1	-	-	20%	62%	18%	<1%	-	-
IMNAHA	100	8%	71	29	71%	29%	176	10	67	18	3	-	-	10%	68%	18%	3%	-	-
LOCLWR	50	4%	34	16	68%	32%	171	13	26	9	-	-	-	27%	54%	19%	-	-	-
LOSALM	16	1%	11	5	69%	31%	182	2	9	4	-	-	-	13%	60%	27%	-	-	-
LSNAKE	192	16%	128	64	67%	33%	175	41	107	35	2	-	-	22%	58%	19%	1%	-	-
MFSALM	60	5%	44	16	73%	27%	180	-	27	28	1	-	-	-	48%	50%	2%	-	-
SFCLWR	107	9%	71	36	66%	34%	176	20	59	18	3	1	1	20%	58%	18%	3%	1%	1%
SFSALM	42	4%	31	11	74%	26%	184	2	19	17	4	-	-	5%	45%	40%	10%	-	-
UPCLWR	75	6%	52	23	69%	31%	183	6	24	38	1	-	-	9%	35%	55%	1%	-	-
UPSALM	182	15%	130	52	71%	29%	175	30	106	33	1	1	-	18%	62%	19%	1%	1%	-
Total:	*1,203*		*803*	*400*	*67%*	*33%*	*178*	*198*	*675*	*266*	*16*	*2*	*1*	*17%*	*58%*	*23%*	*1%*	*<1%*	*<1%*

Table 29. Summary of Lower Granite Dam adult Chinook Salmon samples sampled from SY2022 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.

			Sex					Ocean (Saltwater) age								Length			
			Frequency			Percentage		Frequency				Percentage				Mean length (cm FL) by ocean age			
Genetic stock	**Total assignments**	**% Stock composition**	**F**	**M**	**U**	**F**	**M**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
HELLSC	1,106	39%	555	551	-	50%	50%	43	791	49	1	5%	89%	6%	<1%	51.3	71.2	77.2	92.0
SFSALM	754	27%	343	411	-	45%	55%	20	612	45	-	3%	90%	7%	-	52.9	73.1	81.0	-
UPSALM	392	14%	158	234	-	40%	60%	15	288	25	-	5%	88%	8%	-	50.6	71.5	82.0	-
MFSALM	384	14%	160	223	1	42%	58%	22	230	14	-	8%	86%	5%	-	49.2	73.1	85.6	-
FALL	112	4%	42	70	-	38%	62%	14	11	22	1	29%	23%	46%	2%	49.5	67.6	83.7	97.0
CHMBLN	76	3%	44	32	-	58%	42%	4	43	-	-	9%	91%	-	-	53.2	72.1	-	-
TUCANO	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total:	*2,824*		*1,302*	*1,521*	*1*	*46%*	*54%*	*118*	*1,975*	*155*	*2*	*5%*	*88%*	*7%*	*<1%*	*50.9*	*72.1*	*80.8*	*94.5*

Table 30. Summary of Lower Granite Dam juvenile Chinook Salmon samples from MY2022 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	18	2%	7	11	-	39%	61%	112
FALL	21	2%	8	13	-	38%	62%	121
HELLSC	538	52%	298	240	-	55%	45%	117
MFSALM	154	15%	75	79	-	49%	51%	116
SFSALM	224	22%	132	91	1	59%	41%	111
TUCANO	0	-	-	-	-	-	-	-
UPSALM	86	8%	41	45	-	48%	52%	114
Total:	*1,041*		*561*	*479*	*1*	*54%*	*46%*	*115*

FIGURES

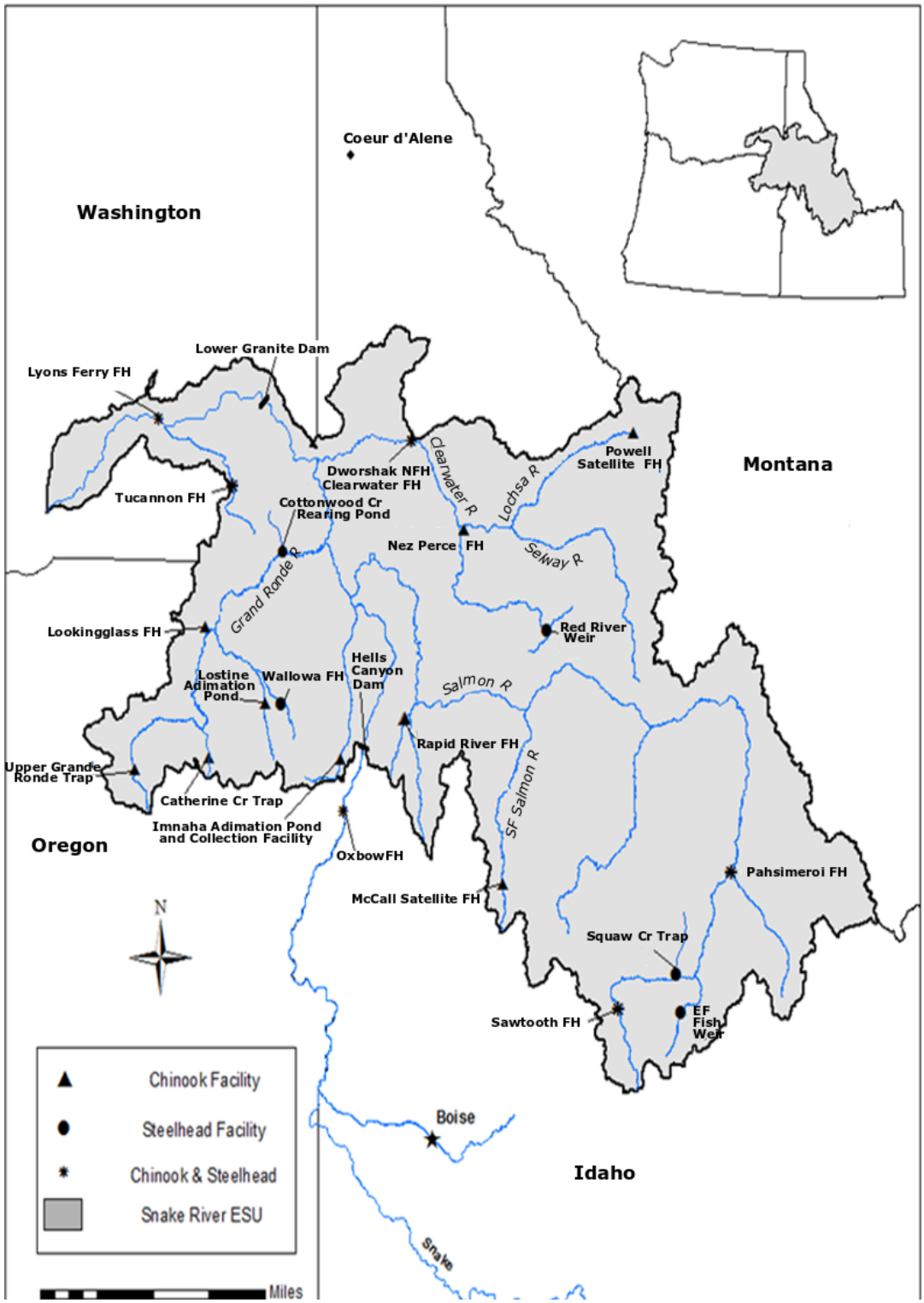

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

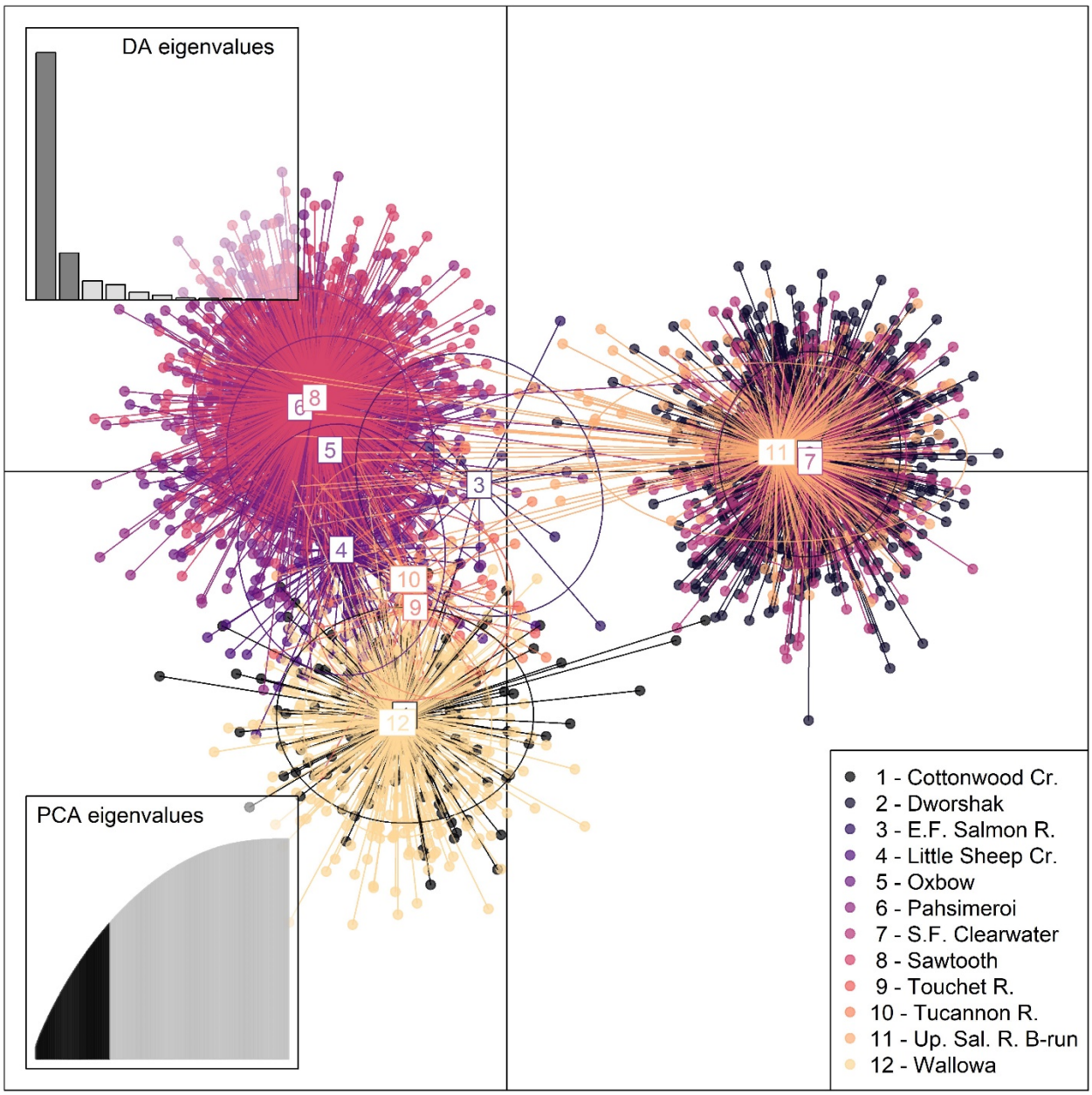

Figure 2. Discriminant analysis of principle components (DAPC) of SY2021 steelhead broodstocks. Each point represents an individual, and the colors of points and inertia ellipses correspond to each steelhead broodstock. Colors for each stock are conserved across DAPC plots and neighbor-joining dendrograms.

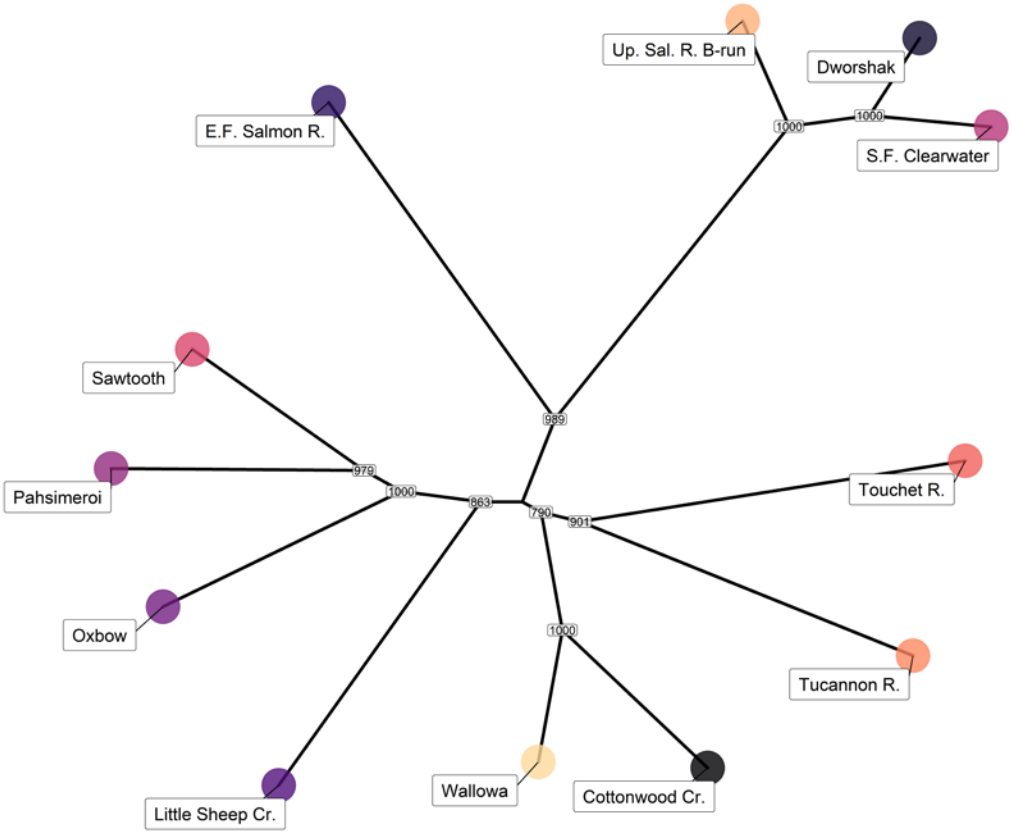

Figure 3. Unrooted neighbor-joining dendrogram of SY2021 steelhead broodstocks, generated using Prevosti's distance and 1,000 bootstrap replicates. Values on the dendrogram represent the number of times a clade was represented within 1,000 bootstrap replicates. Colors for each stock are conserved across DAPC plots and neighbor-joining dendrograms.

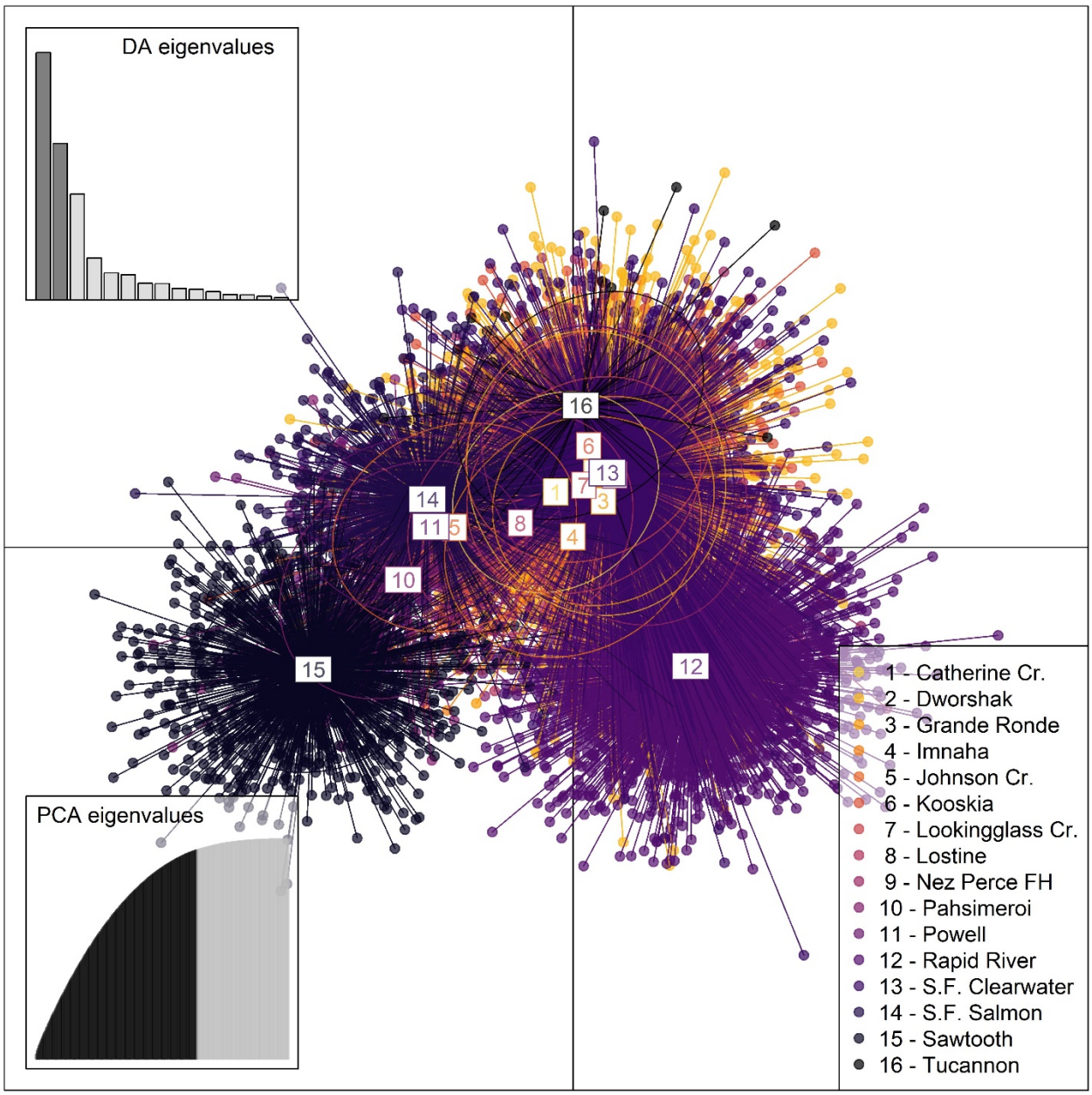

Figure 4. Discriminant analysis of principle components (DAPC) of SY2021 spring-summer Chinook Salmon broodstocks. Each point represents an individual, and the colors of points and inertia ellipses correspond to each Chinook Salmon broodstock. Colors for each stock are conserved across DAPC plots and neighbor-joining dendrograms.

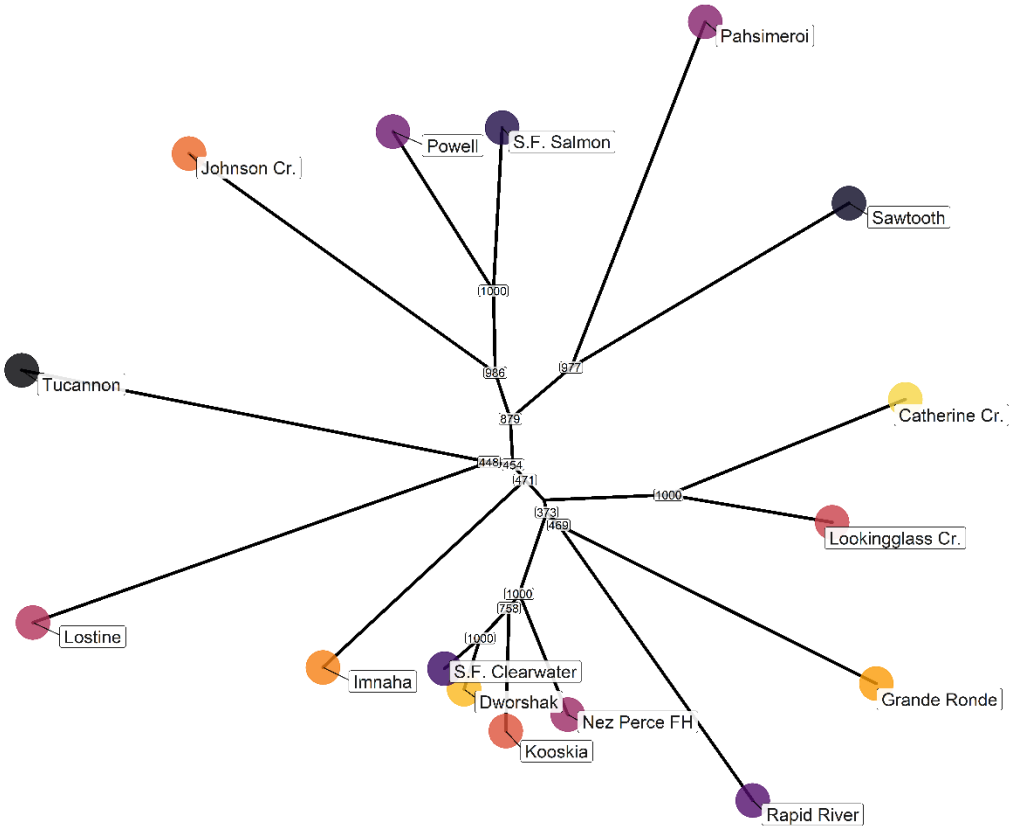

Figure 5. Unrooted neighbor-joining dendrogram of spring-summer SY2021 Chinook Salmon broodstocks, generated using Prevosti's distance and 1,000 bootstrap replicates. Values on the dendrogram represent the number of times a clade was represented within 1,000 bootstrap replicates. Colors for each stock are conserved across DAPC plots and neighbor-joining dendrograms.

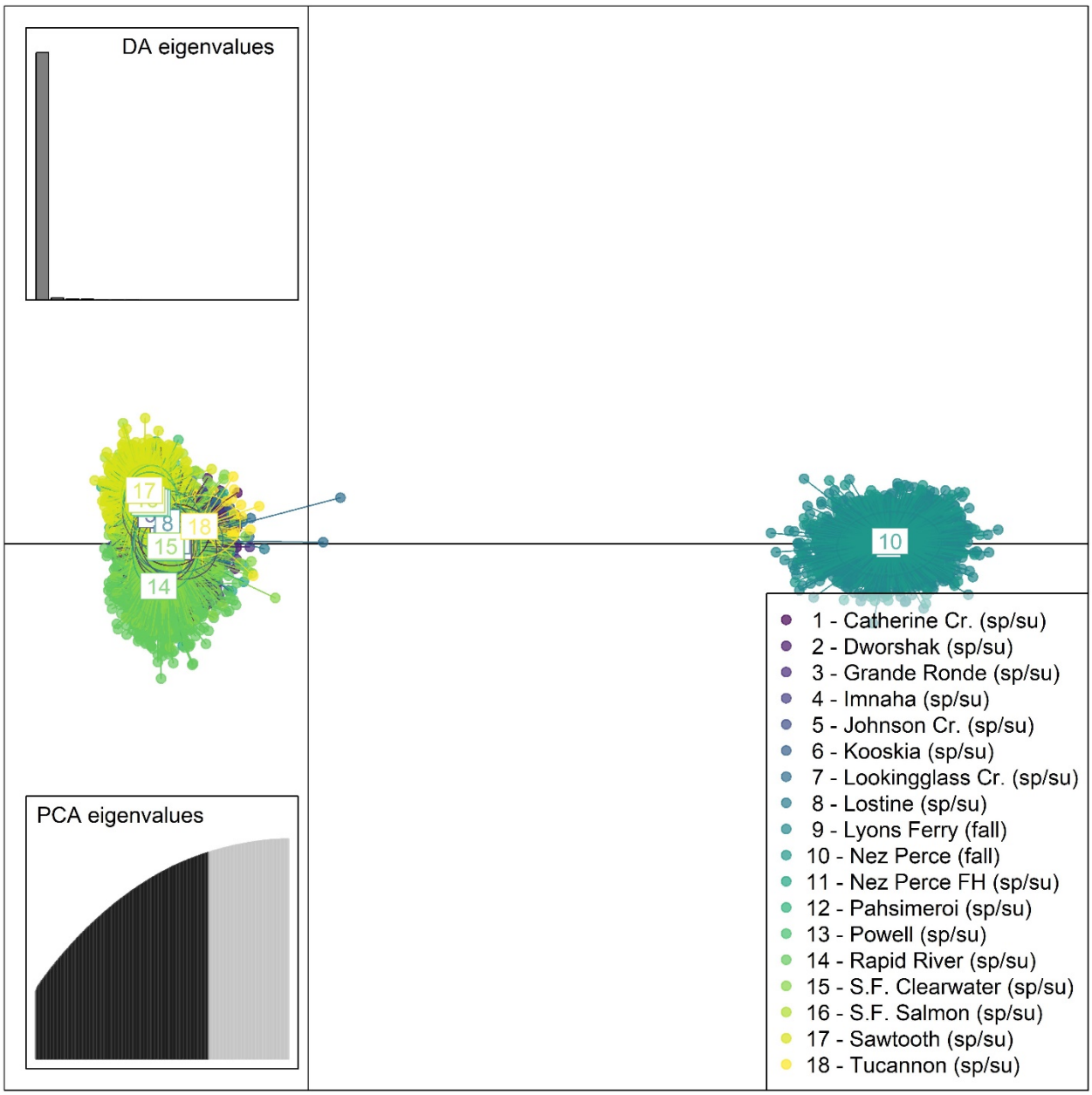

Figure 6. Discriminant analysis of principle components (DAPC) of all SY2021 Chinook Salmon broodstocks (both spring-summer and fall). Each point represents an individual, and the colors of points and inertia ellipses correspond to each Chinook Salmon broodstock. Spring-summer Chinook Salmon broodstocks are designated with (sp/su) and fall Chinook Salmon broodstocks are designated with (fall) in the legend.

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