FISHERY RESEARCH

# CHINOOK SALMON AND STEELHEAD GENOTYPING FOR GENETIC STOCK IDENTIFICATION AT LOWER GRANITE DAM

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**Prepared by:**

**John S. Hargrove, Fishery Biologist 2**
**Thomas A. Delomas, Fishery Biologist 2**
**Jesse McCane, Data Management Specialist 2**
**Micah Davison, Supervisory Fishery Biologist**
**Matthew R. Campbell, Fisheries Genetics Program Coordinator**



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# Chinook Salmon and Steelhead Genotyping for Genetic Stock Identification at Lower Granite Dam

## Project Progress Report

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**By**

**John S. Hargrove**
**Thomas A. Delomas**
**Jesse McCane**
**Micah Davison**
**Matthew R. Campbell**

**Idaho Department of Fish and Game**
**600 South Walnut Street**
**P.O. Box 25**
**Boise, ID 83707**

**and**

**Rebekah L. Horn**
**Shawn R. Narum**

**Columbia River Inter-Tribal Fish Commission**
**Hagerman Fish Culture Experiment Station**
**3059-F National Fish Hatchery Road**
**Hagerman, ID 83332**

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## ABSTRACT

This report summarizes progress in the development and implementation of genetic stock identification (GSI) in the Snake River basin for natural-origin steelhead and spring/summer Chinook Salmon for the 01/01/2020 to 12/31/2020 reporting period. Four objectives for the GSI project are 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) the implementation of GSI to estimate genetic stock composition and life history diversity of steelhead and spring/summer Chinook Salmon passing Lower Granite Dam (LGR); and 4) the summarization of life history and genetic diversity information for steelhead and spring/summer Chinook Salmon detected at PIT tag detection systems. For both species, panels of up to 368 SNPs have been in use for GSI and parentage-based tagging (PBT) at both Idaho Department of Fish and Game's Eagle Fish Genetics Lab, and its collaborating laboratory, the Columbia River Inter-Tribal Fish Commission's Hagerman Genetics Lab. We present an updated Steelhead SNP baseline version 4, which consists of 43 collections and 2,719 individuals. Chinook Salmon SNP baseline v3.1 consists of 30 collections and 4,356 individuals. SNP baselines are used to describe genetic diversity and structure of natural-origin populations throughout the Snake River. Based on population structure we have defined 10 genetic stocks for steelhead and 7 genetic stocks for Chinook Salmon for GSI analysis at LGR. We summarize GSI results for returning adults and emigrating juveniles during 2019-2020 at LGR using v3.1 baselines as reference for both steelhead and Chinook Salmon. Finally, we describe the life history variation and genetic diversity of steelhead and Chinook Salmon detected at IPTDS. The information presented in this report provides critical data for viable salmonid population (VSP) monitoring of the Snake River steelhead DPS and the Snake River spring/summer Chinook Salmon ESU.



Authors:

John S. Hargrove, Fishery Biologist 2

Thomas A. Delomas, Fishery Biologist 2

Jesse McCane, Data Management Specialist 2

Micah Davison, Supervisory Fishery Biologist

Matthew R. Campbell, Fisheries Genetics Program Coordinator



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## INTRODUCTION

Abundance (i.e. number of adults on spawning grounds) is a primary metric needed for monitoring the status of steelhead and salmon populations in the Columbia River basin (McElhany et al. 2000). Estimates of abundance combined with age and sex information over time allows estimation of productivity (e.g. recruits-per-female). Both abundance and productivity metrics provide indicators of the resiliency of populations 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.

Population-level assessments of abundance and productivity for ESA-threatened Snake River steelhead and Chinook Salmon can be particularly difficult due to the wide distribution and location of spawning areas (many populations are present in remote or wilderness areas). Additionally, environmental conditions at the time of spawning often prevent the use of traditional counting methodologies (weirs and redd count surveys). For example, Snake River steelhead monitoring 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.

In lieu of more detailed basin-level and population-specific information, steelhead in the Columbia River basin have traditionally been assigned to two groups (A-run and B-run), based on life history characteristics and bimodal passage timing at Bonneville Dam in the mid-Columbia River (Busby et al. 1996). By definition, A-run steelhead pass Bonneville Dam before August 25 and tend to return after one year in the ocean. B-run steelhead pass Bonneville Dam after August 25 and tend to return after two years in the ocean. B-run steelhead are thought to be larger at age than A-run steelhead. Upstream migrating steelhead adults at Lower Granite Dam (LGR) do not exhibit a bimodal passage distribution. A-run and B-run adults at LGR are enumerated based on length (A-run, ≤78 cm; B-run, >78 cm) as a proxy for ocean age. A-run and B-run steelhead also exhibit differences in spawning distribution. A-run steelhead spawn throughout the Columbia basin, whereas the majority of B-run steelhead originate primarily from the Clearwater, Middle Fork Salmon, and South Fork Salmon rivers in Idaho. The putative differences in migration timing, morphology, and life history characteristics have been used as a surrogate for biodiversity in conservation planning for Snake River steelhead. However, the relationship between the morphological and life history characteristics to time of passage at Bonneville Dam is uncertain (Good et al. 2005). Further, the bimodal passage distribution at Bonneville Dam has become unimodal in recent years (Robards and Quinn 2002).

Two management concerns regarding Snake River steelhead have arisen in the last several years. First, populations classified as “B-run” do not appear to be self-sustaining (NMFS 2007) and their presence in the basin has affected operation of the Columbia River hydrosystem and fisheries management in the lower Columbia River. In particular, harvest of fall Chinook Salmon is constrained in order to limit impacts to B-run steelhead concurrently present in the Columbia River fishery. Secondly, there are substantial data needs to refine population delineations and conservation assessments (ICTRT 2003), but data have been lacking. Although Snake River “B-run” steelhead are currently identified as a biologically significant and distinct component of the Snake River DPS, their management is confounded by the lack of a clear and

detailed understanding of their actual spawning distribution and population structure. Nielsen et al. (2009) found that steelhead in Idaho Snake River tributaries exhibit a complicated pattern of genetic structure with populations clustering according to drainage locality, not simply by "A-run" or "B-run" designations. Additionally, Copeland et al. (2017) identified a gradient of life history characteristics among traditional A- and B-run populations rather than a discrete, binary classification.

The above issues and similar conservation and management questions relating to Snake River steelhead and spring/summer Chinook Salmon may be addressed through genetic stock identification (GSI). 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). GSI 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 that parentage-based tagging and GSI applied to steelhead and Chinook Salmon at Lower Granite Dam can significantly reduce bias associated with estimation of escapement (Hargrove et al. 2020)

Combined, the above studies demonstrate the utility of GSI to obtain genetic stock abundance estimates for steelhead and Chinook Salmon in the Snake River basin. Continuation of GSI at LGR will allow us to 1) monitor genetic structure throughout the basin over time, and 2) estimate abundance, productivity, and life history diversity for genetic stocks throughout the Snake River. Sustained development and evaluation of GSI has been strongly recommended by regional research, monitoring, and evaluation (RME) workgroups. Similar work initiated at Bonneville Dam and in the lower Columbia River has been supported by the Independent Scientific Review Panel (http://www.nwcouncil.org/library/isrp/isrp2008-15.pdf).

## REPORT STRUCTURE

This report contains four sections, one for each of the study objectives. Section 1 discusses the continued use of GT-seq (Genotyping in Thousands by Sequencing) for more efficient and cost effective high-throughput genotyping for GSI in the Snake River basin as well as the addition of novel markers to our current marker panels. Section 2 summarizes efforts to update, maintain, and test SNP baselines for Snake River steelhead and spring/summer Chinook Salmon. These baselines are used to monitor genetic diversity and structure of natural-origin populations and are the reference for GSI at LGR. Section 3 addresses the use of GSI to estimate genetic stock proportions and life history diversity for natural-origin stocks at LGR. Section 4 directs readers to a separate stand-alone report that summarizes the life history and genetic diversity of steelhead and spring/summer Chinook Salmon detected at PIT tag detection systems. This year's report includes several appendices, which detail data and analyses provided to National Oceanic and Atmospheric Administration (NOAA) Fisheries for use in the 5-year status review of Snake River basin steelhead and Biological Opinion (Biop). Appendix A provides a

detailed description of wild steelhead genetic structure and diversity levels in the Snake River basin. Appendix B identifies genetic differentiation and phylogenetic relationships among steelhead hatchery and wild populations in Idaho. Appendix C details the influence of population management strategy (presence or absence of hatchery supplementation) for Snake River basin steelhead and levels of heterozygosity and number of breeders in Idaho.

In this report, we refer to adult steelhead and Chinook Salmon migrating past LGR using spawn years (SY). For steelhead, a spawn year refers to adults that migrate upstream past LGR during the fall of the previous calendar year and the spring of the current calendar year (e.g., SY2020 steelhead are adults that migrated past LGR between 7/1/19 – 6/30/20 and spawned in spring of 2020). For spring/summer Chinook Salmon, a spawn year refers to adults that migrate past the dam 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.

## SECTION 1: EVALUATE AND MAINTAIN SNP MARKER PANELS

### INTRODUCTION

The conclusion of calendar year 2020 marks our fifth full year of genotyping using the 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 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 iteration of SNP panel for Chinook Salmon includes 343 loci and steelhead includes 368 loci. Genetic stock identification and PBT work started in 2017 was performed using the 299 and 269 marker sets for Chinook Salmon and steelhead, respectively. Chinook Salmon and steelhead projects involving GSI and PBT initiated in 2020 have been genotyped at the 343 and 368 loci panels, respectively.

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 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 steelhead GT-seq panel, we have modified the bioinformatics pipeline used to call SNP variants to extract additional information from available sequence data. Microhaplotypes (hereafter microhaps) 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). Steelhead microhaps were discovered by searching the flanking regions 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. Here, we report on the characteristics of 92 microhaps discovered within the steelhead GT-seq process that have been included in the latest steelhead GSI baseline (Section 2). Microhap discovery efforts have begun for the Chinook GT-seq panel as well, but those results will be presented in a future report.

## METHODS

### Microhaplotype discovery process

For a detailed description of the bioinformatics process associated with microhaplotype discovery and calling, please reference Delomas et al. (2021).

### Microhaplotype description

Marker characteristics were summarized for SNPs and microhaps using steelhead samples from the steelhead baseline v4 (described in section 2). Descriptive characteristics including the number of alleles ($N_A$), effective number of alleles ($n_e$), expected heterozygosity ($H_E$), and marker-specific $F_{ST}$ values were generated using the software program GenAlEx v6.5 (Peakall and Smouse 2006). Tests were performed to assess deviation from HWE across all loci for each population using exact test based on Monte Carlo permutations of alleles (Guo and Thompson 1992) as implemented using the 'hw.test' function in the R package *pegas* (Paradis 2010). P-values were calculated based on 1,000 Monte Carlo permutations and were adjusted to account for tests of multiple comparison using the false discovery rate (Benjamini and Yekutieli 2001).

## RESULTS

### Microhaplotype description

Ninety-two microhaps were discovered within the 'CRITFC/IDFG Steelhead GT-seq v5.0 368' SNP panel. The maximum number of alleles per microhap was six (mean $N_A$ per locus = 3.45). When averaged across populations, the number of alleles ($N_A$) and effective number of alleles ($n_e$) were both higher for microhaps (mean $N_A$ = 2.56, mean $n_e$ = 1.59) relative to the 242 bi-allelic SNPs (mean $N_A$ = 1.88, mean $n_e$ = 1.43) (Table 1). Similarly, levels of expected heterozygosity ($H_E$) and genetic differentiation ($F_{ST}$) were higher for microhap markers relative to existing SNP markers (microhaplotypes: mean $H_E$ = 0.32, mean $F_{ST}$ = 0.053; bi-allelic SNPs: mean $H_E$ = 0.26, mean $F_{ST}$ = 0.049). In total, deviations from HWE were observed for 70 SNPs and 41 microhaps. Most frequently, a SNP deviated from HWE in a singular population (n = 41) although deviations from HWE were observed in up to 11 populations for a given SNP. Deviations from HWE among microhaps was most frequently observed in a single population (n = 27), although four microhaps were found to deviate at four separate populations (Table 1).

## 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 show that additional data present in existing marker panels can be extracted and used to increase the accuracy of GSI assignments for steelhead (Section 2). Specifically, the tweaking of bioinformatic pipelines to extract data on microhaps can yield greater levels of information, and the increased levels of variability associated with microhaps can directly benefit GSI and PBT efforts. Future work will include describing microhaps present in the current Chinook Salmon marker panel as well as developing new marker panels with even greater numbers of microhaps.

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. For this reporting period, we document the addition of microhaps to our current steelhead SNP panel and show that these markers contain higher levels of informative content relative to conventional SNP markers. Furthermore, we describe a new GSI steelhead baseline (Section 2), which includes the addition of microhap markers. For Chinook, we continue the use 'CRITFC IDFG Chinook GT-seq 4.0 343' marker panel, and ongoing efforts are being directed towards the discovery and description of microhap markers for this species.

## SECTION 2: UPDATE, MAINTAIN, AND TEST SNP BASELINES FOR STEELHEAD AND CHINOOK SALMON IN THE SNAKE RIVER

### 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 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, Camacho et al. 2018b) and returning adults (e.g. Camacho et al. 2017, Camacho et al. 2018b). 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. 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). To this end, we describe an updated GSI baseline

for steelhead in the Snake River basin that incorporates the addition of microhaps to assist with the assignment of fish sampled at Lower Granite Dam to their genetic stock of origin.

## METHODS

### Sample collection

The list of samples used to generate the steelhead baseline v4 represent a combination of samples used in v3.1 (Powell et al. 2018; see Appendix A for a detailed description), additional samples from previously unsampled populations, and collections from previously sampled populations that were made more recently. Each of these collections were amplified at the Steelhead GT-seq v5.0 368 SNP panel (Table 1)

### Statistical analyses

For sake of consistency, statistical analyses used to describe Steelhead baseline v4 were similar to those presented for the previous baseline versions (e.g., Vu et al. 2015). The first step involved curation of data to retain only samples that amplified at >90% of the SNP panel and removal of duplicate samples which matched at >95% of loci. One individual from each pair of duplicate samples was removed prior to downstream analyses.

We performed tests for deviation from HWE across all loci for each population; tests were conducted across all nuclear SNPs and microhaps for each population using an exact test based on Monte Carlo permutations of alleles following the algorithm of Guo and Thompson (1992) as implemented using the 'hw.test' function in the package *pegas* (Paradis 2010) in R (R Core Team 2020). P-values were calculated based on 1,000 Monte Carlo permutations and were adjusted to account for tests of multiple comparison using the false discovery rate (Benjamini and Yekutieli 2001).

Summary statistics for baseline collections including expected heterozygosity ($H_E$) and population specific $F_{ST}$ were generated using GENALEX v6.5. Higher $H_E$ indicates greater levels of genetic variability within a population; lower $H_E$ may indicate decreased genetic diversity attributable to various factors (population bottlenecks, reduced meta-population dynamics). Population-specific $F_{ST}$ (Weir and Cockerham 1984) is an indicator of the level of differentiation a population exhibits relative to all other baseline populations.

We performed self-assignment tests using the 'self_assign' function in the R package *rubias* (Anderson et al. 2008; Moran and Anderson 2019) to evaluate the accuracy of the Snake River SNP baselines v4 for individual assignment (IA). In self-assignment tests, each individual from the baseline is removed (one at a time), baseline allele frequencies are re-calculated with that individual removed, and the population (and genetic stock) of origin of that individual is then estimated. For each baseline collection, we calculated the proportion of individuals that assigned to each genetic stock. In addition to comparing self-assignment rates from the present baseline (v4) with the previous version (v3.1), we also performed self-assignment tests with and without the use of microhap markers (i.e., we performed tests considering only the original, bi-allelic SNP within each microhap versus the microhap) to quantify changes in assignment rates as a function of marker set. The expectation was that the additional heterozygosity (Section 1) observed with microhap markers would lead to increased self-assignment rates relative to the original bi-allelic SNP.

In addition to testing if adding microhaps to the GSI baseline affected self-assignment rates, we used PIT tag data on fish sampled at LGR to assess concordance rates between assigned genetic stock and the last known location of adult spawning steelhead. Briefly, when adult steelhead are sampled for biological data and genetics at LGR (see section 3 of this report for details) adipose intact adults are implanted with a PIT tag. Then, observations at PIT tag detection arrays located throughout the Snake River basin are used to assign a final location (last known detection site) to each individual, which are assumed to be their population of natal origin. Concordance between PIT tag detections and genetic stock were assessed by determining which PIT tag arrays correspond to different genetic stocks and then calculating the proportion of fish observed at individual arrays that were assigned to the corresponding genetic stock. Concordance rates were estimated using SY2020 adult steelhead and their associated PIT tag detections (section 4 of this report) against baselines v3.1 and v4.

We performed a principal component analysis (PCA) to visualize patterns in genetic structure using a multivariate approach. Whereas programs like STRUCTURE (Pritchard et al. 2000) make underlying assumptions about data (e.g., marker loci are unlinked and at linkage equilibrium with one another within populations) to assign individuals into population membership groups based on their genotypes, multivariate approaches do not. Thus, the inclusion of a PCA approach in addition to Bayesian clustering (STRUCTURE) provides multiple lines of evidence to support (or refute) proposed population structure. The PCA was performed using the “ade4” and “adegenet” packages (Thioulouse et al. 1997; Jombart 2008) in R version 3.3 (R Core Team 2020). Missing values were replaced by zero, and once the PCA was computed a mean value per population was plotted for the first and second components to reduce clutter on the resulting graph. The graphical output displayed the absolute variance (i.e., eigenvalues) explained by each of the principal components (PCs), with the *x*- and *y*-axes representing the percentage of total variance explained by PC1 and PC2, respectively. Samples that clustered together were interpreted as sharing a common ancestry (e.g., similar genotypes), and the distribution of points provided an indication of diversity levels (e.g., tighter clusters denote lower diversity than do widely dispersed clusters).

We used STRUCTURE 2.3.4 (Pritchard et al. 2000) to infer population structure using genetic Bayesian clustering methods. Default parameters of admixture and correlated allele frequencies were used to account for recent gene flow among populations and allow some flexibility for linkage disequilibrium within populations. These default settings are most flexible for dealing with real biological phenomena (Pritchard et al. 2009) and are likely most appropriate for steelhead populations under investigation. Within the admixture model, the LOCPRIOR option was used to include sampling locations as prior information (Hubisz et al. 2009). The LOCPRIOR version of the admixture model works by modifying the prior distribution for each individual’s population assignment; the new prior distributions allow the proportion of individuals assigned to a particular cluster to vary by location. In total, there were a total of 10 ‘sampling locations’ for steelhead, equal to the number of genetic stocks identified in Ackerman et al. (2012); the number of inferred clusters (*K*) was set to 10 for steelhead. A burn-in length of 50,000 with 100,000 repeats of the Monte Carlo Markov Chain (MCMC) was used to capture structure in the data and 10 repetitions were performed. Cluster membership values were averaged across runs.

## RESULTS

The steelhead baseline v4 consisted of 2,719 individuals from 65 sampling events. Samples collected temporally through time from the same populations as well as populations proximate on the landscape were pooled to represent 43 baseline collections (Table 2; Figure 1).

Sample collections were represented by a mix of juveniles (39) and returning adults (26). Twenty-one NMFS populations were represented and each population had an average number of 3.1 sample collections (range: 1–9). The total number of NMFS populations represented in the current baseline was 21 which contrasts with the 23 presented in v3.1. The current baseline does not have samples from the North Fork Salmon River (SRNFS-s), Wallowa River (GRWAL-s), or Pahsimeroi River (SRPAH-s) populations. Whereas baseline v3.1 placed Lolo Creek as part of the lower Clearwater population (CRLMA-s), in the present baseline it was presented as its own population (CRLOL-s). All five MPGs were represented in baseline v4. Following a correction for multiple testing, deviations from Hardy Weinberg equilibrium at the population-level occurred at less than 6% of the total SNP panel for all populations (Table 1).

Levels of expected heterozygosity at the population-level ranged from a low of 0.253 in Boulder Creek (Lower Salmon R) to a high of 0.314 in Morgan Creek (Upper Salmon R). Across all populations, the mean level of expected heterozygosity was 0.273. At the Genetic Stock level, heterozygosity was highest in the Lower Snake R (0.291), Upper Salmon R (0.289), and Grande Ronde R (0.283) and lowest in the South Fork Clearwater R (0.258), Upper Clearwater R (0.262) and Middle Fork Salmon R (0.266).

Population-specific $F_{ST}$ ranged from 0.016 (Asotin Cr, Lower Snake R) to 0.035 (Big Bear Cr, Lower Clearwater R), with an overall population-level mean of 0.025 (Figure 2). When examined at the Genetic Stock level, levels of differentiation were highest for the Middle Fork Salmon R (0.029), South Fork Clearwater R (0.029), and South Fork Salmon R (0.027) and lowest for the Lower Snake R (0.017), Grande Ronde R (0.019), and Imnaha R (0.020).

## Steelhead GSI

Self-assignment rates varied by genetic stock and were lowest in the most downstream stocks including the lower Snake R (0.60), lower Salmon R (0.67), and lower Clearwater R (0.73) (Table 3). The highest rates of self-assignment were observed in the South Fork Salmon R (0.99), followed by the Upper Clearwater R (0.96), and Middle Fork Salmon R (0.93). At the collection-level, self-assignment rates ranged from a low of 0.38 in Slate Creek (lower Salmon R) to a high of 1.00 (four separate collections in the Upper Clearwater R, three in Middle Fork Salmon R, and one each in the lower Salmon R and South Fork Salmon R genetic stocks; Table 3).

Relative to baseline v3.1 (Powell et al. 2018), self-assignment rates made with the current baseline were improved. Across all genetic stocks, the average increase in self-assignment rate was 0.10 (range 0.02–0.19) (Table 4). Greatest increases in assignment rates were observed in the lower Snake R (increase of 0.19), Grande Ronde R (0.18), and lower Salmon R (0.15) while little or no change in self-assignment was observed among the SF Clearwater R (0.02), Upper Clearwater R (0.03), and Middle Fork Salmon R (0.04). In general, terminal portions of rivers managed for wild stocks (e.g., Upper Clearwater R and Middle Fork Salmon R) had the highest rates of self-assignment.

The rate at which individuals were self-assigned to their genetic stock of origin was higher when the marker panel included microhaps relative to the same panel without microhaps (average increase = 0.02, range: 0–0.05). Observed increases in self-assignment rates were highest for the Grande Ronde R and Lower Snake R genetic stocks (both = 0.05) followed by the Upper Salmon R and Imnaha R genetic stocks (both = 0.04). One genetic stock (SFSALM) had equally high self-assignment rates regardless of whether or not microhaps were included (self-assignment rate = 0.99). A total of five genetic stock had self-assignment rates that were minimally

affected (0.01 increase in self-assignment rate) by the inclusion of microhaps (SFCLWR, UPCLWR, LOCLWR, MFSALM, LOSALM).

Concordance rates between PIT tag detections and genetic stock were estimated for 21 steelhead populations for SY2020 (Table 5). Concordance rates were higher for GSI baseline v4 (average = 81.9%, range = 36.4–100.0 %) relative to baseline v3.1 (average = 64.2%, range = 12.3–100.0%). Across all populations, concordance rates increased an average of 17.7% for baseline v4 relative to baseline v3.1 (range: -27.3–56.8%). Only two populations experienced a decrease in concordance (SRNFS-s; -27.3%, SRLEM; -6.9%) and one population was unchanged (SFSEC-s). At the genetic stock level, improvements in concordance were greatest for the Imnaha R and Lower Clearwater R.

The genetic structure of steelhead across the Snake River basin mirrored results from previous baseline characterizations (e.g., Vu et al. 2015 and references therein). Additionally, we observed congruent patterns in genetic structure across different analytical approaches (e.g., PCA, Bayesian clustering) which varied in their underlying model assumptions. Had multivariate and Bayesian models identified dissimilar patterns, this could have been the result of biological processes (e.g., population structure/Wahlund effect) which violate model assumptions (e.g., populations not in HWE) leading to erroneous results. Instead, we observed clear genetic distinction of the South Fork Clearwater (SFCLWR), Upper Clearwater River (UPCLWR), Middle Fork Salmon River (MFSALM), and South Fork Salmon River (SFSALM) genetic stocks based on results from both PCA and STRUCTURE (Figure 2; 3). In contrast, collections from the lower Snake (LSNAKE), Grande Ronde (GRROND), Imnaha (IMNAHA), and lower Clearwater (LOCLWR) rivers showed higher admixture among stocks suggesting either historical or ongoing levels of gene flow. Similarly, the lower Salmon River (LOSALM) and upper Salmon River (UPSALM) showed evidence of admixture (Figure 2; 3), which is not surprising given historical stocking practices which promoted admixture among these two stocks (Powell and Campbell 2020).

## DISCUSSION

For the current reporting period, a new steelhead baseline was characterized to identify the genetic stock of origin of natural origin steelhead returning to the Snake River basin. Steelhead baseline v4 capitalizes on the use of microhaps to add increased resolution to a marker panel. Despite a reduction in the number of collections and samples used to construct baseline v4, self-assignment rates increased relative to previous iterations. This was not necessarily unexpected as previous work has shown that increasing the number of genotyped loci would provide a greater benefit to GSI assignment probabilities relative to increasing the number of individuals genotyped (Powell et al. 2018). In several areas within the Snake River basin, self-assignment rates for steelhead populations were improved considerably relative to previous baselines.

Several minor differences in population coverage were noted for the current baseline relative to previous iterations. Specifically, there was a reduction in the number of NMFS populations in baseline v4 (21) relative to v3.1 (23). As noted in the results, baseline v4 does not have samples from the North Fork Salmon River (SRNFS-s), Wallowa River (GRWAL-s), or Pahsimeroi River (SRPAH-s) populations. Future sampling and sequencing efforts should focus on expanding the number of populations present in the baseline to maximize coverage across the basin. Many of the collections used for steelhead baseline v4 were from more recent sampling efforts which is significant in that it reflects contemporary genetic structure. While genetic structure

is expected to remain consistent over short-time scales, the use of newer collections provides updated allele frequencies across populations.

While we describe a new genetic baseline for steelhead in this chapter, we have maintained the use of 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 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 (Appendix A).

## SECTION 3. IMPLEMENT GSI METHODS TO ESTIMATE PROPORTIONS AND BIOLOGICAL PARAMETERS OF NATURAL-ORIGIN STOCKS AT LOWER GRANITE DAM

### INTRODUCTION

The long-range goal of the Idaho Department of Fish and Game's (IDFG) anadromous fish program is to preserve Idaho's salmon and steelhead runs and recover them to benefit all users (IDFG 2007). This goal is consistent with basin-wide mitigation and recovery efforts. Fisheries management requires an understanding of how salmonid populations function as well as regular status assessments to document progress towards achieving these goals (McElhany et al. 2000). Estimates of abundance, combined with sex and age information over time, allow estimation of population growth rates; and both abundance and productivity metrics provide indicators of the resiliency and viability of populations. Estimates of these metrics at the genetic stock or MPG level can be used by fisheries managers to prescribe sustainable harvest rates for larger populations, while protecting weaker stocks and the genetic diversity within them.

However, population level or MPG assessments of abundance and productivity for ESA-listed Snake River steelhead and spring/summer Chinook Salmon can be particularly difficult (see Report Introduction). Specific data on Snake River steelhead and Chinook Salmon MPGs and populations are lacking, particularly key parameters such as population abundance, age composition, genetic diversity, recruits per spawner, and survival rates (ICTRT 2003). Genetic Stock Identification 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 Appendix A (for steelhead). Here we use complementary sets of genotype data from adults sampled at the Lower Granite Dam (LGR) 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.

In 2020, a global pandemic (COVID-19) resulted in a significant alteration in sampling efforts at LGR. Specifically, trap operations were closed for all sampling efforts between March 25$^{th}$ – July 1$^{st}$, during which a combination of juvenile and adult steelhead and Chinook Salmon would have been otherwise sampled. As a result of limited operations, no adult Chinook Salmon (SY2020) were collected for genetic analysis, and the number of juvenile Chinook Salmon and steelhead (MY2020) were a fraction of normal collections. The number of adult steelhead sampled in SY2020 was less than normal levels. Thus, we present individual genetic assignments and life history diversity information for SY2020 steelhead adults only, small numbers of MY2020 juveniles (both steelhead and Chinook Salmon) sampled at LGR, but no adult Chinook Salmon.

### METHODS

#### Adult Trap Operations

Detailed methods for operation of the LGR adult trap can be found in Lawry et al. (2020) and citations within. Briefly, adult steelhead and spring/summer Chinook Salmon migrating upstream past LGR may be intercepted at a trapping facility, located on the adult fish ladder above

the counting window, according to a predetermined sampling rate. Trapping rates that achieve sample requirements for various projects while balancing fish handling concerns are determined by a committee of collaborating management agencies. Sample rates are typically 10–20%. The sample rate determines how long a trap gate remains open four times per hour; the trap is operational 24 hours per day.

## Juvenile Trap Operations

Detailed methods for operation of the LGR juvenile trap can be found in Camacho et al. (2018b) and citations within. The juvenile trap is located on the LGR juvenile bypass system. The trap captures a systematic sample of fish by operating two trap gates according to a predetermined sample rate. The sample rate determines how long the trap gates remain open, up to six times per hour. The trap is operational 24 hours per day and fish are processed every morning. Sample rate is predetermined daily to collect 250–750 fish per day (all species combined) and is based on the expected number of fish entrained in the bypass system that day.

## Fish Handling Protocols (Adults and Juveniles)

Fish handling procedures are detailed in Lawry et al. (2020) for adults and Camacho et al. (2018b) for juveniles (and citations within both reports). Fish captured at the LGR adult or juvenile trap are anesthetized; identified to species; examined for external marks, tags, and injuries; scanned for an internal CWT or PIT tag; and measured for fork length (FL). All fish are examined for the presence (unclipped) or absence (clipped) of the adipose fin and classified to putative origin (hatchery or natural). All natural-origin fish have an unclipped adipose fin because they spend their entire life cycle in the natural environment. Most hatchery-origin fish have a clipped adipose fin. However, some hatchery fish may be released with an unclipped adipose fin for supplementation or tribal harvest opportunities. Thus, unclipped fish are also examined for a CWT or a PIT tag. The presence of a CWT definitively identifies an unclipped fish as hatchery origin. For unclipped adult steelhead, hatchery origin may also be determined by the presence of dorsal and/or ventral fin erosion, which is assumed to occur only in hatchery-reared steelhead (Latremouille 2003). Captured fish determined to be putatively natural-origin or unclipped hatchery with no CWT are sampled for scales (for age; except juvenile Chinook). 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 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 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). A limited number of MY2020 steelhead and Chinook Salmon juveniles were collected during 2020, and those along with SY2020 steelhead adults were processed at IDFG's Eagle Fish Genetics Lab (EFGL) in Eagle, 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 MY2020 and adult fish sampled in SY2020 using the software SNPPIT (Anderson 2010). 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.

In an effort to confirm that PBT does not falsely assign wild origin salmon and steelhead as hatchery-origin, we calculated a false positive rate by treating GSI collections of wild juveniles and adults as potential offspring of hatchery parents. These individuals should not assign to hatchery parents in the PBT baseline, and include populations of wild Chinook Salmon and steelhead that are managed exclusively for wild populations as well as rivers with ongoing hatchery supplementation efforts. The false assignment rate (wild-origin fish falsely classified as hatchery) was calculated as the number of fish from our GSI baseline that were matched to parents in our PBT collections. For Chinook Salmon, we compared GSI baseline v3.1 against all spring/summer Chinook Salmon hatchery broodstock sampled for spawn years 2014–2017 using 170 SNP markers that overlapped the 179 SNP GSI marker panel and the Ots 298 SNP panel. For steelhead, we compared GSI baseline v3.1 against all hatchery broodstock sampled for spawn years 2013–2018 using 176 SNP markers that overlapped the 179 SNP GSI marker panel and the Omy 379 SNP panel. Parentage analysis was performed using SNPPIT.

## Genetic Stock Identification

Individual assignment (IA) tests were conducted on all unmarked juveniles and adults that did not receive a PBT assignment in MY2020 and SY2020 using the Snake River SNP baselines v3.1 described in Section 2 of Powell et al. (2018) for Chinook Salmon and Appendix A for steelhead. SNP allele frequency estimates from baseline collections serve as the reference information for IA tests. Fish sampled at the LGR adult and juvenile trapping facilities were genotyped at the same SNPs, and multilocus genotype data were used to assign individual fish back to their estimated population (and genetic stock) of origin (Pella and Milner 1987; Shaklee et al. 1999). In IA, the probability that each fish originates from a baseline population is calculated based on the likelihood that the individual's genotype belongs to that population, given baseline allele frequency estimates. Individual population estimates were first calculated and then summed into genetic stock estimates (allocate-sum procedure; Wood et al. 1987). Genetic stocks (aka

reporting groups) 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). 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 three sample groups:

- SY2020 steelhead adults (partial sample)
- MY2020 steelhead juveniles (partial sample)
- MY2020 Chinook juveniles (partial sample)

## RESULTS

We inventoried 2,973 samples (including both steelhead and Chinook Salmon) from SY2020 adults and MY2020 juveniles at LGR (Table 6). All 2,973 inventoried samples were queued for genotyping. We failed to successfully genotype 10 (0.3%) of the queued samples. We assigned 441 (14.9) samples to hatchery parents in our PBT baseline despite all samples being collected from fish with intact adipose fins. For the remaining 2,522 samples, we performed IA, which are summarized below and in Tables 6–8.

### SY2020 Steelhead Adults

Of the 2,800 unclipped adult steelhead sampled in SY2020, 2,579 (92.1%) were phenotypically identified as natural-origin because they had no apparent dorsal or ventral fin erosion (Table 6). The remaining 221 (7.9%) fish were identified as hatchery origin due to the presence of dorsal and/or ventral fin erosion. All 2,800 total samples collected in SY2020 were queued for genotyping, and 2,794 (99.8%) of the queued samples were successfully genotyped. We assigned 234 (9.1%) of the 2,573 successfully genotyped, phenotypically natural-origin fish to hatchery parents. Of the 221 fish that were successfully genotyped and phenotypically identified as hatchery origin fish, 202 (91.4%) individuals were assigned via PBT to hatchery parents. All remaining successfully genotyped, phenotypically natural-origin (2,339), and phenotypically hatchery (19) adult steelhead that failed to assign to hatchery parents were

assigned to a genetic stock via IA. Parentage analysis performed using the steelhead GSI baseline v3.1 as potential offspring of hatchery broodstock sampled for spawn years 2013–2018 identified zero PBT assignments, representing a false assignment rate of 0%.

Life history diversity information (sex, length, and ocean age) for the 2,339 unclipped, phenotypically wild adult steelhead sampled in SY2020 and assigned a genetic stock is summarized in Table 7. Of the 2,339 steelhead assigned a genetic stock, 454 (19.4%) assigned to UPSALM, 129 (5.5%) to MFSALM, 26 (1.1%) to SFSALM, 44 (1.9%) to LOSALM, 74 (3.2%) to UPCLWR, 91 (3.9%) to SFCLWR, 111 (4.7%) to LOCLWR, 174 (7.4%) to IMNAHA, 790 (33.80%) to GRROND, and 446 (19.1%) to LSNAKE.

### MY2020 Steelhead Juveniles

All 123 unclipped juvenile steelhead samples for MY2020 were queued for genotyping (Table 6). A total of 119 (96.7%) juveniles were genotyped successfully, with 1 (0.8%) assigning back to hatchery parents. The remaining 118 (99.2%) were assigned a genetic stock via IA.

Life history diversity information for the 118 emigrating steelhead smolts that were assigned to a genetic stock is summarized in Table 8. Of the 118 steelhead smolts assigned a genetic stock, 1 (0.8%) assigned to UPSALM, 0 to MFSALM, 0 to SFSALM, 0 to LOSALM, 1 (0.8%) to UPCLWR, 4 (3.4%) to SFCLWR, 8 (6.8%) to LOCLWR, 0 to IMNAHA, 74 (62.7%) to GRROND, and 30 (25.4%) to LSNAKE.

### MY2020 Chinook Salmon Juveniles

We inventoried 50 unclipped juvenile Chinook Salmon for MY2020. All inventoried juvenile Chinook Salmon (50) were queued for genotyping and 50 (100%) of those individuals were successfully genotyped (Table 5). Of the juveniles that were successfully genotyped, 4 (8.0%) were assigned back to hatchery parents and the remaining 46 (92.0%) were assigned a genetic stock via IA. Parentage analysis performed using the Chinook Salmon GSI baseline v3.1 as potential offspring of hatchery broodstock sampled for spawn years 2014–2017 identified zero PBT assignments, representing a false assignment rate of 0%.

Life history diversity information for the 46 Chinook Salmon smolts assigned a genetic stock is summarized in Table 9. Of the 46 Chinook Salmon smolts assigned a genetic stock, 1 (2.2%) assigned to UPSALM, 0 to MFSALM, 0 to CHMBLN, 0 to SFSALM, 37 (80.0%) to HELLSC, 0 (0.0%) to TUCANO, and 8 (17.0%) to FALL.

## DISCUSSION

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

Trapped adult fish are also PIT tagged by the Integrated Status and Effectiveness Monitoring Project (ISEMP; BPA Project 2003-017-00). Detection of these PIT-tagged fish throughout the Snake River basin are used in a Bayesian branching model to provide reliable and unbiased estimates of abundance at the tributary scale (QCI 2013; See et al. 2016). A multi-agency collaboration has been initiated to utilize information generated from these two innovative technologies (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 4.

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.

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. 2020). This overestimate is likely the largest potential source of bias in abundance estimation within the Snake River basin. We illustrate the importance of this filtering step with returning steelhead. Hatchery steelhead can potentially be identified through the presence of dorsal and/or ventral fin erosion that is assumed absent from natural-origin individuals (Latremouille 2003) in addition to any physical marks (e.g., coded wire tags, adipose fin clip) applied at the hatchery. Despite this additional “mark,” using PBT we identified an additional 9.1% of the phenotypically natural-origin steelhead trapped at LGR as hatchery origin in SY2020 (Table 5). Because there is no additional phenotypic hatchery “mark” for Chinook Salmon, the rate of adipose-intact hatchery fish identified via PBT-only is higher than for steelhead. 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 4: SUMMARIZE LIFE HISTORY AND GENETIC DIVERSITY OF NATURAL-ORIGIN STEELHEAD AND SPRING/SUMMER CHINOOK SALMON THAT ARE DETECTED AT PIT TAG DETECTIONS SYSTEMS IN THE SNAKE RIVER BASIN SY2020

The life history and genetic diversity of steelhead and Chinook Salmon populations within the Snake River basin were summarized for SY2010–2019 in a separate report (IPTDSW 2020). Hargrove and others are presenting this information for SY2020 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." Because no returning adult Chinook Salmon were sampled for SY2020, this report will focus on returning steelhead adults.

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**TABLES**

Table 1. Details of 334 SNPs (242 number bi-allelic, 92 number microhaplotypes) screened against 44 collections of steelhead (*Oncorhynchus mykiss*) from the Snake River basin used for parentage-based tagging (PBT) and genetic stock identification (GSI). Summary statistics include the number of alleles per locus averaged across populations ($N_A$), the effective number of alleles ($n_e$), expected heterozygosity ($H_E$), and mean fixation index ($F_{ST}$). The column HWE designates the number of populations for which a marker deviated from Hardy-Weinberg equilibrium (HWE) following a p-value correction for tests of multiple comparison using the false discovery rate (FDR).

| **Marker** | **type** | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | **HWE** |
|---|---|---|---|---|---|---|
| OMS00039_mh | microhaplotype | 2.419 | 2.011 | 0.502 | 0.009 | 1 |
| OMS00052_mh | microhaplotype | 3.000 | 2.576 | 0.606 | 0.048 | 0 |
| OMS00077_mh | microhaplotype | 2.488 | 1.927 | 0.477 | 0.047 | 1 |
| OMS00101_mh | microhaplotype | 2.233 | 1.913 | 0.475 | 0.046 | 0 |
| OMS00116_mh | microhaplotype | 2.093 | 1.136 | 0.110 | 0.048 | 2 |
| OMS00118_mh | microhaplotype | 2.721 | 1.927 | 0.476 | 0.071 | 0 |
| OMS00120_mh | microhaplotype | 2.791 | 1.428 | 0.275 | 0.078 | 2 |
| OMS00128_mh | microhaplotype | 2.140 | 1.077 | 0.068 | 0.033 | 0 |
| OMS00129_mh | microhaplotype | 3.860 | 2.457 | 0.575 | 0.041 | 1 |
| OMS00143_mh | microhaplotype | 3.140 | 1.316 | 0.229 | 0.037 | 4 |
| OMS00149_mh | microhaplotype | 2.837 | 2.063 | 0.511 | 0.053 | 0 |
| OMS00151_mh | microhaplotype | 2.209 | 1.437 | 0.291 | 0.041 | 1 |
| OMS00175_mh | microhaplotype | 2.349 | 1.904 | 0.471 | 0.039 | 0 |
| Omy_101832195_mh | microhaplotype | 2.140 | 1.897 | 0.471 | 0.045 | 0 |
| Omy_102867443_mh | microhaplotype | 1.791 | 1.075 | 0.065 | 0.041 | 0 |
| Omy_104519624_mh | microhaplotype | 2.116 | 1.687 | 0.391 | 0.072 | 0 |
| Omy_107336170_mh | microhaplotype | 2.628 | 1.388 | 0.266 | 0.068 | 0 |
| Omy_108007193_mh | microhaplotype | 2.116 | 1.820 | 0.447 | 0.047 | 1 |
| Omy_109243222_mh | microhaplotype | 2.907 | 1.555 | 0.344 | 0.034 | 0 |
| Omy_110201359_mh | microhaplotype | 3.093 | 1.823 | 0.423 | 0.087 | 2 |
| Omy_11138351_mh | microhaplotype | 2.442 | 1.913 | 0.470 | 0.045 | 1 |
| Omy_112301202_mh | microhaplotype | 1.698 | 1.048 | 0.040 | 0.072 | 0 |
| Omy_11282082_mh | microhaplotype | 2.070 | 1.065 | 0.057 | 0.035 | 0 |
| Omy_117540259_mh | microhaplotype | 2.302 | 1.310 | 0.215 | 0.067 | 0 |
| Omy_120255332_mh | microhaplotype | 1.767 | 1.041 | 0.037 | 0.029 | 0 |
| Omy_128923433_mh | microhaplotype | 2.372 | 1.960 | 0.488 | 0.040 | 0 |
| Omy_130524160_mh | microhaplotype | 2.535 | 1.892 | 0.467 | 0.028 | 0 |
| Omy_131460646_mh | microhaplotype | 1.860 | 1.057 | 0.051 | 0.039 | 2 |
| Omy_187760385_mh | microhaplotype | 1.395 | 1.021 | 0.019 | 0.036 | 0 |
| Omy_96222125_mh | microhaplotype | 2.907 | 1.449 | 0.295 | 0.039 | 0 |
| Omy_9707773_mh | microhaplotype | 2.395 | 1.236 | 0.172 | 0.065 | 4 |
| Omy_97954618_mh | microhaplotype | 2.651 | 1.566 | 0.349 | 0.047 | 0 |
| Omy_99300202_mh | microhaplotype | 2.721 | 1.631 | 0.375 | 0.041 | 1 |
| Omy_anp17_mh | microhaplotype | 2.977 | 1.906 | 0.443 | 0.112 | 0 |
| Omy_aromat280_mh | microhaplotype | 2.837 | 1.544 | 0.336 | 0.036 | 1 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| Omy_aspAT123_mh | microhaplotype | 2.419 | 1.722 | 0.411 | 0.031 | 0 |
| Omy_BACF5284_mh | microhaplotype | 2.767 | 1.690 | 0.389 | 0.043 | 0 |
| Omy_ca05064_mh | microhaplotype | 2.814 | 1.866 | 0.459 | 0.042 | 2 |
| Omy_carban1264_mh | microhaplotype | 2.814 | 1.311 | 0.222 | 0.046 | 1 |
| Omy_cox2335_mh | microhaplotype | 2.930 | 1.556 | 0.347 | 0.033 | 1 |
| Omy_cyp17153_mh | microhaplotype | 2.395 | 1.330 | 0.226 | 0.095 | 4 |
| Omy_e1147_mh | microhaplotype | 1.977 | 1.080 | 0.070 | 0.034 | 0 |
| Omy_g1282_mh | microhaplotype | 2.116 | 1.929 | 0.480 | 0.041 | 0 |
| Omy_GHSR121_mh | microhaplotype | 2.767 | 1.354 | 0.236 | 0.061 | 0 |
| Omy_hsc71580_mh | microhaplotype | 3.163 | 2.110 | 0.517 | 0.045 | 1 |
| Omy_IL1b163_mh | microhaplotype | 2.535 | 1.354 | 0.240 | 0.094 | 1 |
| Omy_lpl220_mh | microhaplotype | 3.488 | 1.783 | 0.405 | 0.056 | 1 |
| Omy_mcsf268_mh | microhaplotype | 2.070 | 1.068 | 0.058 | 0.041 | 2 |
| Omy_metA161_mh | microhaplotype | 2.442 | 1.556 | 0.342 | 0.046 | 0 |
| Omy_metB138_mh | microhaplotype | 2.977 | 1.499 | 0.319 | 0.038 | 0 |
| Omy_MYC_2_mh | microhaplotype | 2.233 | 1.703 | 0.407 | 0.024 | 0 |
| Omy_nach200_mh | microhaplotype | 1.860 | 1.043 | 0.037 | 0.052 | 2 |
| Omy_nkef241_mh | microhaplotype | 2.140 | 1.903 | 0.473 | 0.030 | 0 |
| Omy_nxt2273_mh | microhaplotype | 2.907 | 1.258 | 0.190 | 0.045 | 2 |
| Omy_oxct85_mh | microhaplotype | 2.558 | 1.270 | 0.201 | 0.042 | 1 |
| Omy_pad196_mh | microhaplotype | 2.791 | 1.142 | 0.118 | 0.034 | 0 |
| Omy_ppie232_mh | microhaplotype | 2.116 | 1.266 | 0.202 | 0.028 | 1 |
| Omy_RAD1610420_mh | microhaplotype | 2.953 | 2.211 | 0.544 | 0.025 | 0 |
| Omy_RAD1890348_mh | microhaplotype | 1.744 | 1.055 | 0.049 | 0.040 | 0 |
| Omy_RAD2389458_mh | microhaplotype | 3.442 | 2.261 | 0.554 | 0.032 | 1 |
| Omy_RAD25678_mh | microhaplotype | 3.302 | 2.224 | 0.545 | 0.061 | 3 |
| Omy_RAD2608069_mh | microhaplotype | 2.465 | 1.854 | 0.454 | 0.097 | 0 |
| Omy_RAD2669136_mh | microhaplotype | 2.907 | 1.775 | 0.428 | 0.028 | 0 |
| Omy_RAD2970018_mh | microhaplotype | 2.488 | 1.531 | 0.328 | 0.049 | 0 |
| Omy_RAD297626_mh | microhaplotype | 2.465 | 1.207 | 0.153 | 0.064 | 1 |
| Omy_RAD392622_mh | microhaplotype | 1.930 | 1.185 | 0.141 | 0.063 | 1 |
| Omy_RAD4361242_mh | microhaplotype | 3.000 | 2.736 | 0.633 | 0.042 | 1 |
| Omy_RAD4744453_mh | microhaplotype | 2.767 | 1.525 | 0.327 | 0.058 | 1 |
| Omy_RAD4911135_mh | microhaplotype | 2.953 | 2.035 | 0.498 | 0.064 | 0 |
| Omy_RAD5245817_mh | microhaplotype | 2.047 | 1.211 | 0.132 | 0.353 | 1 |
| Omy_RAD5281228_mh | microhaplotype | 2.372 | 1.695 | 0.397 | 0.046 | 0 |
| Omy_RAD537456_mh | microhaplotype | 2.907 | 1.342 | 0.237 | 0.053 | 4 |
| Omy_RAD5821370_mh | microhaplotype | 3.302 | 1.532 | 0.332 | 0.043 | 0 |
| Omy_RAD5883515_mh | microhaplotype | 2.140 | 1.432 | 0.284 | 0.060 | 0 |
| Omy_RAD701631_mh | microhaplotype | 3.000 | 2.769 | 0.637 | 0.042 | 0 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| Omy_RAD72108_mh | microhaplotype | 3.023 | 2.094 | 0.519 | 0.042 | 0 |
| Omy_RAD7252844_mh | microhaplotype | 1.767 | 1.142 | 0.109 | 0.079 | 0 |
| Omy_RAD7320463_mh | microhaplotype | 2.860 | 1.211 | 0.165 | 0.041 | 1 |
| Omy_RAD738450_mh | microhaplotype | 1.791 | 1.050 | 0.042 | 0.053 | 1 |
| Omy_RAD7396373_mh | microhaplotype | 3.860 | 2.246 | 0.542 | 0.052 | 0 |
| Omy_RAD7657062_mh | microhaplotype | 2.186 | 1.111 | 0.093 | 0.040 | 0 |
| Omy_RAD7931458_mh | microhaplotype | 1.326 | 1.016 | 0.015 | 0.027 | 0 |
| Omy_RAD8670672_mh | microhaplotype | 2.279 | 1.639 | 0.348 | 0.208 | 3 |
| Omy_RAD900413_mh | microhaplotype | 2.907 | 2.115 | 0.523 | 0.044 | 1 |
| Omy_RAD9248564_mh | microhaplotype | 4.372 | 2.175 | 0.512 | 0.059 | 0 |
| Omy_sast264_mh | microhaplotype | 2.116 | 1.458 | 0.304 | 0.027 | 0 |
| Omy_SECC22b88_mh | microhaplotype | 2.326 | 1.191 | 0.144 | 0.060 | 0 |
| Omy_sys1188_mh | microhaplotype | 2.395 | 1.296 | 0.211 | 0.062 | 1 |
| Omy_tlr5205_mh | microhaplotype | 2.326 | 1.176 | 0.136 | 0.057 | 1 |
| Omy_txnip343_mh | microhaplotype | 2.535 | 1.635 | 0.380 | 0.032 | 0 |
| Omy_u0956119_mh | microhaplotype | 3.395 | 1.325 | 0.231 | 0.039 | 1 |
| Omy_U11_2b154_mh | microhaplotype | 3.349 | 1.801 | 0.435 | 0.045 | 0 |
| M09AAD076 | biallelic | 2.000 | 1.937 | 0.483 | 0.031 | 0 |
| M09AAJ163 | biallelic | 2.000 | 1.728 | 0.414 | 0.040 | 0 |
| M09AAE082 | biallelic | 2.000 | 1.469 | 0.310 | 0.039 | 1 |
| OMS00002 | biallelic | 2.000 | 1.868 | 0.463 | 0.025 | 0 |
| OMS00006 | biallelic | 2.000 | 1.928 | 0.480 | 0.035 | 0 |
| OMS00024 | biallelic | 2.000 | 1.857 | 0.459 | 0.046 | 0 |
| OMS00053 | biallelic | 2.000 | 1.883 | 0.466 | 0.047 | 0 |
| OMS00057 | biallelic | 2.000 | 1.828 | 0.449 | 0.057 | 0 |
| OMS00058 | biallelic | 2.000 | 1.853 | 0.453 | 0.071 | 0 |
| OMS00062 | biallelic | 2.000 | 1.585 | 0.357 | 0.041 | 0 |
| OMS00064 | biallelic | 2.000 | 1.780 | 0.427 | 0.073 | 0 |
| OMS00068 | biallelic | 2.000 | 1.759 | 0.424 | 0.034 | 1 |
| OMS00070 | biallelic | 2.000 | 1.899 | 0.471 | 0.058 | 0 |
| OMS00071 | biallelic | 2.000 | 1.876 | 0.463 | 0.045 | 0 |
| OMS00072 | biallelic | 2.000 | 1.921 | 0.478 | 0.032 | 0 |
| OMS00074 | biallelic | 2.000 | 1.827 | 0.447 | 0.061 | 0 |
| OMS00078 | biallelic | 2.000 | 1.592 | 0.362 | 0.032 | 0 |
| OMS00079 | biallelic | 2.000 | 1.925 | 0.479 | 0.040 | 0 |
| OMS00111 | biallelic | 2.000 | 1.499 | 0.316 | 0.067 | 0 |
| OMS00089 | biallelic | 2.000 | 1.611 | 0.365 | 0.056 | 0 |
| OMS00090 | biallelic | 2.000 | 1.925 | 0.479 | 0.040 | 0 |
| OMS00105 | biallelic | 2.000 | 1.795 | 0.437 | 0.032 | 0 |
| OMS00106 | biallelic | 2.000 | 1.541 | 0.336 | 0.053 | 0 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| OMS00154 | biallelic | 2.000 | 1.467 | 0.307 | 0.040 | 0 |
| OMS00112 | biallelic | 1.977 | 1.455 | 0.297 | 0.053 | 0 |
| OMS00121 | biallelic | 2.000 | 1.936 | 0.483 | 0.031 | 0 |
| OMS00132 | biallelic | 2.000 | 1.889 | 0.468 | 0.040 | 0 |
| OMS00179 | biallelic | 2.000 | 1.723 | 0.410 | 0.051 | 3 |
| OMS00180 | biallelic | 2.000 | 1.759 | 0.424 | 0.059 | 0 |
| Omy_101993189 | biallelic | 2.000 | 1.576 | 0.352 | 0.053 | 0 |
| Omy_102505102 | biallelic | 2.000 | 1.811 | 0.444 | 0.039 | 0 |
| Omy_105105448 | biallelic | 2.000 | 1.845 | 0.450 | 0.083 | 0 |
| Omy_105385406 | biallelic | 2.000 | 1.839 | 0.453 | 0.042 | 0 |
| Omy_105714265 | biallelic | 2.000 | 1.726 | 0.415 | 0.039 | 0 |
| Omy_10780634 | biallelic | 2.000 | 1.696 | 0.397 | 0.060 | 0 |
| Omy_109894185 | biallelic | 2.000 | 1.793 | 0.436 | 0.050 | 4 |
| Omy_110064419 | biallelic | 2.000 | 1.830 | 0.446 | 0.066 | 0 |
| Omy_113490159 | biallelic | 2.000 | 1.851 | 0.456 | 0.074 | 0 |
| Omy_114315438 | biallelic | 2.000 | 1.843 | 0.449 | 0.078 | 0 |
| Omy_114587480 | biallelic | 2.000 | 1.765 | 0.425 | 0.056 | 0 |
| Omy_129870756 | biallelic | 2.000 | 1.398 | 0.275 | 0.034 | 0 |
| Omy_116733349 | biallelic | 2.000 | 1.679 | 0.396 | 0.031 | 0 |
| Omy_97660230 | biallelic | 2.000 | 1.799 | 0.437 | 0.041 | 0 |
| Omy_arp630 | biallelic | 2.000 | 1.912 | 0.475 | 0.046 | 0 |
| Omy_b1266 | biallelic | 2.000 | 1.659 | 0.392 | 0.023 | 1 |
| Omy_BACB4324 | biallelic | 2.000 | 1.903 | 0.472 | 0.042 | 0 |
| Omy_ada1071 | biallelic | 2.000 | 1.450 | 0.304 | 0.021 | 1 |
| Omy_redd1410 | biallelic | 2.000 | 1.469 | 0.306 | 0.058 | 0 |
| Omy_cd59206 | biallelic | 2.000 | 1.702 | 0.406 | 0.026 | 0 |
| Omy_cox1221 | biallelic | 2.000 | 1.840 | 0.452 | 0.047 | 0 |
| Omy_crb106 | biallelic | 2.000 | 1.859 | 0.460 | 0.067 | 3 |
| Omy_gluR79 | biallelic | 2.000 | 1.933 | 0.481 | 0.027 | 0 |
| Omy_hsf2146 | biallelic | 2.000 | 1.726 | 0.405 | 0.091 | 0 |
| Omy_IL17185 | biallelic | 2.000 | 1.919 | 0.477 | 0.043 | 0 |
| Omy_Il1b_028 | biallelic | 2.000 | 1.365 | 0.258 | 0.029 | 0 |
| Omy_IL6320 | biallelic | 2.000 | 1.485 | 0.316 | 0.032 | 0 |
| Omy_NaKATPa350 | biallelic | 2.000 | 1.654 | 0.386 | 0.038 | 0 |
| Omy_ntl27 | biallelic | 2.000 | 1.767 | 0.425 | 0.059 | 0 |
| Omy_Ogo4212 | biallelic | 2.000 | 1.841 | 0.453 | 0.041 | 0 |
| Omy_bcAKala380rd | biallelic | 2.000 | 1.699 | 0.402 | 0.044 | 0 |
| Omy_Ots249227 | biallelic | 2.000 | 1.680 | 0.396 | 0.038 | 0 |
| Omy_p53262 | biallelic | 2.000 | 1.515 | 0.329 | 0.043 | 0 |
| Omy_rapd167 | biallelic | 2.000 | 1.376 | 0.263 | 0.034 | 0 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| Omy_rbm4b203 | biallelic | 1.884 | 1.356 | 0.238 | 0.090 | 10 |
| Omy_srp0937 | biallelic | 2.000 | 1.693 | 0.401 | 0.040 | 0 |
| Omy_stat3273 | biallelic | 1.977 | 1.482 | 0.310 | 0.047 | 0 |
| Omy_u0953469 | biallelic | 2.000 | 1.831 | 0.451 | 0.072 | 0 |
| Omy_u0954311 | biallelic | 2.000 | 1.753 | 0.421 | 0.048 | 0 |
| Omy_vatf406 | biallelic | 2.000 | 1.745 | 0.417 | 0.070 | 0 |
| OMY1011SNP | biallelic | 2.000 | 1.561 | 0.348 | 0.038 | 2 |
| M09AAC055 | biallelic | 1.977 | 1.160 | 0.127 | 0.054 | 0 |
| OMGH1PROM1SNP1 | biallelic | 1.977 | 1.252 | 0.179 | 0.084 | 0 |
| OMS00003 | biallelic | 2.000 | 1.349 | 0.249 | 0.031 | 0 |
| OMS00008 | biallelic | 1.977 | 1.358 | 0.245 | 0.063 | 0 |
| OMS00013 | biallelic | 1.977 | 1.168 | 0.136 | 0.034 | 1 |
| OMS00014 | biallelic | 1.442 | 1.028 | 0.025 | 0.041 | 0 |
| OMS00015 | biallelic | 1.953 | 1.128 | 0.105 | 0.048 | 0 |
| OMS00017 | biallelic | 2.000 | 1.572 | 0.348 | 0.100 | 1 |
| OMS00018 | biallelic | 1.977 | 1.206 | 0.160 | 0.045 | 1 |
| Omy_cd28130 | biallelic | 1.651 | 1.029 | 0.027 | 0.019 | 1 |
| OMS00030 | biallelic | 1.977 | 1.205 | 0.159 | 0.045 | 0 |
| OMS00048 | biallelic | 1.977 | 1.260 | 0.194 | 0.043 | 0 |
| OMS00056 | biallelic | 2.000 | 1.536 | 0.339 | 0.039 | 0 |
| OMS00061 | biallelic | 1.860 | 1.142 | 0.114 | 0.056 | 0 |
| OMS00092 | biallelic | 2.000 | 1.412 | 0.269 | 0.088 | 0 |
| OMS00096 | biallelic | 2.000 | 1.455 | 0.302 | 0.031 | 1 |
| OMS00087 | biallelic | 2.000 | 1.391 | 0.266 | 0.052 | 4 |
| OMS00119 | biallelic | 2.000 | 1.358 | 0.251 | 0.038 | 0 |
| OMS00133 | biallelic | 1.512 | 1.038 | 0.033 | 0.056 | 1 |
| OMS00138 | biallelic | 1.977 | 1.261 | 0.194 | 0.044 | 0 |
| OMS00095 | biallelic | 1.860 | 1.132 | 0.109 | 0.043 | 6 |
| OMS00169 | biallelic | 1.326 | 1.016 | 0.014 | 0.041 | 0 |
| OMS00173 | biallelic | 2.000 | 1.296 | 0.215 | 0.058 | 0 |
| OMS00176 | biallelic | 1.837 | 1.134 | 0.111 | 0.037 | 0 |
| Omy_impa155 | biallelic | 1.977 | 1.182 | 0.147 | 0.028 | 1 |
| Omy_103705558 | biallelic | 1.953 | 1.220 | 0.170 | 0.037 | 2 |
| Omy_105075162 | biallelic | 1.977 | 1.206 | 0.163 | 0.030 | 1 |
| Omy_107031704 | biallelic | 2.000 | 1.378 | 0.263 | 0.040 | 0 |
| Omy_10728569 | biallelic | 2.000 | 1.339 | 0.246 | 0.023 | 0 |
| OMS00114 | biallelic | 1.977 | 1.214 | 0.167 | 0.037 | 1 |
| OMS00174 | biallelic | 1.977 | 1.101 | 0.088 | 0.021 | 0 |
| Omy_97865196 | biallelic | 1.767 | 1.074 | 0.065 | 0.033 | 1 |
| Omy_128996481 | Biallelic | 2.000 | 1.170 | 0.138 | 0.035 | 9 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| Omy_b9164 | biallelic | 1.977 | 1.325 | 0.214 | 0.110 | 1 |
| Omy_BAMBI2312 | biallelic | 1.930 | 1.232 | 0.175 | 0.049 | 2 |
| Omy_cd59b112 | biallelic | 1.907 | 1.256 | 0.186 | 0.072 | 11 |
| Omy_cin172 | biallelic | 2.000 | 1.486 | 0.312 | 0.050 | 0 |
| Omy_g1103 | biallelic | 1.721 | 1.113 | 0.094 | 0.046 | 1 |
| Omy_G3PD_2371 | biallelic | 2.000 | 1.417 | 0.280 | 0.050 | 0 |
| Omy_gadd45332 | biallelic | 2.000 | 1.275 | 0.195 | 0.080 | 0 |
| Omy_gdh271 | biallelic | 2.000 | 1.219 | 0.170 | 0.037 | 1 |
| Omy_gh475 | biallelic | 2.000 | 1.331 | 0.239 | 0.033 | 0 |
| Omy_hsp4786 | biallelic | 2.000 | 1.522 | 0.337 | 0.020 | 1 |
| Omy_hsp70aPro329 | biallelic | 1.884 | 1.103 | 0.082 | 0.091 | 2 |
| Omy_inos97 | biallelic | 1.953 | 1.139 | 0.114 | 0.040 | 0 |
| Omy_LDHB1_i2 | biallelic | 1.953 | 1.166 | 0.135 | 0.030 | 2 |
| Omy_LDHB2_e5 | biallelic | 2.000 | 1.388 | 0.272 | 0.027 | 0 |
| Omy_LDHB2_i6 | biallelic | 1.419 | 1.019 | 0.018 | 0.038 | 0 |
| Omy_mapK3103 | biallelic | 1.558 | 1.040 | 0.035 | 0.051 | 1 |
| Omy_myoD178 | biallelic | 1.907 | 1.229 | 0.172 | 0.050 | 0 |
| Omy_OmyP9180 | biallelic | 1.977 | 1.221 | 0.172 | 0.035 | 0 |
| Omy_sSOD1 | biallelic | 1.488 | 1.016 | 0.015 | 0.022 | 0 |
| Omy_star206 | biallelic | 1.744 | 1.103 | 0.088 | 0.037 | 0 |
| Omy_tlr3377 | biallelic | 1.860 | 1.198 | 0.151 | 0.057 | 1 |
| Omy_hsf1b241 | biallelic | 1.930 | 1.180 | 0.145 | 0.033 | 0 |
| Omy_u0779166 | biallelic | 1.907 | 1.235 | 0.172 | 0.069 | 0 |
| Omy_hus152 | biallelic | 1.698 | 1.105 | 0.088 | 0.044 | 1 |
| Omy_nips299 | biallelic | 1.930 | 1.131 | 0.110 | 0.030 | 1 |
| Omy_UT16_2173 | biallelic | 1.953 | 1.148 | 0.121 | 0.038 | 2 |
| Omy_vamp5303 | biallelic | 2.000 | 1.484 | 0.316 | 0.033 | 1 |
| Omy_zg5791 | biallelic | 1.860 | 1.189 | 0.144 | 0.064 | 1 |
| Omy_ndk152 | biallelic | 1.674 | 1.050 | 0.045 | 0.026 | 0 |
| Omy_114976223 | biallelic | 2.000 | 1.863 | 0.459 | 0.058 | 9 |
| OMS00041 | biallelic | 1.465 | 1.032 | 0.028 | 0.052 | 0 |
| OMS00103 | biallelic | 1.442 | 1.020 | 0.019 | 0.023 | 0 |
| OMS00127 | biallelic | 1.256 | 1.008 | 0.007 | 0.036 | 0 |
| OMS00134 | biallelic | 1.442 | 1.017 | 0.016 | 0.017 | 1 |
| OMS00153 | biallelic | 1.907 | 1.130 | 0.108 | 0.036 | 0 |
| OMS00156 | biallelic | 2.000 | 1.774 | 0.428 | 0.044 | 0 |
| OMS00164 | biallelic | 1.465 | 1.024 | 0.023 | 0.022 | 0 |
| Omy_1004 | biallelic | 2.000 | 1.431 | 0.290 | 0.038 | 0 |
| Omy_UBA3b | biallelic | 1.977 | 1.353 | 0.244 | 0.058 | 0 |
| Omy_GH1P1_2 | biallelic | 1.442 | 1.025 | 0.022 | 0.048 | 5 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| Omy_11781581 | biallelic | 1.977 | 1.608 | 0.351 | 0.109 | 0 |
| Omy_11865491 | biallelic | 2.000 | 1.554 | 0.343 | 0.053 | 0 |
| Omy_98683165 | biallelic | 1.791 | 1.093 | 0.074 | 0.082 | 0 |
| Omy_ftzf1217 | biallelic | 1.209 | 1.005 | 0.005 | 0.014 | 0 |
| Omy_101554306 | biallelic | 1.860 | 1.101 | 0.086 | 0.037 | 0 |
| Omy_109525403 | biallelic | 2.000 | 1.677 | 0.396 | 0.029 | 0 |
| Omy_110362585 | biallelic | 1.419 | 1.014 | 0.013 | 0.019 | 0 |
| Omy_110689148 | biallelic | 2.000 | 1.334 | 0.238 | 0.043 | 1 |
| Omy_111084526 | biallelic | 2.000 | 1.482 | 0.316 | 0.031 | 0 |
| Omy_111666301 | biallelic | 1.535 | 1.033 | 0.029 | 0.049 | 0 |
| Omy_116938264 | biallelic | 1.791 | 1.153 | 0.119 | 0.067 | 0 |
| Omy_117286374 | biallelic | 1.907 | 1.173 | 0.140 | 0.032 | 1 |
| Omy_117370400 | biallelic | 2.000 | 1.939 | 0.483 | 0.032 | 0 |
| Omy_118175396 | biallelic | 1.233 | 1.009 | 0.008 | 0.031 | 0 |
| Omy_128693455 | biallelic | 1.023 | 1.001 | 0.001 | 0.011 | 0 |
| Omy_BAMBI4238 | biallelic | 1.023 | 1.002 | 0.001 | 0.032 | 0 |
| Omy_G3PD_2246 | biallelic | 1.256 | 1.013 | 0.012 | 0.043 | 0 |
| Omy_RAD354179 | biallelic | 1.953 | 1.271 | 0.192 | 0.076 | 0 |
| Omy_RAD368487 | biallelic | 2.000 | 1.849 | 0.455 | 0.086 | 0 |
| Omy_RAD880287 | biallelic | 2.000 | 1.676 | 0.394 | 0.050 | 0 |
| Omy_u0961043 | biallelic | 1.209 | 1.010 | 0.009 | 0.032 | 0 |
| Omy_RAD1763223 | biallelic | 2.000 | 1.730 | 0.414 | 0.034 | 0 |
| Omy_RAD2357743 | biallelic | 1.116 | 1.003 | 0.003 | 0.025 | 0 |
| Omy_RAD4279359 | biallelic | 2.000 | 1.481 | 0.301 | 0.097 | 0 |
| Omy_RAD4510418 | biallelic | 2.000 | 1.830 | 0.450 | 0.033 | 0 |
| Omy_RAD6259638 | biallelic | 2.000 | 1.896 | 0.470 | 0.028 | 0 |
| Omy_RAD6683417 | biallelic | 2.000 | 1.390 | 0.268 | 0.041 | 0 |
| Omy_RAD7778954 | biallelic | 2.000 | 1.495 | 0.318 | 0.060 | 0 |
| Omy_RAD8812232 | biallelic | 2.000 | 1.480 | 0.313 | 0.055 | 0 |
| Omy_hsp90BA193 | biallelic | 1.837 | 1.165 | 0.129 | 0.061 | 2 |
| Omy_RAD4795551 | biallelic | 2.000 | 1.454 | 0.302 | 0.033 | 1 |
| Omy_RAD4879969 | biallelic | 2.000 | 1.344 | 0.242 | 0.053 | 0 |
| Omy_RAD11659 | biallelic | 1.535 | 1.039 | 0.035 | 0.045 | 1 |
| Omy_RAD191922 | biallelic | 1.140 | 1.005 | 0.005 | 0.026 | 0 |
| Omy_RAD365148 | biallelic | 2.000 | 1.859 | 0.456 | 0.038 | 4 |
| Omy_RAD484814 | biallelic | 1.535 | 1.042 | 0.036 | 0.053 | 0 |
| Omy_RAD1073310 | biallelic | 1.535 | 1.024 | 0.022 | 0.024 | 0 |
| Omy_RAD1256614 | biallelic | 1.186 | 1.002 | 0.002 | 0.006 | 0 |
| Omy_RAD1349913 | biallelic | 1.907 | 1.116 | 0.095 | 0.051 | 0 |
| Omy_RAD1403346 | biallelic | 1.814 | 1.079 | 0.070 | 0.024 | 1 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| Omy_RAD1957859 | biallelic | 2.000 | 1.294 | 0.205 | 0.086 | 4 |
| Omy_RAD2212369 | biallelic | 2.000 | 1.850 | 0.451 | 0.053 | 0 |
| Omy_RAD2774055 | biallelic | 1.767 | 1.076 | 0.066 | 0.039 | 3 |
| Omy_RAD3039217 | biallelic | 2.000 | 1.659 | 0.381 | 0.073 | 1 |
| Omy_RAD3140867 | biallelic | 2.000 | 1.714 | 0.403 | 0.115 | 0 |
| Omy_RAD3915633 | biallelic | 2.000 | 1.362 | 0.255 | 0.034 | 1 |
| Omy_RAD4013255 | biallelic | 2.000 | 1.851 | 0.457 | 0.027 | 0 |
| Omy_RAD4052048 | biallelic | 1.977 | 1.274 | 0.203 | 0.042 | 2 |
| Omy_RAD4246532 | biallelic | 1.767 | 1.058 | 0.053 | 0.026 | 1 |
| Omy_RAD4645251 | biallelic | 2.000 | 1.854 | 0.456 | 0.036 | 0 |
| Omy_RAD5540454 | biallelic | 1.698 | 1.079 | 0.066 | 0.059 | 1 |
| Omy_RAD5599710 | biallelic | 1.116 | 1.002 | 0.002 | 0.007 | 0 |
| Omy_RAD5975841 | biallelic | 1.558 | 1.039 | 0.035 | 0.035 | 0 |
| Omy_RAD6013512 | biallelic | 2.000 | 1.315 | 0.223 | 0.053 | 0 |
| Omy_RAD6580868 | biallelic | 2.000 | 1.409 | 0.275 | 0.056 | 0 |
| Omy_RAD6640236 | biallelic | 2.000 | 1.422 | 0.269 | 0.104 | 1 |
| Omy_RAD6863440 | biallelic | 1.581 | 1.066 | 0.056 | 0.056 | 0 |
| Omy_RAD3379824 | biallelic | 1.884 | 1.108 | 0.089 | 0.053 | 0 |
| Omy_RAD3500513 | biallelic | 1.884 | 1.078 | 0.069 | 0.028 | 1 |
| Omy_RAD3695253 | biallelic | 1.558 | 1.065 | 0.055 | 0.057 | 0 |
| Omy_RAD7814727 | biallelic | 2.000 | 1.757 | 0.422 | 0.047 | 1 |
| Omy_RAD7850257 | biallelic | 1.930 | 1.115 | 0.095 | 0.045 | 0 |
| Omy_RAD7877610 | biallelic | 1.953 | 1.102 | 0.088 | 0.029 | 2 |
| Omy_RAD8513135 | biallelic | 2.000 | 1.575 | 0.338 | 0.097 | 6 |
| Omy_RAD9871553 | biallelic | 2.000 | 1.605 | 0.365 | 0.063 | 1 |
| Omy_RAD10335945 | biallelic | 1.070 | 1.001 | 0.001 | 0.008 | 0 |
| Omy_RAD3667 | biallelic | 2.000 | 1.687 | 0.391 | 0.073 | 0 |
| Omy_RAD61959 | biallelic | 2.000 | 1.631 | 0.378 | 0.034 | 0 |
| Omy_RAD73959 | biallelic | 1.977 | 1.249 | 0.189 | 0.037 | 0 |
| Omy_RAD118659 | biallelic | 2.000 | 1.755 | 0.424 | 0.046 | 0 |
| Omy_RAD320910 | biallelic | 2.000 | 1.811 | 0.436 | 0.091 | 0 |
| Omy_RAD1243964 | biallelic | 2.000 | 1.856 | 0.458 | 0.040 | 0 |
| Omy_RAD1307316 | biallelic | 1.814 | 1.073 | 0.063 | 0.037 | 0 |
| Omy_RAD1570953 | biallelic | 1.884 | 1.360 | 0.228 | 0.412 | 0 |
| Omy_RAD1784916 | biallelic | 2.000 | 1.946 | 0.486 | 0.027 | 0 |
| Omy_RAD1934024 | biallelic | 1.698 | 1.069 | 0.060 | 0.038 | 0 |
| Omy_RAD2091711 | biallelic | 2.000 | 1.266 | 0.198 | 0.041 | 4 |
| Omy_RAD2428774 | biallelic | 2.000 | 1.477 | 0.304 | 0.074 | 1 |
| Omy_RAD2504268 | biallelic | 1.256 | 1.008 | 0.008 | 0.021 | 0 |
| Omy_RAD2823638 | biallelic | 2.000 | 1.623 | 0.355 | 0.181 | 0 |

Table 1. Continued

| Marker | type | $N_A$ | $n_e$ | $H_E$ | $F_{ST}$ | HWE |
|---|---|---|---|---|---|---|
| Omy_RAD3213958 | biallelic | 2.000 | 1.551 | 0.334 | 0.097 | 0 |
| Omy_RAD3312247 | biallelic | 2.000 | 1.687 | 0.396 | 0.059 | 8 |
| Omy_RAD351499 | biallelic | 2.000 | 1.649 | 0.380 | 0.055 | 0 |
| Omy_RAD3781668 | biallelic | 2.000 | 1.504 | 0.324 | 0.047 | 0 |
| Omy_RAD3840619 | biallelic | 1.977 | 1.177 | 0.142 | 0.034 | 7 |
| Omy_RAD4064158 | biallelic | 1.884 | 1.103 | 0.085 | 0.056 | 0 |
| Omy_RAD4159434 | biallelic | 1.395 | 1.023 | 0.021 | 0.034 | 1 |
| Omy_RAD4357337 | biallelic | 2.000 | 1.720 | 0.413 | 0.032 | 0 |
| Omy_RAD4369441 | biallelic | 1.930 | 1.162 | 0.130 | 0.046 | 3 |
| Omy_RAD4667227 | biallelic | 2.000 | 1.803 | 0.441 | 0.043 | 0 |
| Omy_RAD5063221 | biallelic | 2.000 | 1.279 | 0.210 | 0.027 | 0 |
| Omy_RAD5791629 | biallelic | 2.000 | 1.927 | 0.480 | 0.040 | 2 |
| Omy_RAD5995044 | biallelic | 1.907 | 1.195 | 0.153 | 0.040 | 0 |
| Omy_RAD6595969 | biallelic | 2.000 | 1.729 | 0.412 | 0.119 | 0 |
| Omy_RAD7606020 | biallelic | 1.465 | 1.059 | 0.049 | 0.061 | 8 |
| Omy_RAD9358037 | biallelic | 2.000 | 1.646 | 0.384 | 0.045 | 0 |
| Omy_RAD4631435 | biallelic | 1.930 | 1.144 | 0.113 | 0.069 | 1 |
| Omy_RAD1303467 | biallelic | 2.000 | 1.324 | 0.235 | 0.032 | 0 |
| Omy_RAD3061961 | biallelic | 1.814 | 1.080 | 0.070 | 0.032 | 0 |

Table 2. Details associated with Snake River basin steelhead (*Oncorhynchus mykiss*) genetic stock identification (GSI) baseline version 4. Collections are identified by National Marine Fisheries Service (NMFS) designated population, genetic stock, major population group (MPG), sample size, latitude, longitude, expected heterozygosity ($H_E$), mean pairwise fixation index ($F_{ST}$). Map number (#) corresponds to those presented in Figure 1. All collections are summer-run of natural origin and presumed to be of anadromous lineage.

| No | Collection | Population | Genetic Stock | MPG | n | Year Collected | Latitude | Longitude | $H_E$ | $F_{ST}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Big Bear Cr | CRLMA-s | LOCLWR | Clearwater River | 9 | 2012 | 46.6306291 | -116.6562 | 0.266 | 0.035 |
| 2 | Clear Cr | CRLMA-s | SFCLWR | Clearwater River | 23 | 2015 | 46.13291 | -115.9515 | 0.256 | 0.028 |
| 3 | EF Potlach R | CRLMA-s | LOCLWR | Clearwater River | 52 | 2016 | 46.7984445 | -116.4194 | 0.279 | 0.021 |
| 4 | Lapwai Cr | CRLMA-s | LOCLWR | Clearwater River | 176 | 2013 | 46.37267 | -116.70478 | 0.288 | 0.018 |
| 5 | Little Bear Cr | CRLMA-s | LOCLWR | Clearwater River | 57 | 2012 | 46.6372261 | -116.678 | 0.282 | 0.019 |
| 6 | WF Potlach R | CRLMA-s | LOCLWR | Clearwater River | 49 | 2010 | 46.8053764 | -116.4182 | 0.279 | 0.018 |
| 7 | Canyon Cr | CRLOC-s | UPCLWR | Clearwater River | 45 | 2004 | 46.2161351 | -115.5559 | 0.259 | 0.026 |
| 8 | upper Lochsa R | CRLOC-s | UPCLWR | Clearwater River | 46 | 2000 | 46.5251077 | -114.6786 | 0.256 | 0.029 |
| 9 | Lolo Cr | CRLOL-s | SFCLWR | Clearwater River | 46 | 2012 | 46.29056 | -115.93415 | 0.254 | 0.029 |
| 10 | Bear Cr | CRSEL-s | UPCLWR | Clearwater River | 27 | 2012 | 46.01902 | -114.8376 | 0.268 | 0.027 |
| 11 | Gedney Cr | CRSEL-s | UPCLWR | Clearwater River | 51 | 2004 | 46.0582161 | -115.3136 | 0.264 | 0.023 |
| 12 | middle Selway R | CRSEL-s | UPCLWR | Clearwater River | 44 | 2012 | 46.17356 | -114.8861 | 0.259 | 0.025 |
| 13 | Ohara Cr | CRSEL-s | UPCLWR | Clearwater River | 28 | 2013 | 46.04806 | -115.5169 | 0.255 | 0.028 |
| 14 | Three Links Cr | CRSEL-s | UPCLWR | Clearwater River | 33 | 2012 | 46.1112 | -115.0719 | 0.262 | 0.030 |
| 15 | upper Selway R | CRSEL-s | UPCLWR | Clearwater River | 158 | 2008 | 45.70726 | -114.71946 | 0.269 | 0.026 |
| 16 | Crooked R | CRSFC-s | SFCLWR | Clearwater River | 56 | 2013 | 45.8212 | -115.5277 | 0.262 | 0.027 |
| 17 | Newsome Cr | CRSFC-s | SFCLWR | Clearwater River | 48 | 2012 | 45.83656 | -115.6152 | 0.259 | 0.030 |
| 18 | Tenmile Cr | CRSFC-s | SFCLWR | Clearwater River | 51 | 2013 | 45.80419 | -115.6833 | 0.258 | 0.028 |
| 19 | Joseph Cr | GRJOS-s | GRROND | Grande Ronde River | 100 | 2011, 2012 | 46.0277692 | -117.0177 | 0.284 | 0.017 |
| 20 | Wenaha R | GRLMT-s | GRROND | Grande Ronde River | 77 | 2001 | 45.977 | -117.555 | 0.281 | 0.018 |
| 21 | Catherine Cr | GRUMA-s | GRROND | Grande Ronde River | 272 | 2012, 2013, 2014 | 45.307 | -117.866 | 0.289 | 0.017 |
| 22 | upper Grande Ronde R | GRUMA-s | GRROND | Grande Ronde River | 49 | 2011, 2012, 2013, 2014 | 45.248781 | -118.3889 | 0.277 | 0.021 |
| 23 | Gumboot/Mahogany Cr | IRMAI-s | IMNAHA | Imnaha River | 52 | 2011, 2012, 2013 | 45.18838 | -116.8708 | 0.267 | 0.021 |
| 24 | Lightning Cr | IRMAI-s | IMNAHA | Imnaha River | 76 | 2001 | 45.65537 | -116.7265 | 0.276 | 0.019 |
| 25 | Lower Big Cr | MFBIG-s | MFSALM | Salmon River | 47 | 2011 | 45.0925936 | -114.7322 | 0.272 | 0.023 |
| 26 | Elk/Bear Cr | MFUMA-s | MFSALM | Salmon River | 82 | 2011 | 44.41043 | -115.37264 | 0.267 | 0.032 |
| 27 | Pistol Cr | MFUMA-s | MFSALM | Salmon River | 37 | 2012 | 44.72352 | -115.1501 | 0.273 | 0.026 |
| 28 | Rapid R (MF Salmon R) | MFUMA-s | LOSALM | Salmon River | 30 | 2012 | 44.68006 | -115.1523 | 0.276 | 0.030 |
| 29 | EFSF Salmon River | SFMAI-s | SFSALM | Salmon River | 47 | 2009, 2010 | 45.0128622 | -115.7131 | 0.269 | 0.027 |
| 30 | Secesh R | SFSEC-s | SFSALM | Salmon River | 63 | 2011 | 45.0594 | -115.7567 | 0.266 | 0.027 |
| 31 | Alpowa Cr | SNASO-s | LSNAKE | Lower Snake River | 51 | 2010 | 46.4076204 | -117.2198 | 0.290 | 0.019 |
| 32 | Asotin Cr | SNASO-s | LSNAKE | Lower Snake River | 112 | 2010 | 46.3029064 | -117.1168 | 0.293 | 0.016 |

Table 2. Continued

| No | Collection | Population | Genetic Stock | MPG | n | Year Collected | Latitude | Longitude | $H_E$ | $F_{ST}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 33 | Tucannon R | SNTUC-s | LSNAKE | Lower Snake River | 89 | 2010, 2011, 2013 | 46.3097332 | -117.6572 | 0.291 | 0.017 |
| 34 | Bargamin Cr | SRCHA-s | MFSALM | Salmon River | 56 | 2015 | 45.721674 | -115.0345 | 0.260 | 0.031 |
| 35 | Chamberlain Cr | SRCHA-s | MFSALM | Salmon River | 100 | 2016 | 45.36865 | -115.19689 | 0.258 | 0.032 |
| 36 | EF Salmon R | SREFS-s | UPSALM | Salmon River | 64 | 2016 | 44.115434 | -114.43 | 0.276 | 0.019 |
| 37 | Morgan Cr | SREFS-s | UPSALM | Salmon River | 45 | 2012 | 44.66757 | -114.2301 | 0.314 | 0.028 |
| 38 | upper Lemhi R | SRLEM-s | UPSALM | Salmon River | 53 | 2017 | 44.8616 | -113.6319 | 0.299 | 0.027 |
| 39 | Boulder Cr | SRLSR-s | LOSALM | Salmon River | 46 | 2014 | 45.15409 | -116.3954 | 0.253 | 0.033 |
| 40 | Slate Cr | SRLSR-s | LOSALM | Salmon River | 26 | 2013 | 45.63938 | -116.1202 | 0.279 | 0.022 |
| 41 | Whitebird Cr | SRLSR-s | LOSALM | Salmon River | 44 | 2014 | 45.77049 | -116.2907 | 0.285 | 0.019 |
| 42 | Panther Cr | SRPAN-s | UPSALM | Salmon River | 50 | 2013 | 45.03494 | -114.2995 | 0.283 | 0.023 |
| 43 | Sawtooth | SRUMA-s | UPSALM | Salmon River | 52 | 2016, 2018 | 44.15058 | -114.88509 | 0.275 | 0.023 |

Table 3. The numbers and proportions (in parentheses) of Steelhead that were self-assigned to different genetic stocks as determined via leave-one-out tests performed in the program rubias (Moran and Anderson 2019). Each sample in the baseline was removed and then assigned back to the baseline. Rows represent population of origin and columns denote assigned genetic stock. Shaded boxes denote the correct genetic stock for each population.

| Population | n | GRROND | IMNAHA | LOWCLWR | LOWSALM | LSNAKE | MFSALM | SFCLWR | SFSALM | UPCLWR | UPSALM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Catherine Cr | 272 | 248 (0.91) | 3 (0.01) | 2 (0.01) | 2 (0.01) | 14 (0.05) | | | | | 3 (0.01) |
| Joseph Cr | 100 | 69 (0.69) | 2 (0.02) | 7 (0.07) | 2 (0.02) | 18 (0.18) | | | | | 2 (0.02) |
| upper Grande Ronde R | 49 | 43 (0.88) | 1 (0.02) | 2 (0.04) | | 3 (0.06) | | | | | |
| Wenaha R | 77 | 63 (0.82) | 2 (0.03) | 2 (0.03) | | 10 (0.13) | | | | | |
| Gumboot/Mahogany Cr | 52 | 2 (0.04) | 42 (0.81) | 1 (0.02) | 2 (0.04) | 3 (0.06) | 1 (0.02) | | | | 1 (0.02) |
| Lightning Cr | 76 | 7 (0.09) | 58 (0.76) | 2 (0.03) | 1 (0.01) | 8 (0.11) | | | | | |
| Big Bear Cr | 9 | 1 (0.11) | | 7 (0.78) | | 1 (0.11) | | | | | |
| EF Potlach R | 52 | 2 (0.04) | | 49 (0.94) | | | | | | 1 (0.02) | |
| Lapwai Cr | 176 | 21 (0.12) | 1 (0.01) | 122 (0.69) | | 31 (0.18) | | | | | 1 (0.01) |
| Little Bear Cr | 57 | 5 (0.09) | 2 (0.04) | 38 (0.67) | | 11 (0.19) | | 1 (0.02) | | | |
| WF Potlach R | 49 | 8 (0.16) | 1 (0.02) | 33 (0.67) | | 4 (0.08) | | 1 (0.02) | | 1 (0.02) | 1 (0.02) |
| Boulder Cr | 46 | | | | 46 (1) | | | | | | |
| Slate Cr | 26 | 2 (0.08) | 5 (0.19) | | 10 (0.38) | 7 (0.27) | 1 (0.04) | | 1 (0.04) | | |
| Whitebird Cr | 44 | 6 (0.14) | 1 (0.02) | | 22 (0.5) | 9 (0.2) | 1 (0.02) | | 2 (0.05) | | 3 (0.07) |
| Alpowa Cr | 51 | 9 (0.18) | | 4 (0.08) | 3 (0.06) | 34 (0.67) | | | | 1 (0.02) | |
| Asotin Cr | 112 | 22 (0.2) | 1 (0.01) | 14 (0.13) | 1 (0.01) | 72 (0.64) | | | | | 2 (0.02) |
| Tucannon R | 89 | 27 (0.3) | 3 (0.03) | 11 (0.12) | 1 (0.01) | 44 (0.49) | | | | | 3 (0.03) |
| Bargamin Cr | 56 | | | | | | 56 (1) | | | | |
| Chamberlain Cr | 100 | | | | | | 100 (1) | | | | |
| Elk/Bear Cr | 82 | | | | | | 82 (1) | | | | |
| Lower Big Cr | 47 | | 1 (0.02) | | 1 (0.02) | 3 (0.06) | 38 (0.81) | | | | 4 (0.09) |
| Pistol Cr | 37 | 1 (0.03) | 1 (0.03) | | 1 (0.03) | 1 (0.03) | 30 (0.81) | | | | 3 (0.08) |
| Rapid R (MF Salmon R) | 30 | | 1 (0.03) | | 3 (0.1) | | 23 (0.77) | | | 1 (0.03) | 2 (0.07) |
| Clear Cr | 23 | | | | | | | 18 (0.78) | | 5 (0.22) | |
| Crooked R | 56 | | | 1 (0.02) | | | | 52 (0.93) | | 3 (0.05) | |
| Lolo Cr | 46 | | | 1 (0.02) | | | | 43 (0.93) | | 2 (0.04) | |
| Newsome Cr | 48 | | | 1 (0.02) | | | | 46 (0.96) | | 1 (0.02) | |
| Tenmile Cr | 51 | | | | | | | 44 (0.86) | | 7 (0.14) | |
| EFSF Salmon River | 47 | | | | | | | | 47 (1) | | |
| Secesh R | 63 | | | | 1 (0.02) | | | | 62 (0.98) | | |
| Bear Cr | 27 | | | | 1 (0.04) | | | | | 26 (0.96) | |
| Canyon Cr | 45 | | | | | 1 (0.02) | | 7 (0.16) | | 37 (0.82) | |
| Gedney Cr | 51 | | | | | | | 4 (0.08) | | 47 (0.92) | |
| middle Selway R | 44 | | | | | | | | | 44 (1) | |
| Ohara Cr | 28 | | | | | | | 3 (0.11) | | 25 (0.89) | |
| Three Links Cr | 33 | | | | | | | | | 33 (1) | |
| upper Lochsa R | 46 | | | | | | | | | 46 (1) | |

Table 3. Continued

| Population | n | GRROND | IMNAHA | LOWCLWR | LOWSALM | LSNAKE | MFSALM | SFCLWR | SFSALM | UPCLWR | UPSALM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| upper Selway R | 158 | | | | | | | | | 158 (1) | |
| EF Salmon R | 64 | 1 (0.02) | 2 (0.03) | 1 (0.02) | 1 (0.02) | 1 (0.02) | 1 (0.02) | 1 (0.02) | | | 56 (0.88) |
| Morgan Cr | 45 | | | | 3 (0.07) | 3 (0.07) | | | | | 39 (0.87) |
| Panther Cr | 50 | 1 (0.02) | 2 (0.04) | | 1 (0.02) | 3 (0.06) | 1 (0.02) | | | | 42 (0.84) |
| Sawtooth | 52 | 3 (0.06) | | | 4 (0.08) | 1 (0.02) | | | | | 44 (0.85) |
| upper Lemhi R | 53 | | | | 5 (0.09) | 2 (0.04) | | | | | 46 (0.87) |

Table 4. A comparison of self-assignment rates for Snake River basin steelhead baselines as quantified using leave-one-out tests in rubias (Anderson et al. 2008; Moran and Anderson 2019) for v3.1, which contained 5,967 individuals amplified at 179 SNPs (Powell et al. 2018) and v4 (this document), which included 2,719 individuals amplified at 92 microhaps and 242 SNPs.

| | **Self-assignment rate** | |
|---|---|---|
| **Genetic stock** | **Baseline v3.1** | **Baseline v4** |
| SFCLWR | 0.89 | 0.91 |
| UPCLWR | 0.93 | 0.96 |
| MFSALM | 0.90 | 0.93 |
| LOWCLWR | 0.66 | 0.73 |
| SFSALM | 0.91 | 0.99 |
| IMNAHA | 0.68 | 0.78 |
| UPSALM | 0.74 | 0.86 |
| LOWSALM | 0.52 | 0.67 |
| GRROND | 0.67 | 0.85 |
| LSNAKE | 0.41 | 0.60 |

Table 5. Concordance rates between GSI genetic stock assignments and PIT tag detections for SY2020 adult steelhead made using GSI baseline v3.1 and v4.

| | | Concordance rates | |
|---|---|---|---|
| **Genetic Stock** | **Population** | **Baseline v3.1** | **Baseline v4** |
| UPSALM | SRPAN-s | 27.0 | 83.8 |
| | SRNFS-s | 63.6 | 36.4 |
| | SRLEM-s | 79.3 | 72.4 |
| | SRPAH-s | 53.8 | 69.2 |
| | SRUMA-s | 73.0 | 89.2 |
| MFSALM | MFBIG-s | 85.0 | 100.0 |
| | MFUMA-s | 66.7 | 100.0 |
| SFSALM | SFSEC-s | 100.0 | 100.0 |
| | SFMAI-s | 73.7 | 100.0 |
| UPCLWR | CRLOC-s | 87.5 | 100.0 |
| | CRSEL-s | 78.8 | 90.9 |
| SFCLWR | CRLOL-s | 92.3 | 100.0 |
| | CRSFC-s | 62.5 | 87.5 |
| LOCLWR | CRLMA-s | 12.3 | 43.9 |
| IMNAHA | IRMAI-s | 51.7 | 86.2 |
| GRROND | GRLMT-s | 67.1 | 83.6 |
| | GRJOS-s | 58.1 | 77.8 |
| | GRWAL-s | 73.0 | 75.7 |
| | GRUMA-s | 56.1 | 83.5 |
| LSNAKE | SNTUC-s | 35.6 | 57.8 |
| | SNASO-s | 51.1 | 82.2 |

Table 6. Summary of SY2020 adult and MY2020 juvenile steelhead and MY2020 Chinook Salmon samples from Lower Granite Dam (LGR). Summary includes the number of samples that arrived from LGR (inventoried) and the number inventoried that were queued for genotyping. Of queued samples, we show the number that genotyped successfully and the number that failed genotyping. For samples that genotyped successfully, we show the number of individuals with a parentage based tag (PBT; i.e., of hatchery origin) and the number that were assigned a genetic stock based on individual assignment (IA) using SNP baselines v3.1.

| **Sample Group** | **Total Samples Inventoried and Queued for Genotyping** | **Failed Genotyping (NG)** | **Successfully Genotyped** | **PBT Assignments** | **GSI Assignments** |
|---|---|---|---|---|---|
| *Steelhead* | | | | | |
| SY2020 Adults (Natural-origin Phenotype) | 2,579 | 6 (0.2%) | 2,573 (99.8%) | 234 (9.1%) | 2,339 (90.9%) |
| SY2020 Adults (Hatchery Phenotype) | 221 | 0 (0%) | 221 (100%) | 202 (91.4%) | 19 (8.6%) |
| MY2020 Juveniles | 123 | 4 (3.3%) | 119 (96.7%) | 1 (0.8%) | 118 (99.2%) |
| *Chinook* | | | | | |
| MY2020 Juveniles | 50 | 0 (0%) | 50 (100%) | 4 (8.0%) | 46 (92.0%) |
| TOTAL: | 2,973 | 10 (0.3%) | 2,963 (99.7%) | 441 (14.9%) | 2,522 (85.1%) |

Table 7. Summary of Lower Granite Dam (LGR) natural-origin adult steelhead samples from SY2020 assigned to a genetic stock using individual assignment based on the Snake River steelhead SNP baseline v3.1. Summaries of life history diversity information (sex, length, and ocean age) for each genetic stock are shown. The 'Other' saltwater age category includes fish that were not queued to be aged, fish that could not be aged, and fish with spawn checks.

| | | | Sex | | | | | Length | | | | | | | Ocean (Saltwater) Age | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Frequency | | | Percentage | | Mean Length (cm FL) by Ocean Age | | | Frequency | | Percentage | | Frequency | | | | Percentage | | |
| Genetic Stock | Total Assignments | % Stock Composition | F | M | U | F | M | 1 | 2 | 3 | A-Run | B-Run | A-Run | B-Run | 1 | 2 | 3 | Other | 1 | 2 | 3 |
| UPSALM | 454 | 19% | 286 | 168 | - | 63% | 37% | 57.9 | 69.6 | 80 | 444 | 10 | 98% | 2% | 278 | 160 | 1 | 15 | 63% | 36% | 0% |
| MFSALM | 129 | 6% | 95 | 34 | - | 74% | 26% | 60.5 | 71.6 | 77 | 128 | 1 | 99% | 1% | 75 | 48 | 1 | 5 | 60% | 39% | 1% |
| SFSALM | 26 | 1% | 19 | 7 | - | 73% | 27% | 63.1 | 74.8 | 81 | 19 | 7 | 73% | 27% | 8 | 16 | 1 | 1 | 32% | 64% | 4% |
| LOSALM | 44 | 2% | 32 | 12 | - | 73% | 27% | 57.5 | 69.2 | 79 | 42 | 2 | 95% | 5% | 23 | 19 | 1 | 1 | 53% | 44% | 2% |
| UPCLWR | 74 | 3% | 42 | 32 | - | 57% | 43% | 65.7 | 77.2 | 85 | 55 | 19 | 74% | 26% | 30 | 42 | 1 | 1 | 41% | 58% | 1% |
| SFCLWR | 91 | 4% | 54 | 37 | - | 59% | 41% | 61.5 | 76.2 | 77 | 70 | 21 | 77% | 23% | 20 | 61 | 6 | 4 | 23% | 70% | 7% |
| LOCLWR | 111 | 5% | 79 | 32 | - | 71% | 29% | 58.9 | 71.4 | 86 | 106 | 5 | 95% | 5% | 49 | 55 | 1 | 6 | 47% | 52% | 1% |
| IMNAHA | 174 | 7% | 115 | 59 | - | 66% | 34% | 57.4 | 70.2 | - | 172 | 2 | 99% | 1% | 110 | 60 | 0 | 4 | 65% | 35% | 0% |
| GRROND | 790 | 34% | 515 | 275 | - | 65% | 35% | 57.9 | 69.6 | 78 | 781 | 9 | 99% | 1% | 446 | 319 | 1 | 24 | 58% | 42% | 0% |
| LSNAKE | 446 | 19% | 311 | 135 | - | 70% | 30% | 57.7 | 70.1 | - | 440 | 6 | 99% | 1% | 231 | 201 | 0 | 14 | 53% | 47% | 0% |
| *Total:* | *2,339* | | *1,548* | *791* | - | *67%* | *33%* | *58.3* | *70.8* | *79.1* | *2,256* | *82* | *91%* | *9%* | *1,270* | *981* | *13* | *75* | *50%* | *49%* | *2%* |

Table 8. Summary of Lower Granite Dam (LGR) juvenile steelhead samples from MY2020 assigned to a genetic stock using individual assignment based on the Snake River steelhead SNP baseline v3.1. Summaries of life history diversity information (sex, length, and freshwater age) for each genetic stock are shown. The 'Other' freshwater age category includes fish that were not queued to be aged or could not be aged.

| | | | Sex | | | | | Length | Freshwater Age | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Frequency | | | Percentage | | | Frequency | | | | | | Percentage | | | | |
| Genetic Stock | Total Assignments | % Stock Composition | F | M | U | F | M | Mean Length (mm FL) | 1 | 2 | 3 | 4 | 5 | Other | 1 | 2 | 3 | 4 | 5 |
| UPSALM | 1 | 1% | 1 | 0 | 0 | 100% | 0% | 283 | 0 | 1 | 0 | 0 | 0 | 0 | 0% | 100% | 0% | 0% | 0% |
| MFSALM | 0 | 0% | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SFSALM | 0 | 0% | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| LOSALM | 0 | 0% | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| UPCLWR | 1 | 1% | 1 | 0 | 0 | 100% | 0% | 296 | 0 | 1 | 0 | 0 | 0 | 0 | 0% | 100% | 0% | 0% | 0% |
| SFCLWR | 4 | 3% | 1 | 3 | 0 | 25% | 75% | 186 | 0 | 3 | 1 | 0 | 0 | 0 | 0% | 75% | 25% | 0% | 0% |
| LOCLWR | 8 | 7% | 8 | 0 | 0 | 100% | 0% | 248 | 0 | 6 | 2 | 0 | 0 | 0 | 0% | 75% | 25% | 0% | 0% |
| IMNAHA | 0 | 0% | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| GRROND | 74 | 63% | 45 | 29 | 0 | 61% | 39% | 231 | 2 | 50 | 20 | 0 | 0 | 2 | 3% | 69% | 28% | 0% | 0% |
| LSNAKE | 30 | 25% | 22 | 8 | 0 | 73% | 27% | 212 | 0 | 17 | 12 | 1 | 0 | 0 | 0% | 57% | 40% | 3% | 0% |
| *Total:* | *118* | | *78* | *40* | *0* | *77%* | *24%* | *227* | *2* | *78* | *35* | *1* | 0 | *2* | *2%* | *66%* | *30%* | *1%* | *0%* |

Table 9. Summary of Lower Granite Dam (LGR) juvenile Chinook Salmon samples from MY2020 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)** |
| UPSALM | 1 | 2% | 1 | 0 | 0 | 100% | 0% | 90 |
| MFSALM | 0 | 0% | - | - | - | - | - | - |
| CHMBLN | 0 | 0% | - | - | - | - | - | - |
| SFSALM | 0 | 0% | - | - | - | - | - | - |
| HELLSC | 37 | 80% | 19 | 18 | 0 | 51% | 49% | 122 |
| TUCANO | 0 | 0% | - | - | - | - | - | - |
| FALL | 8 | 17% | 4 | 4 | 0 | 50% | 50% | 119 |
| *MY2019 Total:* | *46* | | *22* | *24* | - | *52%* | *48%* | *120* |

**FIGURES**

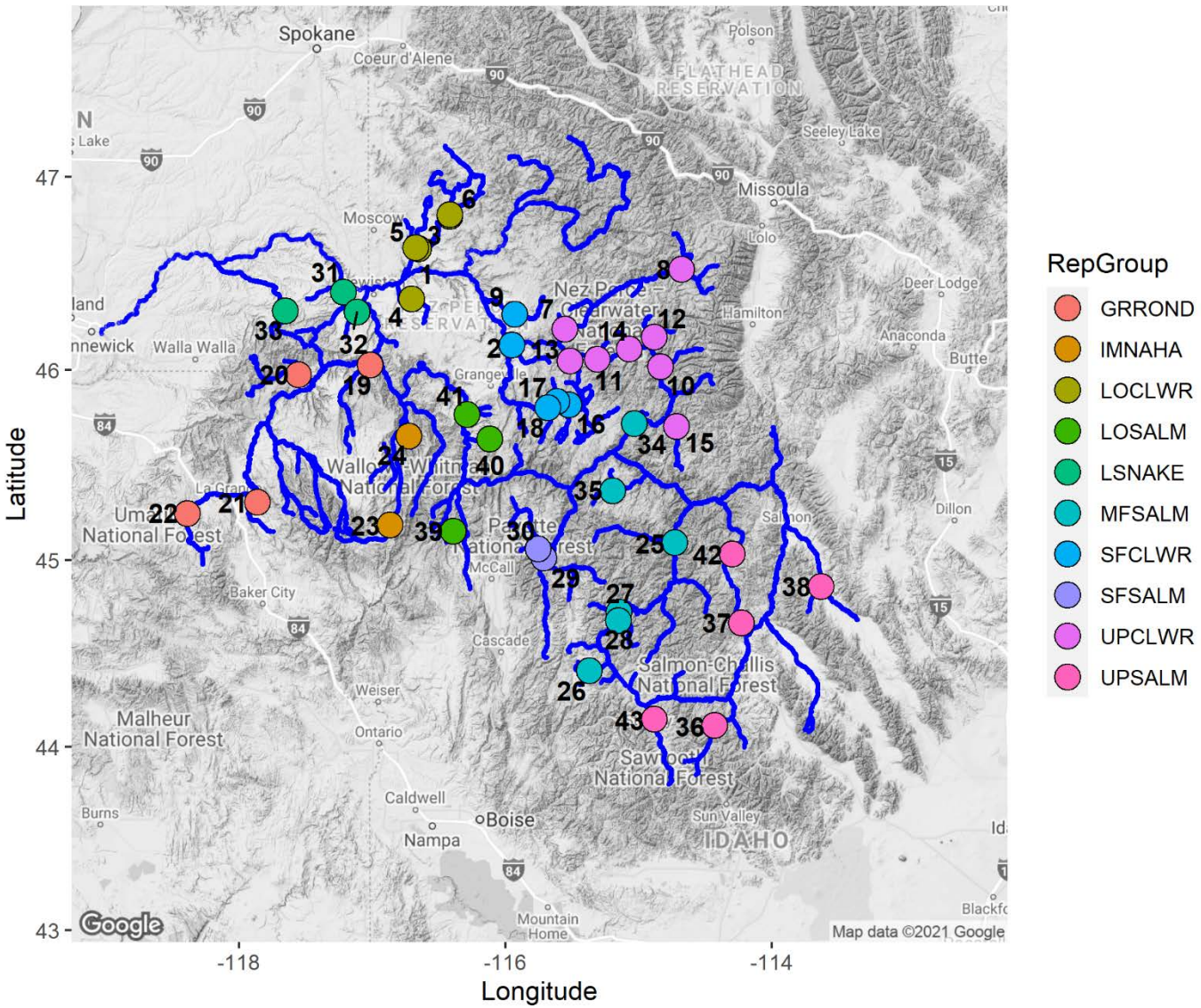


Figure 1. A map of steelhead (*Oncorhynchus mykiss*) collections used for genetic stock identification baseline v4 in the Snake River basin. Details associated with individual collections are presented in Table 2.

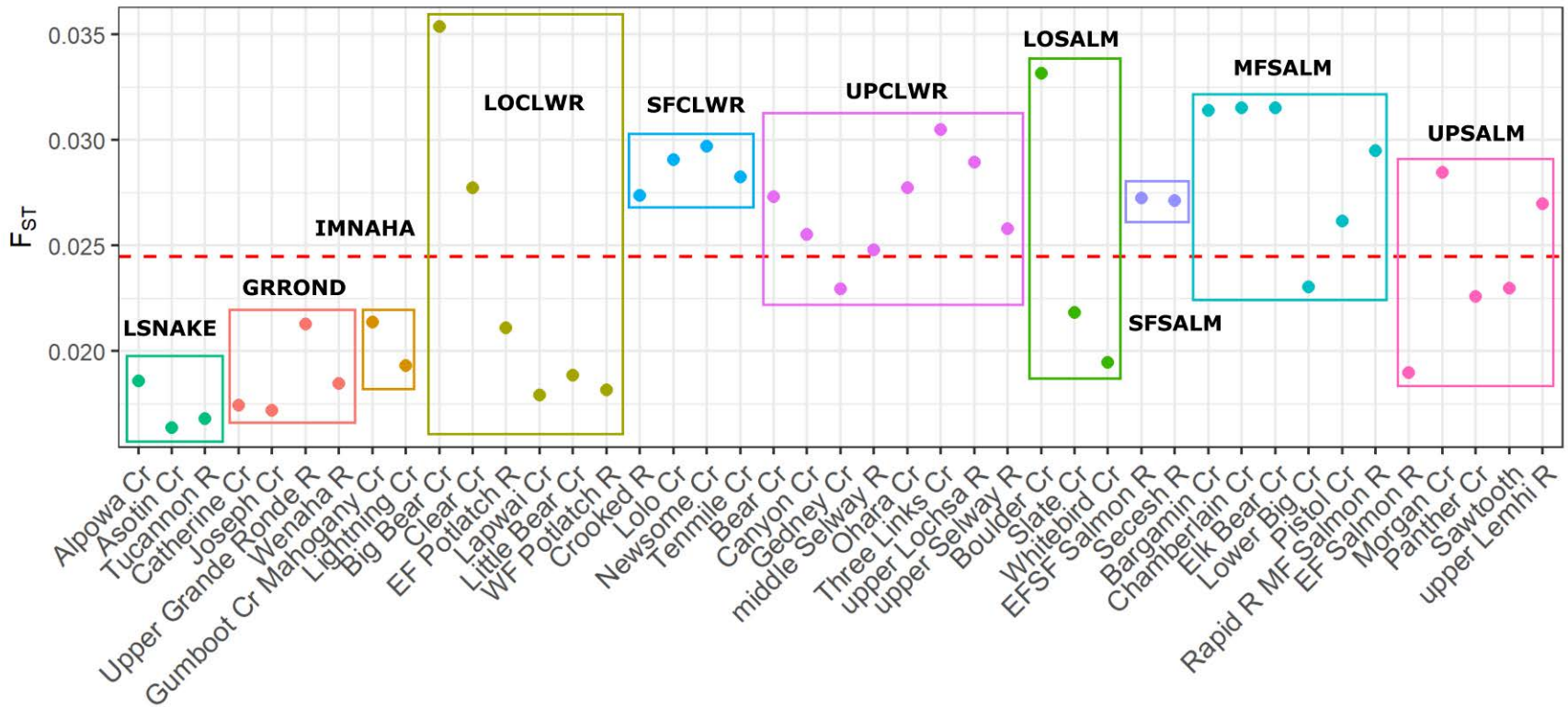


Figure 2. A plot of genetic differentiation values ($F_{ST}$) for steelhead collections included in baseline v4 organized by genetic stock. Values range from zero to one, with an $F_{ST}$ value of zero indicating that two populations share a common set of alleles and an $F_{ST}$ value of one meaning that two populations have no shared alleles. The dotted red line indicates mean $F_{ST}$ across all populations.

Figure 3. A plot displaying the results from a principal components analysis (PCA) of steelhead from baseline 4 for the Snake River basin. The two axes correspond to the first and second principal components (PC1, PC2) and the percentage of variance explained is presented in parentheses. Points correspond to mean PC values for each collection, which are color coded by the genetic stock. For additional collection details please reference Table 2.

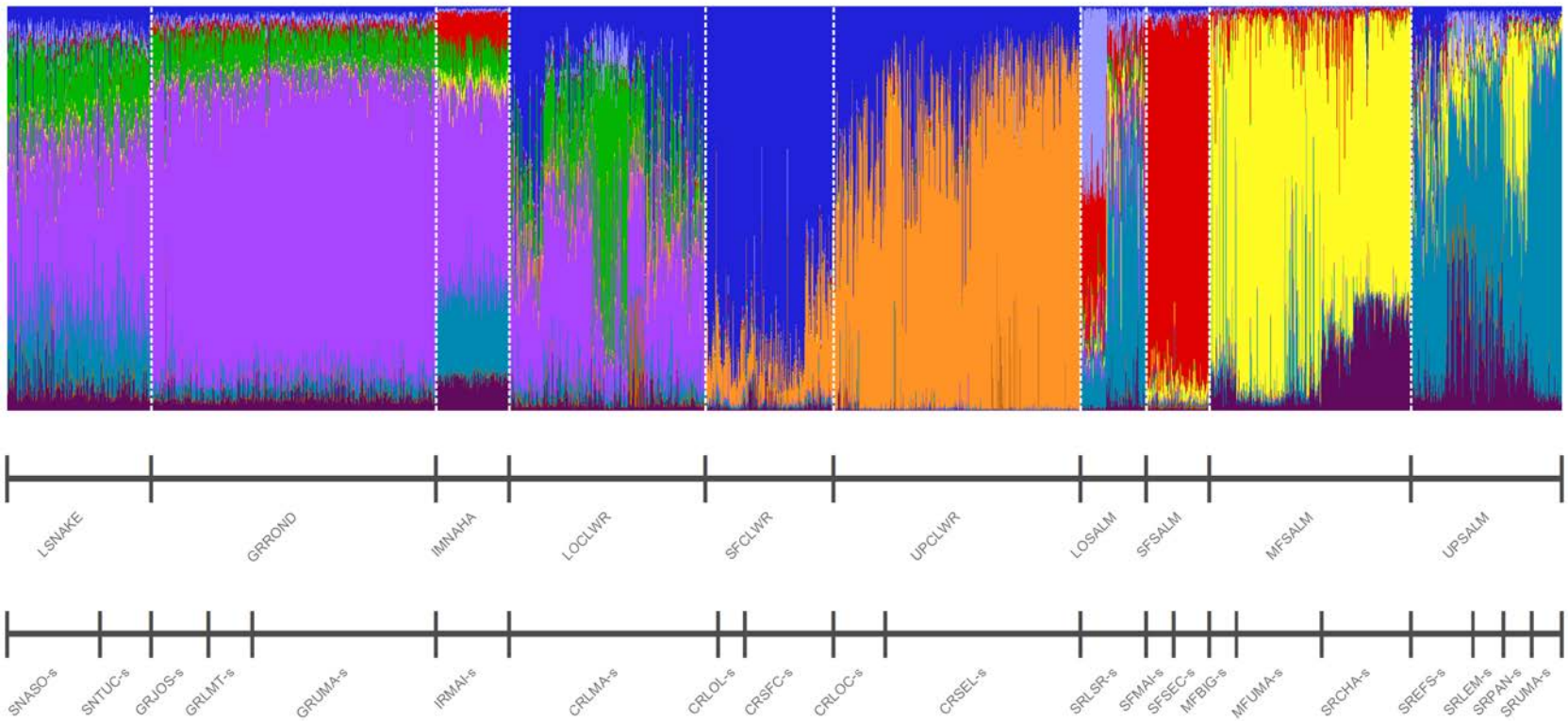


Figure 4. A STRUCTURE plot displaying the results of Bayesian clustering analysis of steelhead baseline v4 for the Snake River basin assuming 10 populations (K = 10). Each vertical bar denotes an individual fish and colors correspond to different genetic clusters. Individuals that contain multiple different colors correspond to admixed individuals (fish with joint ancestry across multiple genetic groups). Fish are organized by genetic stock (top label row) and population (bottom label row).

# APPENDIX A.

## A DESCRIPTION OF STEELHEAD GENETIC DIVERSITY IN THE SNAKE RIVER BASIN

### INTRODUCTION

Long-term recovery efforts for steelhead in the Snake River basin seek to improve their viability to the point of being self-sustaining in the wild. A common set of biological principles are used to assess population viability criteria including four population parameters: abundance, population productivity or growth rate, population spatial structure, and diversity (McElhaney et al. 2000). In this section, we report on metrics related to genetic variation within populations (expected heterozygosity, $H_E$) and levels of differentiation between populations (fixation index, $F_{ST}$) for steelhead populations in Idaho to support ongoing assessments efforts. Specifically, these data were provided to NOAA Fisheries for the 5-year status review of the Snake River basin steelhead DPS scheduled for 2021.

### METHODS

Here, we present on genetic patterns using a collection of steelhead samples used as a genetic baseline (v3.1; Powell et al. 2018) for genetic stock identification in the Snake River basin. These collections include tissues from juveniles and adults collected from across the Snake River basin which are amplified using a panel of 179 single nucleotide polymorphism (SNP) loci. Individual data and genotypes for all samples included in the Snake River SNP baselines are publically available at www.fishgen.net. Powell et al. (2018) provide an overview of different baselines that have been used for annual reports, which have varied in the number of markers and sample collections used for reference. The primary difference between the Snake River basin Steelhead Baseline v3.1 and v3 was that the latter contained adults that were PIT tagged at LGR and subsequently detected at PIT tag instream arrays throughout the Snake River basin. Adults with PIT tags present in baseline v3 were removed from baseline v3.1. For more details on analyses and baseline summaries, we encourage seeing Vu et al. (2015) and Powell et al. (2018).

For this summary, we generated values of expected heterozygosity ($H_E$) and genetic differentiation ($F_{ST}$) using GenAlEx v 6.503 (Peakall and Smouse 2006, 2012) by populations defined initially by the Interior Columbia Technical Recovery Team (2003). As part of this, collections of samples from multiple locations and years within a boundary were pooled and a mean value was generated for these pooled collections. To visualize the genetic relationships among collections, a neighbor-joining (NJ) tree was constructed using our entire Snake River steelhead baseline collections. The NJ tree was generated using Nei's genetic distance (Nei 1972) as calculated in GENDIST (PHYLIP; Felsenstein 1993), with trees constructed in NEIGHBOR (PHYLIP) and topology consistency was estimated via 1,000 bootstrap replicates (across loci) in SEQBOOT (PHYLIP). We show bootstrap support for major nodes that occurred in 700 (70%) or more bootstrap replicates. The final NJ trees was constructed and edited using FigTree v1.31 (Rambaut 2012).

### RESULTS

A total of 5,967 samples collected from 62 locations across the Snake River basin were used to investigate patterns of genetic diversity and differentiation among populations of steelhead (Table A1; Figure A1). Here, we report exclusively on populations from Idaho (4,609 individuals from 46 locations; Table A2). Important to note, metrics of genetic differentiation ($F_{ST}$) reflect values generated using the entire Snake River Steelhead DPS collection (5,967 samples).

Mean levels of expected heterozygosity across all populations in the Upper Salmon River were greater (30.6%) relative to the Clearwater River (28.9%) MPG (Table A2; Figure A2). Within the Upper Salmon River, the highest and lowest values of heterozygosity were recorded in the Lemhi River (32.6%) and Chamberlain Creek (28.6%) populations, respectively. In the Clearwater River, the highest value of heterozygosity was observed in the Lower Clearwater River (30.8%) population and lowest value in Lolo Creek (27.9%).

Overall levels of genetic differentiation across MPG were lower and less variable in the Salmon River MPG than in the Clearwater River MPG (Table A3; Figure A3). Specifically, mean differentiation across all sites in the Salmon River was 0.015 and 0.019 in the Clearwater River. Within the Salmon River, the most highly differentiated sites were the Secesh and South Fork Salmon Rivers (both with $F_{ST}$ = 0.19). In contrast, the sites with lowest $F_{ST}$ in the Salmon River were the North Fork Salmon River and Little Salmon and Lower Salmon tributaries (both 0.011). Within the Clearwater River, the most differentiated population was Lolo Creek (0.025) and the least differentiated was the Lower Clearwater River (0.010) population.

Patterns of genetic relatedness displayed in our neighbor-joining tree identified both the Clearwater and Salmon River MPGs as forming monophyletic clades with strong support (Figure A4). With few exceptions, genetic differentiation among collections of samples conformed to delineated populations (Figure A4).

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Table A1. Summary of sample collections included in the current Snake River steelhead SNP baseline (version 3.1; Powell et al. 2018). Summaries include the NMFS population that the collection was taken in, the genetic stock (reporting group) used for GSI at Lower Granite Dam, major population group (MPG), sample size (n), the years(s) that samples were collected, the lab where samples were genotyped, latitude/longitude, expected heterozygosity ($H_E$), and fixation indices ($F_{ST}$).

| Map# | Collection Name | Population | Genetic Stock[A] | MPG | n | Year Collected | Genotype Agency[B] | Latitude[C] | Longitude | $H_E$[D] | $F_{ST}$[E] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sawtooth | SRUMA-s | UPSALM | Salmon | 108 | 05, 10 | IDFG | 44.1506 | -114.8849 | 29.9% | 0.018 |
| 2 | Valley Cr | SRUMA-s | UPSALM | Salmon | 94 | 00, 10 | IDFG/NWFSC | 44.2181 | -114.9338 | 30.7% | 0.017 |
| 3 | WF Yankee Fork | SRUMA-s | UPSALM | Salmon | 117 | 00 | IDFG | 44.3494 | -114.7295 | 30.5% | 0.017 |
| 4 | Herd Cr | SREFS-s | UPSALM | Salmon | 85 | 10, 11 | NWFSC | 44.1091 | -114.2595 | 30.7% | 0.019 |
| 5 | Morgan Cr | SREFS-s | UPSALM | Salmon | 61 | 00, 12 | IDFG | 44.6611 | -114.2265 | 32.4% | 0.020 |
| 6 | Pahsimeroi R | SRPAH-s | UPSALM | Salmon | 98 | 06, 10 | IDFG | 44.6823 | -114.0396 | 32.1% | 0.018 |
| 7 | upper Lemhi R | SRLEM-s | UPSALM | Salmon | 86 | 09, 10 | IDFG | 44.8675 | -113.6281 | 32.6% | 0.018 |
| 8 | NF Salmon R | SRNFS-s | UPSALM | Salmon | 100 | 10 | IDFG | 45.4094 | -113.9918 | 31.2% | 0.014 |
| 10 | Capehorn/Marsh Cr | MFUMA-s | MFSALM | Salmon | 195 | 00, 09, 10 | IDFG/NWFSC | 44.3949 | -115.1709 | 30.5% | 0.019 |
| 11 | Elk/Bear Cr | MFUMA-s | MFSALM | Salmon | 173 | 10, 11 | IDFG/NWFSC | 44.4104 | -115.4685 | 29.6% | 0.024 |
| 12 | Sulphur Cr | MFUMA-s | MFSALM | Salmon | 94 | 00, 11 | IDFG/NWFSC | 44.5437 | -115.3034 | 29.6% | 0.024 |
| 13 | Rapid R (MF Salmon R) | MFUMA-s | MFSALM | Salmon | 75 | 00, 12 | IDFG | 44.679 | -115.149 | 29.8% | 0.025 |
| 14 | Pistol Cr | MFUMA-s | MFSALM | Salmon | 58 | 00, 12 | IDFG | 44.7635 | -115.1565 | 30.6% | 0.023 |
| 15 | Loon Cr | MFUMA-s | MFSALM | Salmon | 131 | 00, 11 | IDFG/NWFSC/CRITFC | 44.5983 | -114.8083 | 28.7% | 0.023 |
| 16 | Camas Cr | MFBIG-s | MFSALM | Salmon | 97 | 00, 10 | IDFG/NWFSC | 44.824 | -114.7194 | 29.2% | 0.023 |
| 17 | upper Big Cr | MFBIG-s | MFSALM | Salmon | 87 | 00, 11 | IDFG/NWFSC | 45.1506 | -115.307 | 28.7% | 0.028 |
| 18 | lower Big Cr | MFBIG-s | MFSALM | Salmon | 137 | 00, 11 | IDFG/NWFSC | 45.1072 | -114.7429 | 29.9% | 0.019 |
| 19 | Chamberlain Cr | SRCHA-s | MFSALM | Salmon | 189 | 00, 10, 11 | IDFG/NWFSC | 45.3877 | -115.1896 | 28.6% | 0.021 |
| 21 | upper SF Salmon R mainstem | SFMAI-s | SFSALM | Salmon | 45 | 00 | IDFG/CRITFC | 44.6069 | -115.6799 | 29.0% | 0.026 |
| 22 | Johnson Cr | SFMAI-s | SFSALM | Salmon | 89 | 10, 11 | IDFG/NWFSC | 44.9341 | -115.4867 | 30.0% | 0.023 |
| 23 | EFSF Salmon R | SFMAI-s | SFSALM | Salmon | 47 | 00 | IDFG | 44.9553 | -115.7127 | 29.9% | 0.027 |
| 24 | Lake Cr (SF Salmon R) | SFSEC-s | SFSALM | Salmon | 50 | 10, 11 | IDFG | 45.2756 | -115.9184 | 28.9% | 0.033 |
| 25 | Lick Cr | SFSEC-s | SFSALM | Salmon | 63 | 10, 11 | IDFG | 45.0591 | -115.851 | 29.2% | 0.023 |
| 26 | Secesh R | SFSEC-s | SFSALM | Salmon | 95 | 00, 11 | IDFG/NWFSC | 45.2188 | -115.8076 | 29.4% | 0.023 |
| 27 | Boulder Cr/Rapid R | SRLSR-s | LOSALM | Salmon | 147 | 00, 03, 09 | IDFG | 45.3546 | -116.3907 | 30.7% | 0.016 |
| 28 | Slate Cr | SRLSR-s | LOSALM | Salmon | 75 | 00, 13 | IDFG | 45.6392 | -116.2804 | 30.9% | 0.016 |
| 29 | upper Lochsa R | CRLOC-s | UPCLWR | Clearwater | 129 | 00 | IDFG | 46.5082 | -114.6775 | 28.2% | 0.025 |
| 30 | Lake Cr | CRLOC-s | UPCLWR | Clearwater | 47 | 00 | IDFG | 46.4144 | -114.9943 | 28.2% | 0.029 |
| 31 | Fish Cr | CRLOC-s | UPCLWR | Clearwater | 100 | 10, 11 | IDFG | 46.3558 | -115.3483 | 28.5% | 0.024 |
| 33 | upper Selway R | CRSEL-s | UPCLWR | Clearwater | 137 | 08 | IDFG | 45.6921 | -114.7175 | 29.1% | 0.024 |
| 34 | Whitecap Cr | CRSEL-s | UPCLWR | Clearwater | 110 | 11, 12 | IDFG | 45.8689 | -114.7205 | 29.2% | 0.026 |
| 35 | Bear Cr | CRSEL-s | UPCLWR | Clearwater | 70 | 00, 12 | IDFG | 46.0357 | -114.8376 | 29.3% | 0.026 |
| 36 | middle Selway R | CRSEL-s | UPCLWR | Clearwater | 138 | 00, 12 | IDFG | 46.0978 | -114.8842 | 28.6% | 0.021 |
| 37 | Three Links Cr | CRSEL-s | UPCLWR | Clearwater | 81 | 00, 12 | IDFG | 46.1451 | -115.072 | 28.5% | 0.026 |
| 38 | Gedney Cr | CRSEL-s | UPCLWR | Clearwater | 45 | 00 | IDFG | 46.0583 | -115.3141 | 29.4% | 0.023 |
| 39 | O'Hara Cr | CRSEL-s | UPCLWR | Clearwater | 85 | 00, 13 | IDFG | 46.0449 | -115.5177 | 28.9% | 0.019 |
| 40 | Crooked R | CRSFC-s | SFCLWR | Clearwater | 136 | 07, 08, 11 | IDFG | 45.7656 | -115.528 | 27.7% | 0.025 |
| 41 | Newsome Cr | CRSFC-s | SFCLWR | Clearwater | 99 | 12 | IDFG | 45.8345 | -115.6164 | 27.9% | 0.027 |
| 42 | Tenmile Cr | CRSFC-s | SFCLWR | Clearwater | 47 | 00 | IDFG | 45.8057 | -115.6833 | 27.9% | 0.031 |
| 43 | Clear Cr | CRLMA-s | SFCLWR | Clearwater | 45 | 00 | IDFG | 46.0486 | -115.7737 | 28.8% | 0.025 |
| 44 | Lolo Cr | CRLOL-s | SFCLWR | Clearwater | 94 | 12 | IDFG | 46.2906 | -115.9228 | 27.9% | 0.027 |
| 45 | WF Potlatch R | CRLMA-s | LOCLWR | Clearwater | 84 | 09, 10 | IDFG | 46.8638 | -116.419 | 30.5% | 0.016 |
| 46 | EF Potlatch R | CRLMA-s | LOCLWR | Clearwater | 158 | 08, 10, 11 | IDFG | 46.8049 | -116.4247 | 30.3% | 0.017 |
| 47 | Little Bear Cr | CRLMA-s | LOCLWR | Clearwater | 151 | 07, 08, 10, 11 | IDFG | 46.6532 | -116.6594 | 30.6% | 0.015 |
| 48 | Big Bear Cr | CRLMA-s | LOCLWR | Clearwater | 99 | 07, 08, 10, 11 | IDFG | 46.6752 | -116.6595 | 31.5% | 0.015 |

Table A1. Continued

| Map# | Collection Name | NMFS Population | Genetic Stock[A] | MPG | n | Year Collected | Genotype Agency[B] | Latitude[C] | Longitude | $H_E$[D] | $F_{ST}$[E] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 49 | Lapwai Cr | CRLMA-s | LOCLWR | Clearwater | 158 | 13 | IDFG | 46.6191 | -116.6316 | 30.5% | 0.016 |
| 50 | Gumboot/Mahogany Cr | IRMAI-s | IMNAHA | Imnaha | 53 | 11, 12, 13 | IDFG | 45.2033 | -116.8773 | 29.0% | 0.019 |
| 51 | Little Sheep Cr | IRMAI-s | IMNAHA | Imnaha | 93 | 00 | IDFG | 45.4723 | -116.9572 | 30.0% | 0.020 |
| 52 | Big Sheep Cr | IRMAI-s | IMNAHA | Imnaha | 91 | 00 | IDFG/CRITFC | 45.5551 | -116.8391 | 29.8% | 0.018 |
| 53 | Lightning Cr | IRMAI-s | IMNAHA | Imnaha | 39 | 00 | NWFSC | 45.6847 | -116.7265 | 29.6% | 0.019 |
| 54 | upper Grande Ronde R | GRWAL-s | GRROND | Grande Ronde | 65 | 09, 10, 11 | CRITFC | 45.5065 | -117.933 | 30.7% | 0.017 |
| 55 | Catherine Cr | GRWAL-s | GRROND | Grande Ronde | 91 | 11 | CRITFC | 45.2406 | -117.9223 | 30.8% | 0.016 |
| 56 | Little Minam R | GRWAL-s | GRROND | Grande Ronde | 48 | 00 | NWFSC | 45.3991 | -117.674 | 30.2% | 0.024 |
| 57 | Wallowa R | GRWAL-s | GRROND | Grande Ronde | 73 | 00, 09, 11 | NWFSC | 45.436 | -117.3319 | 32.0% | 0.017 |
| 58 | Lostine R | GRWAL-s | GRROND | Grande Ronde | 45 | 00 | CRITFC | 45.5508 | -117.4892 | 31.1% | 0.024 |
| 59 | Wenaha R | GRLMT-s | GRROND | Grande Ronde | 191 | 00 | NWFSC | 45.9455 | -117.4516 | 30.7% | 0.015 |
| 60 | Menatchee Cr | GRLMT-s | GRROND | Grande Ronde | 73 | 00 | CRITFC | 46.0081 | -117.3655 | 31.7% | 0.018 |
| 61 | Elk Cr (Joseph Cr) | GRJOS-s | GRROND | Grande Ronde | 45 | 00 | CRITFC | 45.9727 | -117.1534 | 28.9% | 0.025 |
| 62 | Joseph Cr | GRJOS-s | GRROND | Grande Ronde | 53 | 11 | CRITFC | 46.0446 | -117.0177 | 30.4% | 0.017 |
| 64 | Asotin Cr | SNASO-s | LSNAKE | Lower Snake | 194 | 08, 10 | IDFG | 46.3224 | -117.1379 | 31.4% | 0.014 |
| 65 | Alpowa Cr | SNTUC-s | LSNAKE | Lower Snake | 98 | 10 | IDFG | 46.4076 | -117.2198 | 31.4% | 0.015 |
| 66 | Tucannon R | SNTUC-s | LSNAKE | Lower Snake | 106 | 05, 09, 10 | IDFG | 46.3228 | -117.6557 | 31.4% | 0.015 |

Footnotes:

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

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

C. WGS84 Datum

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

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

Table A2. Sample collections from Idaho steelhead populations and genetic variation present within populations (expected heterozygosity, $H_E$) and between populations (fixation index, $F_{ST}$) as summarized by major population group (MPG) and population.

| | | | Samples Collected | | | | |
|---|---|---|---|---|---|---|---|
| **MPG** | **Population** | **Description** | **Sites** | **Years** | **Individuals** | **$H_E$** | **$F_{ST}$** |
| Clearwater | CRLMA-s | Lower Clearwater River | 6 | 7 | 695 | 30.8% | 0.012 |
| Clearwater | CRSEL-s | Selway River | 7 | 5 | 666 | 29.3% | 0.019 |
| Clearwater | CRLOC-s | Lochsa River | 3 | 3 | 276 | 28.4% | 0.023 |
| Clearwater | CRSFC-s | South Fork Clearwater River | 3 | 5 | 282 | 28.3% | 0.020 |
| Clearwater | CRLOL-s | Lolo Creek | 1 | 1 | 94 | 27.9% | 0.025 |
| Salmon | SRLEM-s | Lemhi River | 1 | 2 | 86 | 32.6% | 0.016 |
| Salmon | SRPAH-s | Pahsimeroi River | 1 | 2 | 98 | 32.1% | 0.016 |
| Salmon | SREFS-s | East Fork Salmon River | 2 | 4 | 146 | 31.7% | 0.014 |
| Salmon | SRNFS-s | North Fork Salmon River | 1 | 1 | 100 | 31.2% | 0.012 |
| Salmon | SRLSR-s | Little Salmon and Lower Salmon tributaries | 2 | 4 | 222 | 30.9% | 0.012 |
| Salmon | SRUMA-s | Upper Mainstem Salmon River | 3 | 3 | 319 | 30.5% | 0.014 |
| Salmon | MFUMA-s | Upper Middle Fork Salmon River | 6 | 5 | 726 | 30.1% | 0.017 |
| Salmon | SFMAI-s | South Fork Salmon River | 3 | 3 | 181 | 30.0% | 0.019 |
| Salmon | MFBIG-s | Lower Middle Fork Salmon River | 3 | 3 | 321 | 29.6% | 0.018 |
| Salmon | SFSEC-s | Secesh River | 3 | 3 | 208 | 29.6% | 0.019 |
| Salmon | SRCHA-s | Chamberlain Creek | 1 | 3 | 189 | 28.6% | 0.019 |

Table A3. A pairwise matrix of genetic differentiation among Idaho steelhead as quantified by FST values (fixation index). Values closer to zero represent lower levels of genetic differentiation and larger values imply populations are more genetically distinct. Values are color coded with green and red implying lower and higher levels of differentiation, respectively. Numbers in columns match populations in rows and collections of samples used to estimate populations-level values of FST are detailed in Table A1.

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Avg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1) SRUMA-s** | - | 0.006 | 0.005 | 0.007 | 0.004 | 0.015 | 0.016 | 0.018 | 0.020 | 0.020 | 0.006 | 0.023 | 0.020 | 0.019 | 0.024 | 0.008 | **0.014** |
| **2) SREFS-s** | 0.006 | - | 0.006 | 0.007 | 0.005 | 0.015 | 0.016 | 0.016 | 0.018 | 0.017 | 0.007 | 0.023 | 0.019 | 0.019 | 0.025 | 0.009 | **0.014** |
| **3) SRPAH-s** | 0.005 | 0.006 | - | 0.006 | 0.006 | 0.017 | 0.018 | 0.019 | 0.021 | 0.021 | 0.008 | 0.028 | 0.024 | 0.024 | 0.029 | 0.010 | **0.016** |
| **4) SRLEM-s** | 0.007 | 0.007 | 0.006 | - | 0.006 | 0.017 | 0.018 | 0.020 | 0.021 | 0.020 | 0.010 | 0.026 | 0.022 | 0.023 | 0.028 | 0.010 | **0.016** |
| **5) SRNFS-s** | 0.004 | 0.005 | 0.006 | 0.006 | - | 0.012 | 0.013 | 0.014 | 0.017 | 0.016 | 0.005 | 0.020 | 0.017 | 0.018 | 0.023 | 0.006 | **0.012** |
| **6) MFUMA-s** | 0.015 | 0.015 | 0.017 | 0.017 | 0.012 | - | 0.004 | 0.011 | 0.013 | 0.014 | 0.010 | 0.029 | 0.025 | 0.029 | 0.035 | 0.016 | **0.017** |
| **7) MFBIG-s** | 0.016 | 0.016 | 0.018 | 0.018 | 0.013 | 0.004 | - | 0.010 | 0.013 | 0.015 | 0.011 | 0.029 | 0.025 | 0.028 | 0.034 | 0.016 | **0.018** |
| **8) SRCHA-s** | 0.018 | 0.016 | 0.019 | 0.020 | 0.014 | 0.011 | 0.010 | - | 0.016 | 0.017 | 0.012 | 0.029 | 0.026 | 0.028 | 0.034 | 0.017 | **0.019** |
| **9) SFMAI-s** | 0.020 | 0.018 | 0.021 | 0.021 | 0.017 | 0.013 | 0.013 | 0.016 | - | 0.007 | 0.012 | 0.029 | 0.026 | 0.028 | 0.033 | 0.017 | **0.019** |
| **10) SFSEC-s** | 0.020 | 0.017 | 0.021 | 0.020 | 0.016 | 0.014 | 0.015 | 0.017 | 0.007 | - | 0.012 | 0.026 | 0.022 | 0.027 | 0.032 | 0.017 | **0.019** |
| **11) SRLSR-s** | 0.006 | 0.007 | 0.008 | 0.010 | 0.005 | 0.010 | 0.011 | 0.012 | 0.012 | 0.012 | - | 0.022 | 0.019 | 0.019 | 0.024 | 0.008 | **0.012** |
| **12) CRLOC-s** | 0.023 | 0.023 | 0.028 | 0.026 | 0.020 | 0.029 | 0.029 | 0.029 | 0.029 | 0.026 | 0.022 | - | 0.004 | 0.013 | 0.019 | 0.015 | **0.023** |
| **13) CRSEL-s** | 0.020 | 0.019 | 0.024 | 0.022 | 0.017 | 0.025 | 0.025 | 0.026 | 0.026 | 0.022 | 0.019 | 0.004 | - | 0.011 | 0.017 | 0.011 | **0.019** |
| **14) CRSFC-s** | 0.019 | 0.019 | 0.024 | 0.023 | 0.018 | 0.029 | 0.028 | 0.028 | 0.028 | 0.027 | 0.019 | 0.013 | 0.011 | - | 0.008 | 0.010 | **0.020** |
| **15) CRLOL-s** | 0.024 | 0.025 | 0.029 | 0.028 | 0.023 | 0.035 | 0.034 | 0.034 | 0.033 | 0.032 | 0.024 | 0.019 | 0.017 | 0.008 | - | 0.015 | **0.025** |
| **16) CRLMA-s** | 0.008 | 0.009 | 0.010 | 0.010 | 0.006 | 0.016 | 0.016 | 0.017 | 0.017 | 0.017 | 0.008 | 0.015 | 0.011 | 0.010 | 0.015 | - | **0.012** |

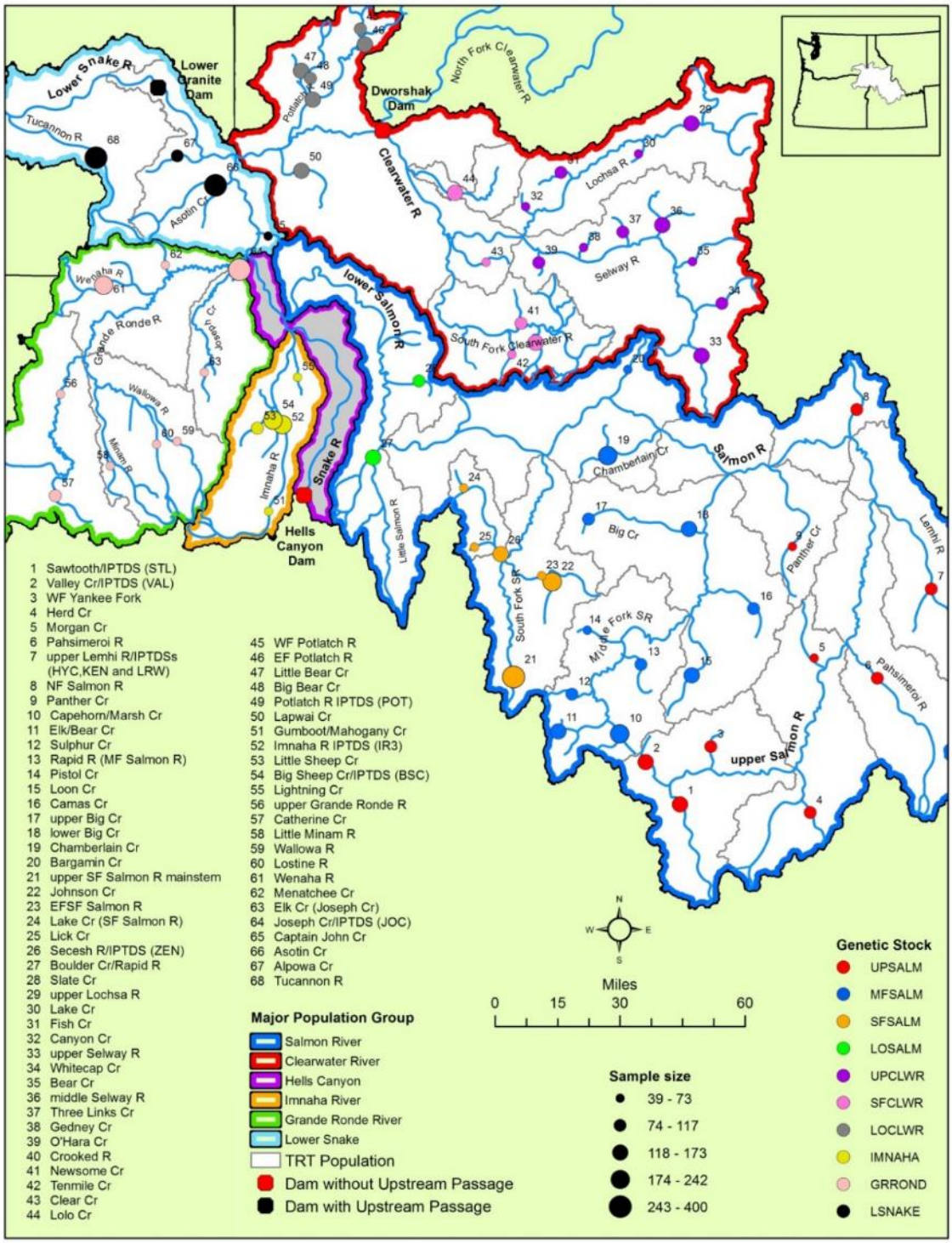


Figure A1. A map of steelhead collections used in the steelhead baseline v3.1 (Powell et al. 2018) and in the current summary of genetic diversity and genetic differentiation among Idaho steelhead populations (taken from Vu et al. 2015). For a detailed description of collection characteristics see Table A1. The Hells Canyon Tributaries major population group (shaded gray) does not support independent populations and is considered extirpated.

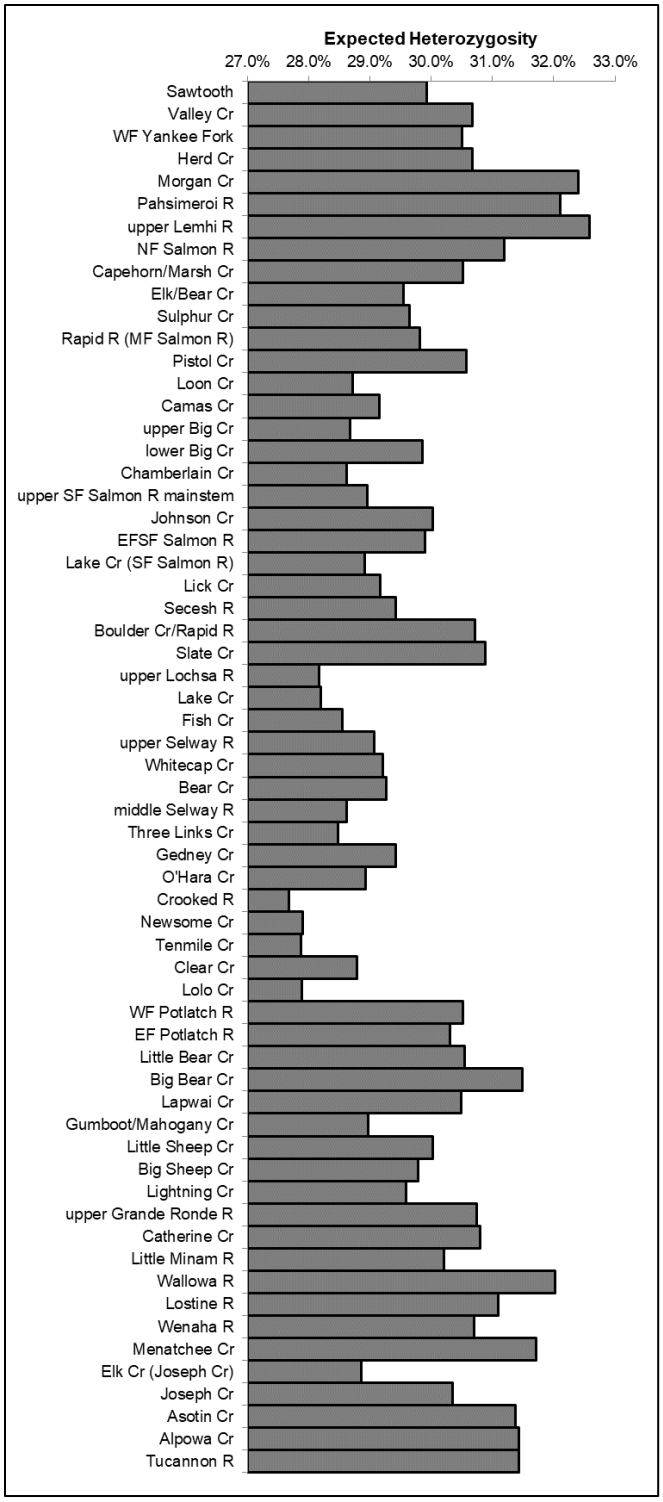


Figure A2. Mean expected heterozygosity for 179 SNPs assayed across collections of Snake River basin steelhead populations used in baseline v3.1. See Table A1 for population details.

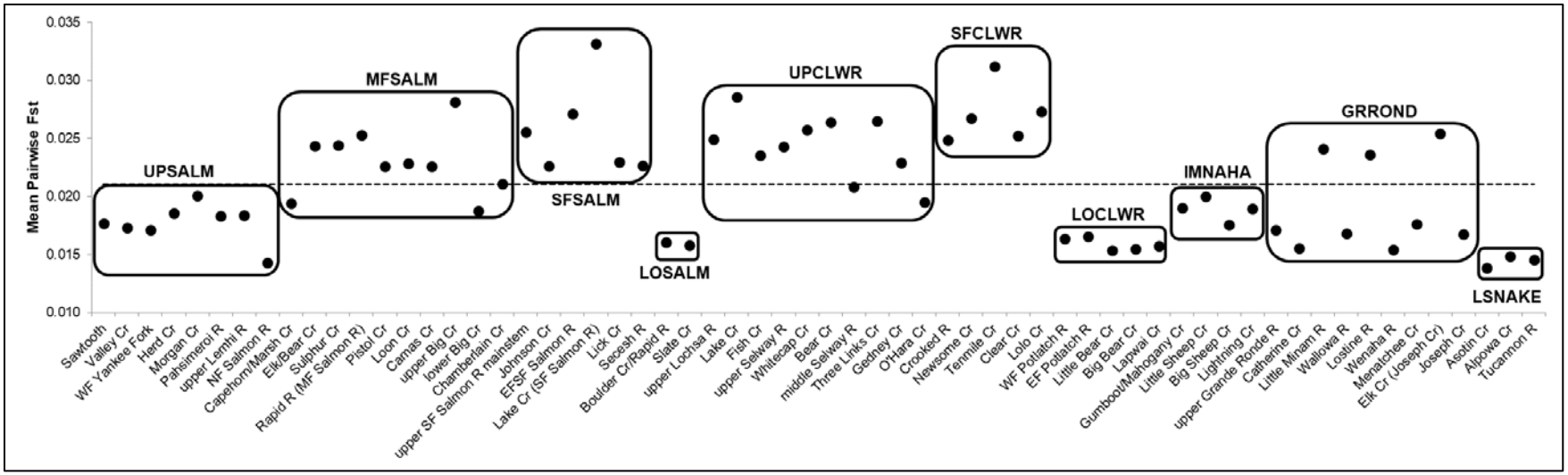


Figure A3. Mean pairwise FST estimates for the Snake River basin steelhead baseline v3.1 (Powell et al. 2018). The dashed line is the average pairwise FST estimate across all collections. High mean FST estimates suggest high genetic differentiation relative to other collections in the baseline. Each genetic stock is circumscribed and population-level details can be found in Table A1.

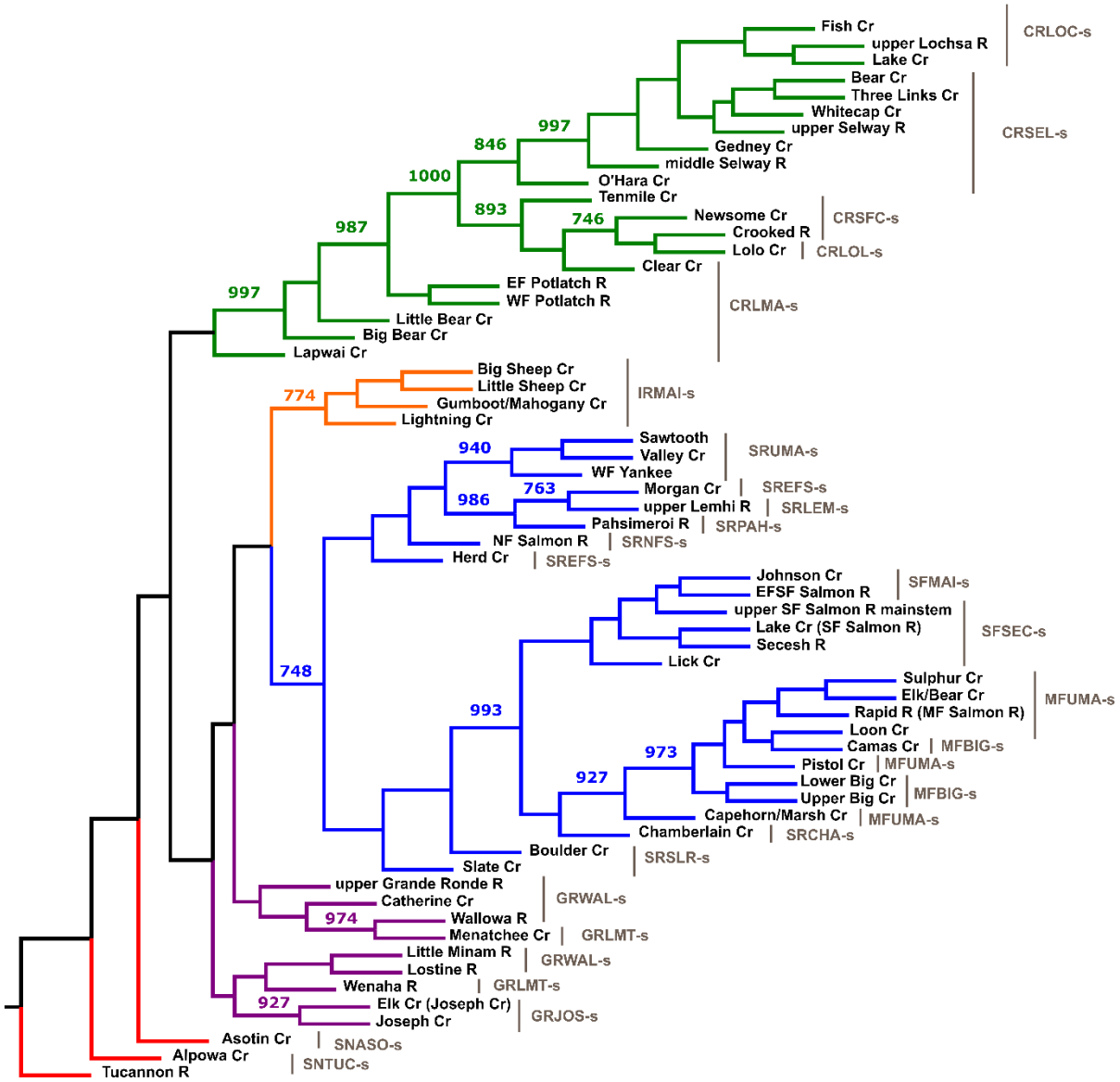


Figure A4. Genetic relationships among populations of steelhead collected from the Snake River basin. Labels in black denote individual collections and labels in grey denote the population for each collection with additional site and collection specific details provided in Table A1. The tree is based on Nei's genetic distance and numbers along branches display bootstraps values supporting the grouping greater than 70% (of 1,000 replicates).

**APPENDIX B.**

## GENETIC DIFFERENTIATION ($F_{ST}$) AND PHYLOGENETIC RELATIONSHIPS AMONG IDAHO STEELHEAD POPULATIONS

### INTRODUCTION

A 2015 status review of steelhead in the Snake River basin identified uncertainty in the proportion of hatchery fish in natural-origin spawning areas near major hatchery release sites within individual populations (NWFSC 2015). In this section, we present information on the genetic relatedness of wild and hatchery stocks in the Snake River basin to address the above mentioned data gap. Specifically, we quantified levels of genetic differentiation ($F_{ST}$) among hatchery and wild steelhead stocks in the Snake River basin. Additionally, we generated a neighbor-joining tree to estimate the phylogenetic relationships among hatchery and wild populations of steelhead (*Oncorhynchus mykiss*). These data were provided to NOAA Fisheries in 2020 to facilitate an upcoming Biological Opinion.

### METHODS

We quantified the genetic relationships among hatchery and wild stocks of Steelhead in the Snake River basin using three complimentary datasets. The data used to calculate these comparisons were as follows:

1) Juvenile and adult steelhead collections included in the most recent genetic stock identification (GSI) baseline described in Vu et al. (2015). In tables and figures, these collections are identified by population names (e.g., SRUMA-s is the Salmon River upper mainstem population) adopted in the ESA Recovery Plan for Snake River steelhead and spring/summer Chinook Salmon (NMFS 2017).

2) PIT-tagged wild adults sampled at Lower Granite Dam and subsequently detected at PIT arrays on the landscape. These data were used in a report submitted to NOAA for the 2020 ESA status review (IPTDSW 2020). These data are labeled according to the population in which the PIT tag array was placed and identified with the prefix 'PIT-tag'.

3) Steelhead hatchery broodstock used in spawn years 2017 and 2018 from all hatchery facilities in the Snake River basin (including satellite hatchery release sites). These data are labeled with the suffix 'Hatchery'. Detailed descriptions of individual collections can be found in Delomas et al. (2019; 2020).

We generated population-level estimates of genetic differentiation ($F_{ST}$) using Genepop (Rousset 2008) and tested the significance of differentiation tests using the following parameters: dememorization = 10,000, batches = 100, iterations per batch = 5000 as implemented in R (R Core Team 2019).

A neighbor-joining (NJ) tree was constructed using pairwise Cavalli-Sforza Edwards chord distance (Cavalli-Sforza and Edwards 1967) calculated using GENDIST (PHYLIP v3.5; Felsenstein 1993). Pairwise genetic distances were used to construct NJ trees in NEIGHBOR (PHYLIP v3.5). The NJ tree was visualized using Figtree v1.3.0 (https://github.com/rambaut/figtree/) and manually edited in Inkscape v0.92.3 (https://inkscape.org/).

## RESULTS

Levels of pairwise genetic differentiation of natural-origin adults (GSI and PIT-tagged adults) averaged across all collections were highest in the Upper Clearwater River, Middle Fork Salmon River, South Fork Clearwater River, and South Fork Salmon River (Table B1). Values of Fst for all pairwise comparisons are provided in a separate spreadsheet (available upon request) and comparisons among hatchery and wild stocks are broken down by major river drainage in separate tabs. In general, levels of genetic differentiation between collections (hatchery and wild) were low but statistically significant. Among hatchery and wild collections in Lower Snake River, most collections of wild fish (GSI baseline and PIT-tagged adults) were not statistically different, but all comparisons between Tucannon River hatchery stock and wild fish were significant. In the Grande Ronde and Imnaha rivers, nearly all pairwise comparisons (63 of 66) were significantly different regardless of source. Similarly, in the Clearwater River, 75 of 78 comparisons were statistically significant. In the Upper Salmon River, comparisons between both the Sawtooth and Pahsimeroi hatchery broodstock and wild stocks were nearly all significant (21 of 22 comparisons). In contrast, broodstock from the East Fork Salmon River were only significantly different from 6 of 11 comparisons with wild stocks.

Overall, we observed the presence of well-resolved clades in the our phylogenetic analysis for the Grand Ronde, Upper Clearwater, South Fork Clearwater, South Fork Salmon and Middle Fork Salmon river GSI reporting groups (Figure B1). In contrast, the Lower Snake, Lower Clearwater, Lower Salmon, and Upper Salmon River reporting groups were less well resolved, either sharing populations across clades (Upper Salmon River) or not forming monophyletic clades (Lower Clearwater River). In general, collections of PIT-tagged adults were most closely related to baseline collections from the same population (e.g., GRJOS-s and PIT-tag-GROJS-s). Hatchery collections formed clades sister to populations from the same reporting group with the exception of the Oxbow, East Fork Salmon River, and Upper Salmon B-run programs which were most closely related to likely broodstock sources.

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Table B1. Average levels of pairwise genetic differentiation ($F_{ST}$) across different collection sources (GSI = samples in GSI baseline, PIT-tag = adults sampled at Lower Granite Dam and PIT-tagged then later detected at an instream detection array, and Hatchery = hatchery broodstock) and populations of Steelhead from the Snake River basin.

| Reporting Group | GSI | PIT-tag | Hatchery |
|---|---|---|---|
| GRROND | 0.021 | 0.020 | 0.018 |
| IMNAHA | 0.021 | 0.021 | 0.023 |
| LOCLWR | 0.017 | 0.015 | -- |
| LOSALM | 0.019 | 0.023 | 0.024 |
| LSNAKE | 0.017 | 0.017 | 0.018 |
| MFSALM | 0.036 | 0.035 | -- |
| SFCLWR | 0.041 | 0.034 | 0.031 |
| SFSALM | 0.039 | 0.034 | -- |
| UPCLWR | 0.041 | 0.040 | -- |
| UPSALM | 0.020 | 0.021 | 0.026 |

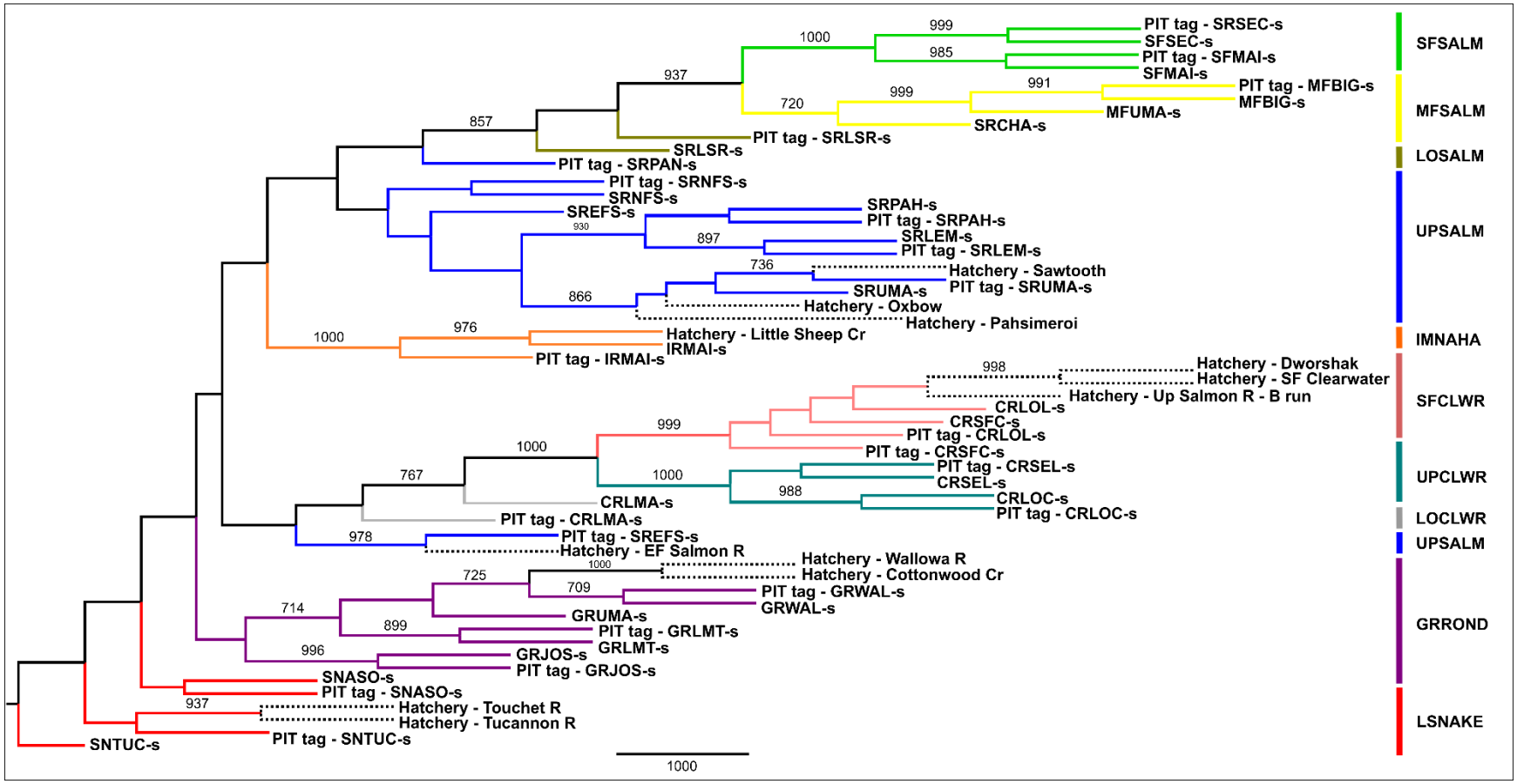


Figure B1. A neighbor-joining tree based on Cavalli-Sforza Edwards chord distance for Snake River steelhead populations included in the GSI baseline version 3.1, collections of PIT tagged returning adults for SY2010-2019 (indicated with prefix 'PIT tag'), and steelhead broodstock used in production hatcheries for SY2017-2018. Bootstrap support greater than 70% based on 1,000 replicated are reported. Colors and labels right of the NJ tree correspond to the genetic stock identification (GSI) reporting groups.

# APPENDIX C.

## THE INFLUENCE OF POPULATION MANAGEMENT STRATEGY (WILD VERSUS HATCHERY) ON LEVELS OF EXPECTED HETEROZYGOSITY (HE) AND NUMBER OF BREEDERS (NB) FOR STEELHEAD (ONCORHYNCHUS MYKISS) POPULATIONS IN IDAHO

### INTRODUCTION

As noted in the previous section, interactions between hatchery-origin and wild steelhead in the Snake River basin represents a management concern. In particular, the interbreeding of wild and hatchery stocks may ultimately lead to decreased levels of native diversity and an increased prevalence of genetic diversity of domestic origin (Waples 1994). A detailed description of genetic patterns of steelhead in the Snake River basin was generated by Nielsen et al. (2009), and part of this work involved comparing levels of genetic diversity and differentiation among watersheds with and without hatchery supplementation. Genetic diversity within watersheds managed exclusively for wild fish appeared to remain largely unaffected by hatchery stocks. Nielsen et al. (2009) noted that levels of genetic diversity were higher in populations proximate to hatchery operations, but these changes are not apparent at the watershed scale. In this section, we perform similar analyses as Nielsen et al. (2009); however, we do so using contemporary samples collected from spawn years 2009-2019 included in a report presented to NOAA Fisheries for the upcoming 5-Year ESA status review (IPTDSW 2020).

### METHODS

For specific samples and SNP markers used for analysis please see IPTDSW (2020). Expected heterozygosity ($H_E$) was calculated for each population as a proxy measure of genetic diversity. Expected heterozygosity is an estimate of the percentage of individuals in the population that are heterozygotes (average across SNPs) based on the allele frequency estimates from the population. Unlike genetic metrics such as effective population size, there is currently no specified value of expected heterozygosity that is used to assess whether a population is at elevated risk. Nonetheless, genetic diversity represents a fundamental population genetic parameter, and significant changes to levels of genetic diversity may reflect changes in population size (e.g., population bottlenecks; Peery et al. 2012) or be driven by changes in gene flow between populations (Slatkin 1995). Estimates of genetic diversity were made for populations with a minimum sample size of twenty individuals.

Effective population size ($N_E$) represents a measure of the relative contribution of individuals in a population that contribute offspring to the next generation, and when individuals from a single brood year are analyzed $N_E$ is the same as $N_B$. Minimum effective population sizes of 50 and 500 have been proposed as goals to prevent short-term inbreeding and to maintain sufficient long-term genetic diversity, respectively (Franklin 1980).

We used two programs to estimate the effective number of breeders ($N_B$) by parental brood year (BY) for each population. The program COLONY 2 (Jones and Wang 2010) implements the sibship assignment (SA) method for calculating effective population size ($N_E$) and $N_B$ proposed by Wang (2009). The SA method is a single-sample approach that uses sibship assignments to determine full-sibling and half-sibling relationships within the sample; estimates of $N_E$ are then acquired from frequencies of full- and half-sibling dyads. The SA method has been shown to perform well both with simulated and empirical data (e.g., Ackerman et al. 2017). When offspring from the same cohort (brood year) are analyzed as a single sample, estimates of $N_E$ from the SA method are equivalent to $N_B$. We also used the program NeEstimator version 2.1 (Do

et al. 2014) to estimate $N_B$ using the bias corrected linkage disequilibrium (LD) method of Waples (2006) excluding all alleles with frequencies less than 0.03. The LD method is the most widely used method for estimating $N_E$ from a single collection (Waples and England 2011), and provides estimates of $N_B$ in a population when used on a single BY (Waples 2005).

We used scale age data (methods described in IPTDSW 2020) to assign each fish detected at a PIT tag array back to a brood year. Offspring from the same population and BY were then analyzed as a single sample to estimate $N_B$. Because steelhead and Chinook Salmon reproductive strategies fall on a spectrum between monogamy and random mating, we calculated $N_B$ assuming random mating. The unweighted harmonic mean of the SA and LD estimates of $N_B$ within a BY were calculated. Because the unweighted harmonic mean is the sample size divided by the sum of the reciprocal values, infinite estimates of $N_B$ were replaced with the limit of the reciprocal of $N_B$, as $N_B$ approaches infinity in the calculation of the harmonic mean $N_B$ within a BY. The SA and LD methods were combined to increase precision of the estimated $N_B$ (Waples and Do 2010).

## RESULTS

Population-level estimates of expected heterozygosity were generated for each site and year combination with more than 20 samples using the groupings presented in Table C1. We observed a significant deviation from normality in mean values of $H_E$ (Shapiro-Wilk test, W = 0.964, p-value = 0.015) and mean estimates of $H_E$ differed significantly among management groups (Mann-Whitney test, W = 1249, p-value <0.001). Mean values of $H_E$ were higher among populations managed with hatchery releases ($H_E$ = 0.303) than populations managed for wild steelhead ($H_E$ = 0.291; Figure C1).

Population-level estimates of $N_B$ were generated for each site and year combination with more than 20 samples using the groupings presented in Table C2. Estimates of $N_B$ were not normally distributed (Shapiro-Wilk test, W = 0.957, p-value = 0.009). Mean estimates of $N_B$ did not differ significantly among management groups (Mann-Whitney test, W = 762.5, p-value = 0.277). Mean values of $N_B$ were higher among populations managed with hatchery releases ($N_B$ = 276.0) than populations managed for wild steelhead ($N_B$ = 244.0.; Figure C2).

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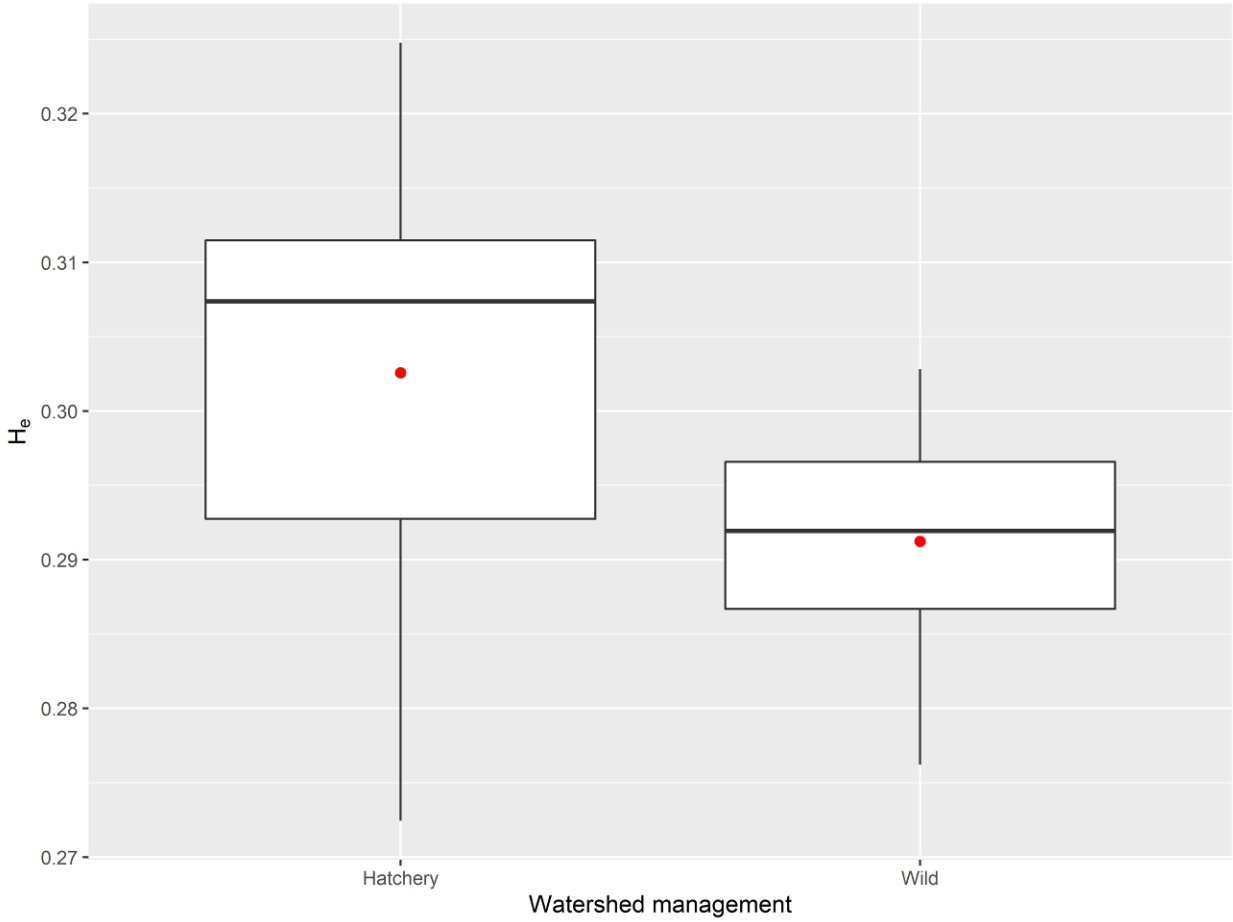


Figure C1. A boxplot displaying mean levels (red dot) of $H_E$ by watershed management practice for Idaho populations of steelhead (*Oncorhynchus mykiss*). The boxplot displays the 25th and 75th quantiles, horizontal bar denotes median value, and whiskers display minimum and maximum values.

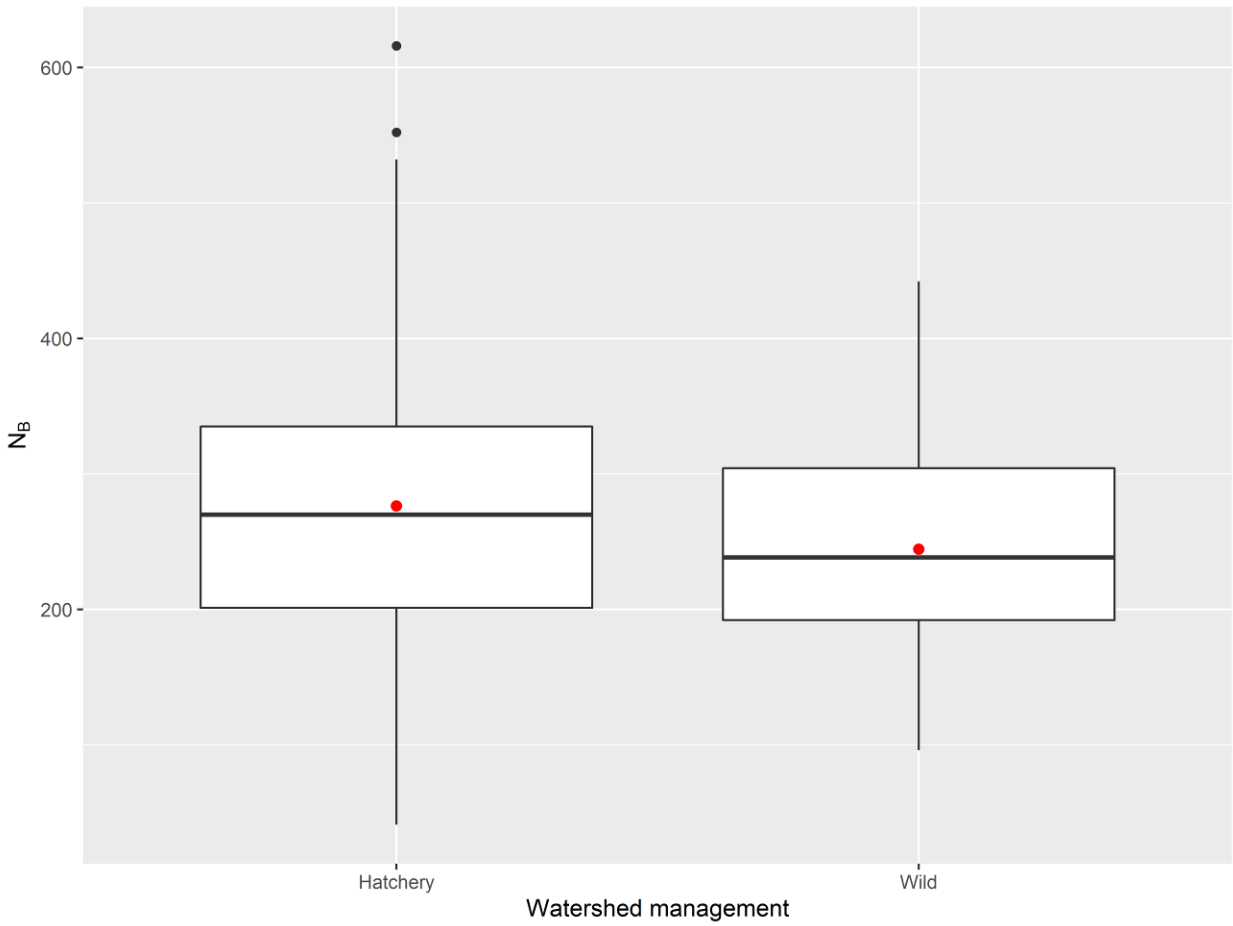


Figure C2. A boxplot displaying mean levels (red dot) of $N_B$ by watershed management practice for Idaho populations of steelhead (*Oncorhynchus mykiss*). The boxplot displays the 25th and 75th quantiles, horizontal bar denotes median value, and whiskers display minimum and maximum values. Black circles represent values more than 1.5 times greater than the maximum quantile (outliers).

Table C1. Average estimates of Expected Heterozygosity ($H_E$) at the population, watershed, and management levels for populations of steelhead (*Oncorhynchus mykiss*) in Idaho. The number of estimates per population (i.e. number of years in which there were sufficient samples to estimate $H_E$) is denoted by n.

| Management | Watershed | Population | n | Avg. $H_E$ | $H_E$ range |
|---|---|---|---|---|---|
| Hatchery | Lower Clearwater R | CRLMA-s | 10 | 0.308 | 0.303 – 0.311 |
| Hatchery | Lower Snake R | SNASO-s | 10 | 0.309 | 0.298 – 0.314 |
| Hatchery | Lower Snake R | SNTUC-s | 10 | 0.311 | 0.305 – 0.318 |
| Hatchery | Main Salmon R | SRLEM-s | 9 | 0.319 | 0.315 – 0.325 |
| Hatchery | Main Salmon R | SRNFS-s | 1 | 0.307 | 0.307 – 0.307 |
| Hatchery | Main Salmon R | SRPAN-s | 1 | 0.296 | 0.296 – 0.296 |
| Hatchery | Main Salmon R | SRUMA-s | 6 | 0.296 | 0.291 – 0.299 |
| Hatchery | SF Clearwater R | CRLOL-s | 7 | 0.280 | 0.272 – 0.284 |
| Hatchery | SF Clearwater R | CRSFC-s | 7 | 0.283 | 0.278 – 0.287 |
| Wild | Lochsa R | CRLOC-s | 4 | 0.280 | 0.276 – 0.283 |
| Wild | MF Salmon R | MFBIG-s | 8 | 0.293 | 0.289 – 0.303 |
| Wild | Selway R | CRSEL-s | 3 | 0.285 | 0.283 – 0.287 |
| Wild | SR Salmon R | SFMAI-s | 10 | 0.297 | 0.294 – 0.300 |
| Wild | SR Salmon R | SFSEC-s | 2 | 0.287 | 0.286 – 0.287 |

Table C2. Estimates of the Effective number of breeders ($N_B$) at the population, watershed, and management levels for populations of steelhead (*Oncorhynchus mykiss*) in Idaho. The number of estimates per population (i.e. number of years in which there were sufficient samples to estimate $N_B$) is denoted by n.

| **Management** | **Watershed** | **Population** | **n** | **Avg.** $N_B$ | $N_B$ **range** |
|---|---|---|---|---|---|
| Hatchery | Lower Clearwater R | CRLMA-s | 10 | 276 | 166 – 532 |
| Hatchery | Lower Snake R | SNASO-s | 10 | 354 | 270 – 552 |
| Hatchery | Lower Snake R | SNTUC-s | 11 | 346 | 154 – 616 |
| Hatchery | Main Salmon R | SRLEM-s | 7 | 209 | 161 – 293 |
| Hatchery | Main Salmon R | SRUMA-s | 5 | 272 | 154 – 404 |
| Hatchery | SF Clearwater R | CRLOL-s | 5 | 180 | 111 – 291 |
| Hatchery | SF Clearwater R | CRSFC-s | 7 | 195 | 41 – 296 |
| Wild | Lochsa R | CRLOC-s | 5 | 266 | 183 – 319 |
| Wild | MF Salmon R | MFBIG-s | 7 | 178 | 96 – 265 |
| Wild | Selway R | CRSEL-s | 2 | 324 | 207 – 442 |
| Wild | SF Salmon R | SFMAI-s | 9 | 281 | 195 – 431 |
| Wild | SF Salmon R | SFSEC-s | 1 | 108 | NA |

**Prepared by:**

John S. Hargrove
Fishery Biologist 2

Thomas A. Delomas
Fishery Biologist 2

Jesse McCane
Data Management Specialist 2

Micah Davison
Supervisory Fishery Biologist

Matthew R. Campbell
Fisheries Genetics Program Coordinator

**Approved by:**
IDAHO DEPARTMENT OF FISH AND GAME

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Matthew P. Corsi
Fisheries Research Manager

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J. Lance Hebdon, Chief
Bureau of Fisheries