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Population Genetic Analysis of Chehalis River Basin Chinook Salmon (*Oncorhynchus tshawytscha*)

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Executive summary:

Understanding the population structure of wild salmonids in the Chehalis River is an important part of the Chehalis Flood and Aquatic Species Project and contributes to the Chehalis Basin Flood Hazard Project and Aquatic Species Enhancement Plan (The Aquatic Species Enhancement Plan Technical Committee 2014). Habitat conditions for salmon and steelhead in the Chehalis River are projected to change substantially over the next decade. The relative influence of these actions on salmon and steelhead will partially depend on the population structure within the watershed. Here we examined the population structure of wild Chinook salmon (*Oncorhynchus tshawytscha*) in the Chehalis river basin. Our objectives were to 1) identify population structure within the Chehalis River and its tributaries, 2) investigate if run timing (spring and fall runs) influenced the population structure within the Chehalis basin, and 3) determine the population structure of the Chehalis basin in relation to baseline populations from Washington state and British Columbia.

Genetic data indicate, that within the Chehalis basin, the population structure consists of two general clusters, 1) a downstream group (Wynoochee River, Wishkah River, Satsop River, Black River and the Chehalis mainstem) and 2) an upstream group (South Fork and Upper Chehalis River, Newaukum River and Skookumchuck River). This finding is supported by clustering analysis and low degrees of differentiation between downstream and upstream collections. This pattern of slight differentiation between downstream and upstream collections, appears to be largely driven by isolation by distance, which is a common driver of population structure in salmonid populations.

Generally, Chinook salmon run types display limited genetic differentiation, as population structure is typically driven by geographical proximity. This pattern holds true in the Chehalis River. Clustering and tests of genetic differentiation revealed that fall and spring runs were not genetically distinct. Similar patterns were found in other Washington Rivers with multiple Chinook salmon run types. The Hoh River showed little distinction between run types, displaying a similar structure to the Chehalis basin. Skagit River summer and fall Chinook salmon were not differentiated, but distinct population structure existed between spring and fall and spring and summer Chinook salmon. The Satsop River, Chehalis River mainstem, and Black River spring runs clustered more closely with the lower river fall runs than they did with upstream spring runs, indicating they likely belong to the same population. This may indicate that criteria used to label fish in the field are inaccurate, or that in the Chehalis, spring run Chinook salmon do not all migrate upriver.

Analysis of the population structure of the Chehalis basin and Washington state Chinook salmon baseline populations revealed three major branches 1: Puget Sound and British Columbia, 2) Lower Columbia, and 3) Washington Coast. Within the Washington Coast group, the Chehalis basin clustered most closely with Willapa Bay Rivers (North River, Fall River, Naselle River, Nemah River and Forks Creek). The results of Chehalis basin Chinook salmon population structure are largely in concordance with previous research. Other populations of salmonids, including Chinook salmon, tend to have population structure that is largely driven by geography (e.g. populations tend to be closely related to their nearest neighbors).

Introduction:

Understanding the population structure wild salmon and steelhead (*Oncorhynchus* spp.) in the Chehalis River is an important part of the Chehalis Flood and Aquatic Species Project and contributes to the Chehalis Basin Flood Hazard Project and Aquatic Species Enhancement Plan (The Aquatic Species Enhancement Plan Technical Committee 2014; http://chehalisbasinstrategy.com/wp-content/uploads/2015/09/Aquatic-Species-Restoration-Program-Report_Final.pdf). Habitat conditions for salmon and steelhead in the Chehalis River are projected to change substantially over the next decade. Habitat may be lost due to the construction of a flood reduction dam planned at river mile (RM) 108. Habitat may also be gained due to restoration and protection activities planned throughout the watershed. The relative influence of these actions on salmon and steelhead will partially depend on the population structure within the watershed.

Future investment in flood reduction strategies and habitat restoration is currently the focus of extensive planning efforts and is likely to impact large portions of the basin. Current models (Ecosystem Diagnostic Treatment, NOAA Watershed Assessment) that predict salmon and steelhead responses to these habitat changes partition species into geospatial units, defined by their location and similar habitat and landscape characteristics. In reality, fish populations are defined by the exchange (or lack thereof) of genetics over space and time and could encompass multiple geospatial units currently included in the modelling efforts (Holsinger and Weir 2009). If future habitat is enhanced, long-term numerical responses may differ if the populations have limited versus extensive genetic exchange among sub-basins. If future habitat is depleted, overall numbers of fish in the basin may be less resilient over time if populations in the depleted sub-basin(s) have limited genetic exchange with other populations and represent a unique component of the genetic diversity for the entire basin. Thus, understanding the genetic structure of salmon and steelhead in the Chehalis River is a critical component to predicting long-term impacts of flood reduction strategies and habitat restoration actions.

A previous population genetic study was conducted in the Chehalis basin of 813 Chinook salmon, at 58 Single Nucleotide Polymorphisms (SNPs). Results suggested slight population structure between the upper and lower watershed, however, more work was warranted because of the low number of SNP markers (unpublished data, Sewall Young WDFW). The purpose of this project is to determine the genetic population structure of Chinook salmon in the Chehalis River watershed. Here, we determine the population genetic structure throughout the Chehalis River basin for Chinook salmon using a panel of 286 SNP markers.

Study Objectives:

1) Determine population structure of Chinook salmon of the Chehalis River sub-basins
2) Determine population structure of the different run timings of Fall/Spring Chinook salmon
3) Determine population structure of the Chehalis River Chinook salmon in relation to the surrounding coast-wide region

Methods:

Study Site

The Chehalis River is a large (6,889 $km^{2)}$ and diverse watershed with multiple sub-basins that drain from three mountain ranges (Willapa Hills, foothills of the Cascade Mountains, foothills of the Olympic Mountains). Chinook salmon (*O. tshawytscha*) spawn throughout the watershed and exhibit both a spring- and fall-run life history. A map of sampling locations (Figure 1) was created by converting river miles (located on the WACEY River miles map, (http:/geo.wa.gov/datasets) to Latitude/Longitude. These points of Latitude/longitude were then plotted onto a google map of Washington, using the R library *ggmap* (Kahle and Wickham 2013).

In the Chehalis River basin, hatchery production of Chinook salmon is of fall-run Chinook salmon only. There are a total of four fall Chinook salmon hatchery programs which employ on-station releases. Two of the program are operated by cooperative entities and two are from WDFW-operated facilities, Lake Aberdeen hatchery and Bingham Creek hatchery. The Grays Harbor Fisheries Task Force (a non-profit organization) operates the WDFW-owned Satsop Springs facility and releases fall-run Chinook salmon into the Satsop River annually. The Mayr Brothers Hatchery facility is operated by Grays Harbor Poggie Club and is located on the Wishkah River. Hatchery production of fall Chinook salmon varies annually and is determined by the availability of broodstock. Hatchery fall Chinook salmon are reared in the Lake Aberdeen hatchery and released annually into Van Winkle Creek, a right bank tributary that enters the Chehalis River at RM 2.7. The Bingham Creek Hatchery releases fall Chinook salmon from their facility located on the East Fork Satsop River. Over the past 10 years, annual production of hatchery fall Chinook salmon in the Chehalis River basin has averaged 370,883 subyearling smolts. All fish released from these programs are adipose fin-clipped to indicate they are hatchery-origin Chinook salmon. The annual releases from these facilities are depicted in Table 1. Although there are no hatchery programs for spring–run Chinook salmon and there are no releases of hatchery produced fall-run Chinook salmon in the upper Chehalis River basin upstream of the Chehalis Tribe Reservation (RM 54), hatchery-produced Chinook salmon are occasionally caught in tribal in-river fisheries (M. White, Chehalis Tribe Department of Natural Resources, personal communication).

Chehalis River Tissue Collections of Chinook Salmon

Based on previously published and unpublished *O. tshawytscha* population genetic studies, we assumed that population structure, if it existed, would likely be ordered by spawning location, i.e., by major tributaries within the watershed. Thus, our collection efforts were focused on known spawning tributaries of the Chehalis River and not on the mainstem Chehalis River. Those tributaries were the Wishkah River, Wynoochee River, Satsop River, Skookumchuck River, Newaukum River, South Fork Chehalis River (SF Chehalis), and the upper Chehalis River. Samples were taken from the Humptulips River, but were not included in this analysis. Sampling was also conducted in the mainstem Chehalis river between Elma and Oakville which is also used by Chinook salmon for spawning. Fin tissue was collected from Chinook salmon carcasses throughout the Chehalis River watershed (Figure 1).

From each captured fish, biological data including run type (spring, fall), origin (hatchery or wild), sex (if possible), and fork length were obtained. Run type was assigned in the field based on the collection date as well as carcass characteristics (Table 2). Between the second and

fourth week of October, spring and fall-run Chinook salmon overlap in many of the spawning areas; collection date alone is inadequate to assign run-type during this time frame and carcass characteristics are used to make this determination in the field. Origin was determined by the presence (wild or naturally-produced) or absence (hatchery-produced) of the adipose fin. Scales were taken for aging, and a small section of caudal fin was excised and immediately placed in 100% ethanol along with a label that uniquely identified the fish and associated genetic data with the collection and biological data. Fin clips in ethanol were accessioned to the Washington Department of Fish and Wildlife Molecular Genetics Laboratory (WDFW MGL) archive and stored at room temperature.

Statewide Tissue Collections of Chinook salmon

Biological data and fin tissue was taken from natural-origin populations of Chinook salmon throughout Washington State. These were collected for previous studies using similar field collection methods as described for the Chehalis River collection. For hatchery population collections, fin tissue and biological data were taken from broodstock, usually during spawning. Statewide collections chosen for inclusion in analysis were randomly chosen from among collections at the WDFW Molecular Genetics Lab with available genetic data within the major genetic lineages, including spring and fall run types.

Laboratory Methods:

A total of ~900 Chinook salmon tissue samples were collected from throughout the Chehalis River basin between 2001 and 2016; of these, 432 were selected for genetic analysis based on their spatial representation of the basin and the quality of tissue sample available for analysis (Figure 1). Genomic DNA was isolated from fin tissue with, 30uL of 10% Chelex (Sigman Aldrich, C7901) and 5uL of Proteinase K solution (Qiagen, 1018332), which were then incubated overnight at 55°C.

A total of 299 Chinook salmon-specific Single Nucleotide Polymorphisms (SNPs) were genotyped using a cost effective method based on custom amplicon sequencing called Genotyping in Thousands (GTseq) (Campbell et al. 2015). One marker was a sex-linked sex identification locus. The rest were designed for general use in population genetic studies of Chinook salmon. GTseq is an efficient genotyping method that amplifies pools of targeted SNPs and then indexes individual samples. The pools are then sequenced, de-multiplexed, and genotyped by generating a ratio of allele counts for each individual. The entire process can be broken down into four segments; extraction, library preparation, sequencing, and genotyping.

To start the library preparation, an ExoSAP cleanup was performed on10uL of extracted DNA. 1.3uL of Exonuclease I (New England BioLabs, M0293L), 0.3 uL of SAP (New England BioLabs, M0371L), 0.15uL of Exonuclease 1 Buffer (New England BioLabs, B0293S), and 1.25uL of nuclease free water were added to the extracted DNA for a combined volume of 13uL. Thermal cycling was conducted in 96-well PCR plates for all reactions and had the following conditions for the ExoSAP reaction: 37°C-60 min, 80°C-20 min, 4°C-hold. Following the ExoSAP reaction, amplification of the multiplexed pool of targeted loci was performed. The multiplex PCR cocktail reaction was 2uL of cleaned DNA extract, 3.5uL of Qiagen Multiplex PCR Plus mix (Qiagen, 10672201), and 1.5uL pooled primer mix (IDT, final volume = 7uL; final primer concentrations at each locus = 54nM). Thermal cycling conditions were as follows:

95°C-15 min; 5 cycles [95°C – 30 s, 5% ramp down to 57°C – 30 s, 72°C – 2 min]; 10 cycles [95°C – 30 s, 65°C – 30 s, 72°C – 30 s]; 4°C hold. Following the multiplex PCR, the amplified samples were diluted 20-fold. 3uL of diluted multiplex PCR product was then used in the barcoding PCR. The barcoding PCR is used to add indexes that identify each sample by well and by plate. For the barcoding PCR, 1uL of 10uM well-specific i5 tagging primer (IDT) and 1uL of 10uM plate-specific i7 tagging primer were added to the 3uL of amplified sample. 5uL of Qiagen Multiplex PCR Plus mix (Qiagen, 10672201) was then added for a final reaction volume of 10uL. Thermal cycling conditions were: 95°C – 15 min; 10 cycles [98°C – 10 s, 65°C – 30 s, 72°C – 30 s]; 72°C – 5 min; 4°C hold. Following the barcode PCR, each plate of samples (library) was normalized using the SequalPrep™ Normalization Plate Kit (Applied Biosystems, A1051001) according to the manufacturer's instructions. Upon completion of normalization, 10uL of each sample per 96-well plates was pooled into a 1.5mL tube constituting a library.

A purification step was then performed on each library with Agencourt AMPure® XP magnetic beads (Agencourt, A63881) according to the manufacturer's instructions for size selection with a 2:1 and 1.43:1 ratio of library to beads. The purified libraries were then eluted with 15uL of TE pH 8.0. In order to complete the final process of library preparation, each library was quantified and normalized. The libraries were quantified using a Qubit 3 Fluorometer (Invitrogen), with the Qubit™dsDNA HS Assay Kit reagents (Invitrogen, Q32854) according to the manufacturer's instructions. Following the quantification, the concentration of each library was calculated using the molecular weight specific to the multiplex pool used. Then each library was normalized to 4nM and pooled with other libraries that were sequenced on the same sequencing run. Pooled libraries were then sequenced at a 2.5pM loading concentration on an Illumnia NextSeq 500 instrument of a single-end read flow cell using 111 cycles with dual-index reads of six cycles each. To genotype the samples a bioinformatics pipeline was used. This pipeline is explained and available online at https://github.com/GTseq/GTseq-Pipeline (Campbell et al. 2015). Briefly, there are a series of custom PERL scripts that ultimately create individual fastq files and genotype files for every individual that can be compiled a number of ways for further analysis. The genotyping is performed with a simple PERL script which counts amplicon-specific sequences for each allele, and allele ratios are used to determine the genotypes.

Evaluation of Diversity/Loci Metrics

Three analysis packages- *splitstackshape*, *tibble*, and *dplyr*, were used to convert GTseq formatted data into the proper format for analysis in *adegenet* (Jombart 2008) and *strataG* (Archer et a. 2017) in the R software v. 3.3.3 (R Core Team, 2013). The R software packages *adegenet*, *strataG* and *poppr* (Kamvar et al. 2014) were then used to calculate per locus diversity statistics (number genotyped, proportion genotyped, allelic richness, and expected and observed heterozygosity), remove individuals with 30% or more missing data, identify and remove matching individuals, and convert data to Genalex (Peakall and Smouse, 2006) and Genepop (Raymond and Roussett, 2008) formats for use in external programs. Related individuals were identified and removed from further analysis, (r = .20; half sibling relationships and greater), using the program ML-Relate (Kalinowski et al. 2006). Departures from Hardy-Weinberg equilibrium (HWE) and Linkage dis-equilibrium (LD) were assessed in Genepop v4.2.1 (Raymond and Roussett, 2008), using sub-options 1.3 and 2.1, respectively, with default parameters. Significance of probability values were adjusted for multiple tests using false

discovery rate (FDR; Verhoeven et al. 2005). We calculated expected (H_E) and observed (H_O) heterozygosity, alleles per locus, alleles per polymorphic locus, proportion of polymorphic loci, and F_{IS}, in GDA v1.0 (Lewis and Zaykin 2001). Estimates of effective population size (N_e) were calculated from patterns of linkage disequilibrium (LD) with the software NeEstimator (Do et al. 2014; Waples and Do 2010). LDN_e was calculated using a minimum allele frequency of 0.02, with random mating assumed.

Species other than Chinook salmon are occasionally mistakenly sampled in the field. Although the genetic markers used are meant to be specific to Chinook salmon, other salmon species sometimes produce genotypes at enough loci to meet our threshold for missing data. However, non-target species tend to be homozygous at all amplified loci (WDFW MGL, unpublished data). Thus, we identified non-Chinook salmon species by within-individual homozygosity > 0.95 (greater than 95% of amplified loci in an individual were homozygous), as calculated using the MS Excel add-on Genalex (Peakall and Smouse, 2006).

Population Structure Within the Chehalis Basin

Spatial population genetic structure was investigated, with the aim of identifying clusters of genetically related individuals within the study area. We used two approaches, 1) the Bayesian clustering method (Pritchard et al. 2000), implemented with the software STRUCTURE 2.3.4, and 2) discriminant analysis of principal components (DAPC; Jombart et al. 2010), implemented in the R package *adegenet* (Jombart et al. 2008). Both of these methods cluster the data without *a priori* population membership information, allowing population structure to be revealed based on individual level information. STRUCTURE clusters individuals in order to minimize Hardy-Weinberg and linkage disequilibrium (Pritchard et al. 2000), whereas, DAPC does not utilize any population genetic models in finding clusters (Jombart et al. 2010).

Using STRUCTURE, we performed 10 iterations of each K (number of populations) = 1 – 12, with 50,000 MCMC repetitions and a 5,000 burin-in period. The optimal K was identified by plotting the log probability of the data for each value of K (ln Pr(X|K)) using the web-based software, STRUCTURE HARVESTER (Earl and vonHoldt 2012). We chose the number of populations, based on where the K value likelihood plateaued. Multiple iterations for each K analyzed were concatenated using CLUMPP (Jackobsson and Rosenberg 2007), using default parameters. STRUCTURE plots were produced with DISTRUCT (Rosenberg 2004). The output of STRUCTURE includes ancestry coefficients, which identifies the proportion of membership an individual has to each population cluster. In order to better visualize the membership proportion across the study area, we performed spatial interpolation of individual ancestry coefficients by using the kriging method with the R libraries "*fields*", "*tess3r*", "*maps*" and the script "*plot.admixture.r*" (http://membres timc.imag.fr /Olivier.F rancois/tess.html).

In the R package *adegenet* (Jombart 2008), we used the *find.clusters* and *chose.n.clust* functions and determined the most likely number of clusters (K), using the Bayesian Information Criterion (BIC), which is a method of model selection which weighs the fit of the model versus its complexity. The optimal K value was selected as the K after which further BIC values decreased only subtly (as per Jombart et al. 2010).

Population structure of collections was evaluated by estimating pairwise F_{ST} estimates. F_{ST} is an estimate of the reduction of heterozygosity in a subpopulation, in relation to the population as a whole, and is typically used as a measure of genetic differentiation. Values range from 0 to 1, where 0 indicates the populations are panmitic (no differentiation), and 1 indicates

that the populations are fully differentiated (separate populations). Pairwise F_{ST} estimates were calculated and statistical significance estimated by permutation tests, for all sampling locations in *strataG* (Archer et al. 2017; Weir and Cockerham 1984). We also calculated pairwise Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967), another measure of population differentiation. Distances were calculated and bootstrapped 1,000 times, with the software POPULATIONS 1.2.32 (Langella 1999). A neighbor joining tree of the genetic distances was visualized using the software FIGTREE v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/; Rambaut, 2007).

Isolation by distance, where populations in closer proximity tend to be genetically more similar, is common in salmonids (Primmer et al. 2006; Pearse et al. 2007; Narum et al. 2010). We tested for a pattern of isolation by distance among sampling locations in the Chehalis basin, by testing for correlation between F_{ST} and geographic distance matrices, using Mantel's test with the *Isolde* option in GENEPOPv4.2.1. (Raymond and Roussett, 2008). Given that genetic differentiation among fall and spring Chinook salmon sampled in the same location was very low (see Results), spring and fall Chinook salmon collections were pooled per location for this analysis. Geographic distances were calculated via river miles between pairs of sampling locations and were not log transformed.

Do Fall and Spring Runs Influence Population Structure?

As an additional test of populations structured by run timing, , we ran STRUCTURE as above but utilized the "*locprior*" model. This model uses sampling location of individuals to assist in the clustering process. In this case, we substituted run timing for location information. As a comparison, we also performed STRUCTURE analysis (without the "*locprior"* model) and estimated F_{ST} values using collections from the Hoh and Skagit rivers, where multiple run-types of Chinook salmon populations also exist.

Chehalis Basin in Relation to Other Populations:

In order to determine how the Chehalis Basin Chinook salmon are related to other populations in Washington State, we compared Chehalis Chinook salmon to baseline collections from the major Distinct Population Segments (DPS), Washington Coast (N = 1,022), Lower Columbia river (N = 1,131), Puget Sound (N. Puget Sound, N = 2,402; Puget Sound White River springs = 95; Puget Sound Fall Aggregate = 1,838), Strait of Juan de Fuca (N = 254), and British Columbia (N = 190) (Table 3). All individuals had previously been genotyped at 192 SNP loci (Warheit et al. 2014), except the Washington Coast baseline samples, which were genotyped at the same 299 SNP loci as the Chehalis samples. Collections were assessed for HWE and LE in Genepop v4.2.1 (Raymond and Roussett, 2008), to determine if there were departures from equilibrium. Population structure was investigated as above, via pairwise F_{ST}, neighbor joining trees, STRUCTURE and DAPC. Location data was only available for the Chehalis Basin samples, so analysis of Isolation by Distance and spatial interpolation were not performed.

Results:

Tissue Collections:

A total of 432 unmarked Chinook salmon carcass samples from the Chehalis basin were collected and processed. The final dataset included 341 individuals. A total of 91 individuals were removed due to 1) complete lack of data (N = 6), 2) matching genotypes (N = 2), 3) missing 30% or more of the data (N = 65), and 4) relatedness 0.20 or higher (N = 18) (Table 3).

An additional 6,932 baseline collection samples were added to the genetic dataset (Table 3). These samples represented all major Distinct Population Segments (DPS; British Columbia, Lower Columbia Fall, Lower Columbia Spring, North Puget Sound, Puget Sound White River, Puget Sound Fall Aggregate, Strait of Juan de Fuca, and the Washington Coast).

Evaluation of Loci and Genetic Diversity:

Loci that are out of HWE could be an indication of genotyping errors, population stratification, or non-random association of loci. Therefore, loci that departed from HWE were removed from the dataset. We identified and removed three loci that were out of HWE in Chehalis basin collections. The locus *Ots*_CCR7 was out of HWE in multiple collections, including; Skookumchuck spring, Upper Chehalis fall, Newaukum spring and fall, Skookumchuck fall, Satsop fall, and Wynoochee River fall. Addtionally, the loci *Ots*_MHC1, and *Ots*_unk3513-49 were out of HWE in the Upper Chehalis fall, and Satsop fall, respectively. A total of four pairs of loci were in Linkage Disequilibrium, and for similar reasons as above with HWE, were removed. Additionally, we removed a locus in which only 38% of the individuals were successfully genotyped, *Ots*_crRAD5061-27. No individuals had homozygosity higher than 95%, thus, all individuals sampled were Chinook salmon. Our complete dataset for within the Chehalis basin was 341 individuals analyzed at 286 loci.

The majority of the baseline populations were genotyped with the statewide Chinook salmon 192 SNP panel, therefore combined analyses of the Washington Coast and Chehalis collections with other baseline used the subset of 192 loci in common with both panels. An additional 44 loci were removed due to 1) lack of data in all collections, and/or 2) loci were not in HWE or linkage equilibrium.

Average estimates of expected (H_e) and observed (H_o) heterozygosity in the Chehalis basin Chinook salmon (Table 4) were within the range of baseline Chinook salmon populations (Appendix 1). However, the Newaukum River spring, Black River spring and Chehalis mainstem fall were slightly lower than baseline populations, and were closer to those seen in some hatchery populations (e.g. Big Qualicum Hatchery, Nooksack Kendall creek Hatchery; Appendix 1). The Wishkah River and Wynoochee Rivers, the only collections in our Chehalis basin sample to have hatcheries associated with them, had the highest levels of heterozygosity, equivalent to larger baseline populations. The average effective population size (LDN_e) and average number of alleles (N^A) was lower than both the wild and hatchery baseline populations, most likely due to relatively low sample sizes in the Chehalis samples (Table 4; Appendix 1).

Population Genetic Structure Within Chehalis Basin:

STRUCTURE analysis revealed that two populations (K = 2) were likely given the data (Figure 2). Support for additional populations decreased with increasing values for K greater than 2 (Figure 2A). At K = 2, individuals appear to be admixed between two clusters, cluster 1 (red) and cluster 2 (yellow) (Figure 2B). The clusters are roughly geographically structured with cluster 1 (red) being more prevalent in the downstream sampling locations (Wynoochee River, Wishkah River, Satsop River, Chehalis main stem, Black River, and Skookumchuck falls), and cluster 2 (yellow) being more prevalent in the upstream sampling locations (Skookumchuck springs, Newaukum springs, SF Chehalis, and Upper Chehalis). Spatial interpolation of the ancestry coefficients show this pattern of a relative cline between the upper and lower watershed (Figure 3). The map shows a break between cluster 1 and 2, near the Black River sampling locations (ancestry coefficients to either cluster 1 or cluster 2 are 0.50; Figure 3). Cluster 1 has

the highest proportions (~70%-55%) in the Wynoochee River, Wishkah River, Satsop River and Chehalis mainstem River, whereas, cluster 2 has the highest proportions (~70%-55%) in the Upper and South Fork Chehalis, and Newaukum (Figure 3). DAPC did not reveal population structure within the Chehalis and only one large population (K = 1) was supported by the BIC values.

Pairwise F_{ST} estimates ranged from 0.038 (highly differentiated) to 0.0008 (low degree of differentiation), across all collections (Table 5; Figure 4). Extremely low degrees of differentiation in F_{ST} values between Wynoochee fall, Satsop River fall, Satsop River spring, Chehalis River main fall/spring, and Black River spring (F_{ST} ranging between 0.0024-0.0052), corroborate the pattern of clustering these lower watershed groups together seen in the STRUCTURE results. Similarly, there is a low degree of differentiation between collections in the upper watershed (Upper Chehalis River fall and spring, Skookumchuck River spring, SF Chehalis River fall and spring, and Newaukum River fall (F_{ST} ranging between 0.088 – -0.0008). These patterns of low differentiation can be visualized in Figure 4, where light blue indicates low population differentiation, and dark blue indicates high population differentiation. The Newaukum spring collection is highly differentiated (F_{ST} ranging between 0.0342 – 0.0126) from most other collections, with the lowest degree of differentiation seen from the Newaukum fall, and Skookumchuck spring collections (Table 4; Figure 5). A significant isolation by distance effect ($R^2 = 0.6614$; $P < 0.0001$) was detected across the Chehalis basin (Figure 5). The neighbor joining dendrogram shows low support for any major subdivisions within the Chehalis basin by collection location (Figure 6). Moderate support exists for a node separating downstream collections (Wynoochee fall, Satsop River spring and fall, and the Chehalis mainstem fall/spring) from upstream collections. Additionally, higher bootstrap support existed for a relationship between the Newaukum River fall and Skookumchuck River fall.

Do Fall and Spring Runs Influence Population Structure?

Including a run-timing type prior in STRUCTURE analysis did not reveal any major population structure attributed to fall or spring run timing (Figure 7). The Satsop River spring, Chehalis mainstem spring and Black River spring had roughly the same proportion belonging to cluster 1 (red), that the fall run samples did. STRUCTURE analysis on the Hoh River spring/summer/fall runs revealed K = 2 as the most likely population structure (Figure 8), however the level of admixture across individuals did not vary as much as in the Chehalis basin (Figure 2 and 8). Skagit River Chinook salmon were split into three clusters (Figure 8). The Lower Skagit fall run was largely clustered with the Skagit summer runs (both the Upper Skagit and the hatchery populations), whereas the Skagit spring hatchery population was a single cluster. F_{ST} estimates in the Hoh River ranged from 0.0008 – 0.0148 (Table 6). The Hoh River fall run was most distinct from the Hoh River spring/summer run and the South Fork Hoh River spring run (0.01 and 0.015). There was little differentiation between the Hoh River fall and spring run (0.0091), and similarly little differentiation between the Hoh River spring and summer runs (F_{ST} = 0.0008 – 0.0043). F_{ST} estimates in the Skagit River ranged from 0.0002 – 0.0343 (Table 6). The Skagit summer hatchery and Upper Skagit summer runs showed very little differentiation (F_{ST} = 0.0002), and the Lower Skagit fall run showed more differentiation between the Skagit spring hatchery run (F_{ST} = 0.034), than the Skagit summer hatchery (F_{ST} = 0.0126) and the Upper Skagit summer run (F_{ST} = 0.0118).

Chehalis Basin in Relation to Other Populations:

DAPC and STRUCTURE analysis of statewide baseline samples and the Chehalis basin supported four populations (K = 4; Figure 9). In both the STRUCTURE and DAPC analysis, the baseline individuals are divided into a Washington coast group and a Lower Columbia group. The Puget Sound fall aggregate DPS falls out as its own cluster in the STRUCTURE analysis, whereas, an underlying level of additional substructure is present across all Puget Sound groups in the DAPC analysis. The Chehalis group clusters with the Washington Coast group in both the STRUCTURE and DAPC analysis.

A neighbor joining dendrogram provided further resolution of the Chehalis basin population in relation to other Washington coast populations. Two major branches represent the Puget sound and British Columbia and the Lower Columbia and Washington Coast (Figure 10). The Washington Coast/ Lower Columbia branch is further divided into two clusters, encompassing the Lower Columbia populations and the Washington Coast. The Chehalis basin clustered, with high bootstrap support (97/100), with Willapa bay Chinook salmon populations (Nemah River, Naselle River, Forks Creek, Fall River and North River). F_{ST} estimates ranged from -0.0007 to 0.052. The Fall River collection (Willapa basin) had low divergence other Willapa basin populations; Forks creek, Naselle River, Nemah River, and the North River. Additionally, the Fall River had low divergence with the Black River spring (F_{ST} = 0.001), Satsop River fall (F_{ST} = 0.007), Satsop River spring (F_{ST} = 0.002), and the Chehalis mainstem fall (F_{ST} = 0.001) and spring (F_{ST} = 0.005). Similar low values of divergence were estimated for the North River and Black River springs (F_{ST} = 0.009; Table 7).

Discussion:

We used SNP genotypes and a combination of population genetic analyses to characterize the population genetic structure of the Chehalis basin Chinook salmon. Our objectives were to 1) identify population structure of Chinook salmon within the Chehalis River and its tributaries, 2) investigate if run timing (spring and fall runs) influenced the population structure within the Chehalis basin, and 3) determine the population structure of the Chehalis basin Chinook salmon in relation to baseline populations from Washington state and British Columbia. Our results indicate, that within the Chehalis basin, the population structure consists of two general clusters, 1) a downstream group (Wynoochee River, Wishkah River, Satsop River, Black River, and the Chehalis mainstem) and 2) an upstream group (South Fork and Upper Chehalis River, Newaukum River and Skookumchuck River). The population structure appears to be largely driven by isolation by distance. There is no apparent distinction between run types in the Chehalis basin. Analysis of the population structure of the Chehalis basin and Washington state Chinook salmon baseline populations revealed three major groups with the Chehalis basin Chinook salmon grouping most closely with Willapa Bay Rivers (North River, Fall River, Naselle River, Nemah River and Forks Creek).

Population Structure within the Chehalis Basin:

The Chehalis basin Chinook salmon displayed a weak signal of population structure that roughly clustered the individuals into a lower basin (Wishkah River, Wynoochee River, Satsop River, Black River, and Chehalis main stem) group, and an upper basin (SF/Upper Chehalis, Newaukum and Skookumchuck) group. This result was supported by concordant patterns in F_{ST}, neighbor joining dendrograms, and Bayesian clustering analysis. Both F_{ST} estimates and neighbor joining dendrograms supported clustering of the Chehalis mainstem, Satsop fall and

spring run, and the Wynoochee River fall. Additionally, F_{ST} estimates supported the Wishkah River fall run clustering with this group, however, the sample size was too small to be included in the neighbor joining tree. Previously published allozyme data supported a close relationship between the Wishkah River and Wynoochee River (Busack and Shaklee 1995), and indicated that Satsop River clustered most closely with the Wynoochee River, Wishkah River and Naselle River (Myers et al. 1998). The upper basin cluster appeared to be less of a cohesive unit, than the lower basin cluster. The dendrogram only had high bootstrap support values for the Newaukum fall and Skookumchuck fall populations. The Newaukum River spring collection appears stand out from most every other collection in the Chehalis basin. The F_{ST} estimates are nearly double other with-in Chehalis estimates. Diversity statistics do not indicate deviations from HWE or systematic issues with genotyping. This result warrants further investigation into the apparent differentiation of the Newaukum River spring run.

Aside from local clustering in the lower basin, the major driver of population structure within the Chehalis basin appears to be isolation by distance, where populations in closer proximity tend to be genetically more similar. This finding is supported by the mantel test and the spatial interpolation map which shows a cline between the upper and lower watershed. A pattern of isolation by distance is common among anadromous salmonids, particularly in long river systems, like the Chehalis (Primmer et al. 2006; Pearse et al. 2007; Narum et al. 2010). It is unclear if distance was the only factor preventing gene flow between the upper and lower watersheds, or if habitat differences were also playing a role.

Is Population Structure Due to Fall/Spring Runs?

As early migrators, spring Chinook salmon tend to migrate and subsequently spawn in upriver locations (Berman and Quinn 1991; Quinn 2005). In contrast, fall Chinook salmon, typically arrive later in the year, and migrate to and spawn in downriver/mainstem locations (Berman and Quinn 1991; Quinn 2005). This pattern has generally been observed in the Chehalis basin. The genetic results support genetic differentiation of upriver collections (particularly the spring run samples), from the rest of the basin. However, the genetic results also bring into question whether the spring collections from the lower portions of the basin (Sastop River spring, Chehalis main stem spring, and Black River spring) are truly spring Chinook salmon. These collections clustered most closely with other lower basin collections, which tended to be fall run Chinook salmon. The genetic relationships seen between spring and fall Chinook salmon in the Chehalis River were also seen in Hoh River Chinook and between fall and summer Chinook salmon in the Skagit. Generally, in salmonids, run types do not display much genetic differentiation, as seen in Chinook salmon of the Klamath River and Feather of California (Kinzinger et al. 2013; O'Malley et al. 2007). Population structure is typically driven by geographical proximity, and genetic distinction of run-types is present in only a few populations, such as the Central Valley of California, various populations in the Puget Sound, the Lower Columbia River, and some hatchery populations (Waples et al. 2004; Narum et al. 2007; Kinziger et al. 2013; Moran et al. 2013).

The lack of differentiation between spring and fall Chehalis Chinook salmon suggests that the criteria used in the field to characterize spring and fall Chinook may be sufficient to identify run-type, but not population. When sampling fish/carcasses in the Chehalis basin, run type is assigned based on collection date (spring run occurs before October 7th, and fall run occurs after October 15th) and morphological characteristics (Table 2). The genetic results suggest that either the "spring" run Chinook salmon in the Satsop, mainstem Chehalis and Black

Rivers were mischaracterized as fall Chinook salmon, or that the general pattern of spring Chinook salmon tending to migrate and spawn upriver might not present in the Chehalis basin. It should be noted that previous work and the present study were conducted utilizing neutral, non-coding loci. A recent study identified a locus, *GREB1L*, which was able to distinguish early (i.e., spring) from late (i.e., fall) migration timing in Chinook salmon (Prince et al. 2017). Analysis with this marker would be an interesting comparison to our findings with neutral markers.

Chehalis Basin in Relation to Other Populations

Genetic diversity of the Chehalis basin populations is within the range of baseline populations, although the Chehalis basin tends to be on the lower end of genetic diversity, most likely due to low sample sizes. Low heterozygosity in the Chehalis basin collections (Newaukum River spring, Black River spring and Chehalis mainstem fall) was most likely due to the low sample size in these collections.

Population genetic analysis revealed the Chehalis basin Chinook salmon have a close genetic affinity with populations along the Washington Coast. During the last glacial maximum, the Chehalis River basin remained ice free, and was a glacial refugium for salmon populations, and served as a drainage for the Puget Sound (McPhail and Lindsey 1986). Initial hypotheses suggested that due to this connectivity the Puget Sound and Chehalis basin stocks could be related (McPhail and Lindsey 1986). However, genetic studies have shown that the Puget Sound populations are most likely derived from populations on the Olympic Penninsula and Vancouver Island (Waples et al. 2004). This study provides additional evidence that the Chehalis basin Chinook salmon are not most closely related to the Puget Sound populations, and instead show an affinity to the Willapa River populations.

Using ecological, geographic and genetic data (Busack and Shaklee 1995; Meyers et al. 1998; Seeb et al. 2007), NOAA has partitioned Chinook salmon populations into several Distinct Population Segments (DPS). Chinook salmon south of the Elwha River and north of the Columbia River are included in the Washington Coast DPS. Some Chehalis River populations (Wishkah River, Wynoochee River, Satsop and Skookumchuck) to the Chehalis were included in the initial genetic analysis (Myers et al. 1998), which was conducted with allozymes and microsatellites (Busack and Shaklee 1995; Seeb et al. 2007). The SNP data from the tributaries previously analyzed, plus the Black River, Newaukum River, and the Chehalis mainstem, south fork, and upper reaches, confirmed the placement of Chehalis basin in the Washington coast DPS.

Both the North and Fall River (a tributary of the North River) fell in the Chehalis basin clade, and showed low F_{ST} divergence from lower basin tributaries (Black River, Satsop River) and the Chehalis main stem. This alignment of the North and Fall Rivers with the lower basin populations could further support the hypothesis that population structure is driven by geographical proximity (Waples et al. 2004; Moran et al. 2013), or could indicate a close relationship with the fall run types.

Summary

In summary, the Chehalis basin Chinook salmon display a population structure largely driven by isolation by distance between the lower watershed and upper watershed. Clustering and tests of genetic differentiation (F_{ST}) revealed that fall and spring runs were not genetically distinct, similar to patterns observed in other systems. Overall, the Chehalis basin Chinook salmon populations have a close genetic affinity to other populations along the Washington

Coast. Though this represents a comprehensive survey of the population genetic structure of the Chehalis basin Chinook salmon, some minor holes do exist. In this study, samples were collected across multiple years, however they were analyzed as one collection due to low sample sizes. Future studies that can add robust temporal sample sizes would provide additional power. Lastly, increasing the spatial scale to include proximate river systems (such as the Humptulips) would provide insight into the watershed as a whole.

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Table 1. Hatchery production of fall Chinook salmon in the Chehalis River basin

Release year	Satsop Springs	Mayr Brothers (Wishkah)	Van Winkle Cr. (Wynoochee)	Bingham Cr. Hatchery (Satsop)	Chehalis Basin Totals
2006	94,900	36,300	50,000		183,206
2007	45,600	62,000	50,000	46,600	206,207
2008	186,300	9,000	48,080	186,300	431,688
2009	330,000	27,000	52,000	330,000	741,009
2010	47,800	18,975	50,500	47,800	167,085
2011	104,100		50,000	104,100	154,100
2012	476,000	91,900	66,734	201,000	837,646
2013	234,100	26,000	53,150	234,100	549,363
2014	160,000	7,000	50,500	NA	219,514
2015	140,000	21,000	56,000	NA	219,015
Avg.	**181,880**	**36,475**	**52,696**	**90,350**	**370,883**

Table 2. Description of spring-run Chinook salmon vs. fall-run Chinook salmon characteristics used to distinguish between run-type during their overlapping spawning period around October 15th.

Pre-overlap	Fish seen prior to October 7th are spring-run.	
Overlap		
	Spring Chinook	Fall Chinook
Fish[a]	Grey, olive, or black/dark in color; Dull and/or dusky appearance, not bright and shiny colors;	Red, green, or purple in color; Bright, shiny colors, vivid
	Low energy level, lethargic, exhibiting an unwillingness to be spooked off of redds (for females) or into quick currents; [b]	High energy level, spooking easily and powering through riffles and low water areas, exhibiting a frantic behavior when spooked or scared
	Fungus present on fish and edges of snout, and fins showing wear;	No or minimal amounts of fungus and/or wear
	Have a soft caudal peduncle	Have a firm caudal peduncle
Post-overlap	After Oct. 15th live fish are fall-run type unless the observation is different from the rest of the observations in the survey	

[a]: Justify decision with at least two characteristics

Table 3. Collections of* O. tshawytscha *used in genetic analysis

Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	WDFW code	N processed
Wild	BC	Fraser River	U. Fraser	Fall	2001	KMORK01	48
Wild	BC	Harrison	HarrisonR	Fall	2010	10NM	47
Hatchery	BC	Qualicum River	Big Qualicum Hat	Unknown	2010	10NN	48
Hatchery	BC	Thompson River	South Thompson	Spring	1997	KMSHU97	47
Hatchery	Lower Columbia Fall	Cowlitz	Cowlitz H. fall	Fall	2004	04IT	57
Wild	Lower Columbia Fall	Cowlitz	Cowlitz fall	Fall	2006, 2011, 2012, 2014, 2015	06EJ, 11IY, 11IZ, 12IG, 12IH, 14EQ, 14QK, 14QL, 15KM, 15KO, 15KX, 15MJ	247
Wild	Lower Columbia Fall	Elochoman	Elochoman fall	Fall	1995, 1997, 2013, 2014, 2015	95EP, 97EY, 13OV, 14LJ, 15LT	158
Hatchery	Lower Columbia Fall	Lewis River	Speelyai hatSp	Spring	2015, 2016	15LU, 16IV	22
Wild	Lower Columbia Fall	Washougal River	Washougal fall	Fall	2013	13PB, 15LX	17
Wild	Lower Columbia Fall	Washougal River	Washougal R Fall	Fall	1995, 1996, 2006	06EK, 95ER, 96EA	96
Wild	Lower Columbia Fall	Grays River	Grays fall	Fall	2010, 2011, 2012, 2013, 2015	10HP, 11HW, 12GR, 13OX, 15EV, 15LP, 15QL,	110
Wild	Lower Columbia Fall	Green (NF Toutle River)	Green fall	Fall	2000, 2014	00IC, 14KW	88
Hatchery	Lower Columbia Fall	Kalama River	Kalama falls fall	Fall	2015	15LW	12

Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	WDFW code	N processed
Wild	Lower Columbia Fall	Lewis River	Lewis	Fall	2003	03IN	57
Wild	Lower Columbia Fall	Lewis River	L. Lewis R Su	Summer	2004	04KY	45
Wild	Lower Columbia Fall	Lewis River	Lewis R Su	Summer	2004	04KZ	58
Wild?	Lower Columbia Fall	Lewis River	LewisR-EF fall	Fall	2015	15LM	22
Hatchery	Lower Columbia Spring	Cowlitz	Cowlitz H. spring	Fall	2004	04FJ	52
Hatchery	Lower Columbia Spring	Cowlitz	Cowlitz spr	Spring	2015, 2016	15MW, 16LW	11
Hatchery	Lower Columbia Spring	Kalama River	Kalama H. spring	Spring	2004	04FK	54
Hatchery	Lower Columbia Spring	Kalama River	Kalama falls spr	Spring	2015	15LV	25
Wild	North Puget Sound	Cascade River	Upper Cascade Sp	Spring	1998, 1999	98DO, 99EC	8
Wild	North Puget Sound	Nooksack River	NFMFNooksack	Spring	1980, 1981, 1982, 1998, 1999,	80AC, 81AD, 81AF, 82AB, 8SAC, 82AD, 82AE, 82AF, 82AG, 85AE, 86AB, 98BA, 98DI, 99CF	274
Hatchery	North Puget Sound	Nooksack River	Nooksack KendallCkH	Fall	2010	10NJ	111
Wild	North Puget Sound	Nooksack River	S.F. Nooksack Sp	Spring	1980, 1981, 1984, 1985, 1986, 1993, 1995, 1998	80AD, 81AE, 84AC, 85AF, 86AC, 93EI,	224

Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	WDFW code	N processed
						94DS, 95DL, 98DK	
Wild	North Puget Sound	Sauk River	Upper Sauk	Fall	2006, 1994, 1998, 1999	06FG, 94EL, 98DN, 99ED	144
Wild	North Puget Sound	Skagit River	L. Skagit Fall	Fall	2006, 1998	06EN, 98EC	92
Hatchery	North Puget Sound	Skagit River	Skagit Marblemount SpH	Spring	2006, 2008, 2010	06EO, 08HC, 10NG	469
Hatchery	North Puget Sound	Skagit River	Skagit Marblemount SuH	Summer	1994	94DV	92
Wild	North Puget Sound	Skagit River	Upper Skagit Su	Summer	2006, 1995, 1998	06EM, 95DN, 98FJ	216
Wild	North Puget Sound	Skykomish River	Skykomish Su	Summer	2012, 2013	12NT, 13NX	188
Wild	North Puget Sound	Snoqualmie River	Snoqualmie Fall	Fall	2012, 2013	12NU, 13NV, 13NY	148
Wild	North Puget Sound	Stillaquamish River	N.F. Stillaguamish Su	Summer	2007, 2009, 2011	07NI, 09NB, 10NW, 11BO	171
Hatchery	North Puget Sound	Stillaquamish River	S.F. Stillaguamish Fall	Fall	2011, 2012	11MK, 12CM	92
Wild	North Puget Sound	Suiattle River	Suiattle Sp	Spring	1989, 1998, 1999	89AE, 98DL, 99DJ	173
Wild	Puget Sound White River	White River	White Sp	Fall	2006	06KK	95
Wild	Puget Sound Fall Aggregate	Bear	Bear	Fall	2003, 2004	03NU, 04IP, 04IQ	91
Wild	Puget Sound Fall Aggregate	Cedar	Cedar	Fall	2003, 2004	03NT, 04HS	95

Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	WDFW code	N processed
Wild	Puget Sound Fall Aggregate	Green (Duwamish)	Green	Fall	2012	12IP	92
Hatchery	Puget Sound Fall Aggregate	Green (Duwamish)	Green SoosCkH	Fall	2004, 2010, 1998	04HW, 10JX, 98HB	208
Wild	Puget Sound Fall Aggregate	HammaHamma	HammaHammaFa	Fall	1999, 2000	00HJ, 99EP	79
Wild	Puget Sound Fall Aggregate	Nisqually River	Nisqually	Fall	2005, 2006, 1998, 2000,	00FO, 00FP, 06EL, 98ED, 99EH, 99FB	79
Hatchery	Puget Sound Fall Aggregate	Nisqually River	Nisqually ClearCkH	Fall	2005	05KB	88
Hatchery	Puget Sound Fall Aggregate	Puyallup River	Puyallup	Fall	2008	08HZ	94
Hatchery	Puget Sound Fall Aggregate	Samish River	Samish Fall	Fall	1986, 1998	86QJ, 86QK, 98AZ, 98HK	262
Hatchery	Puget Sound Fall Aggregate	Sammamish River	Issaquah	Fall	2004	04HV	80
Wild	Puget Sound Fall Aggregate	Skokomish River	N.F. Skokomish Fall	Fall	2004, 2006, 1998, 2000	00GL, 04HH, 05IT, 06DP, 98FH, 99FG	96
Wild	Puget Sound Fall Aggregate	Skokomish River	S.F. Skokomish Fall	Fall	2005, 2006	05IS, 05JZ, 06DO	107
Hatchery	Puget Sound Fall Aggregate	Skokomish River	Skokomish George Adams H	Fall	2008	08HV	467
Wild	Strait Juan de Fuca	Dungeness	Dungeness	unknown	2004	04FI, 04HP	131
Hatchery	Strait Juan de Fuca	Elwha	Elwha	unknown-possible fall	1996	96AF, 96AG	123
Wild	Washington Coast	Fall River	Fall River	Fall	2015	15OZ	12

Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	WDFW code	N processed
Hatchery/Wild	Washington Coast	Hoh	Hoh-SF spr	Spring	2009	09MG	17
Wild	Washington Coast	Hoh	Hoh River Fall	Fall	2004, 2005	04GE, 05MW,	90
Wild	Washington Coast	Hoh	Hoh R SpSu	Fall	2005, 2006, 1995, 1996, 1997	05EN, 06CK, 95EC, 96BQ, 97DL, 97DZ	131
Wild	Washington Coast	Hoh	Hoh spr	Spring	2007, 2009	07DV, 09AJ	42
Hatchery	Washington Coast	Hoko	Hoko H Fa	Fall	2004, 2006	04AAQ, 06GO	56
Wild	Washington Coast	Naselle River	Naselle River	Fall	2014	14NT	100
Hatchery	Washington Coast	Naselle River	Naselle WDFW Hatchery	Fall	2014	14NQ	99
Wild	Washington Coast	Nemah River	Nemah River	Fall	2014	14NT	32
Hatchery	Washington Coast	Nemah River	Nemah WDFW Hatchery	Fall	2014	14NP	100
Wild	Washington Coast	North River	North River	Fall	2015	150Z, 15PB	28
Hatchery	Washington Coast	Quillayute River	Sol Duc (Quillayute) Hat su	Su	2006	06BZ	22
Hatchery	Washington Coast	Quinault River	Quinault NFH	Fall	2001, 2006, 2010	01EO, 06BV, 10NJ	111
Wild	Washington Coast	Willapa River	Forks Creek	Fall	2014	14NT	83
Hatchery	Washington Coast	Willapa River	Forks Creek WDFW Hatchery	Fall	2014	14NR	99

Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	WDFW code	N processed
Wild	Washington Coast	Chehalis River	Black River	Spring	2015	15NT	9
Wild	Washington Coast	Chehalis River	Chehalis main fall	Fall	2002, 2009	02AAT, 09IR	4
Wild	Washington Coast	Chehalis River	Chehalis main spring	Fall	2001, 2005,2009, 2015	01ABB, 05OT, 09IR, 15PF	9
Wild	Washington Coast	Chehalis River	SF Chehalis fall	Fall	2002, 2003, 2005	02AAT, 03AAS, 05OY	11
Wild	Washington Coast	Chehalis River	SF Chehalis spring	Spring	2001, 2002, 2004, 2009	01ABB, 02AAP, 04ABH, 09IR	7
Wild	Washington Coast	Chehalis River	Upper Chehalis fall	Fall	2002, 2015	02AAT, 15PV	45
Wild	Washington Coast	Chehalis River	Upper Chehalis spring	Spring	2002, 2003, 2005, 2009, 2014	02AAP, 03AAJ, 05OT, 09IR, 14SU	18
Wild	Washington Coast	Chehalis River	Newaukum fall	Fall	2001, 2002, 2003, 2004, 2005	01ABE, 02AAV, 03AAR, 04ABL, 05OX	36
Wild	Washington Coast	Chehalis River	Newaukum spring	Spring	2003, 2005	03AAL, 05OS,	29
Wild	Washington Coast	Chehalis River	Satsop fall	Fall	2004, 2005	04ABI, 05OU	37
Wild	Washington Coast	Chehalis River	Satsop spring	Spring	2002, 2004, 2005, 2009	02AAO, 04ABE, 05OP,09IW	20
Wild	Washington Coast	Chehalis River	Skookumchuck fall	Fall	2003, 2005	03AAQ, 05OW	35

Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	WDFW code	N processed
Wild	Washington Coast	Chehalis River	Skookumchuck spring	Spring	2002, 2004	02AAQ, 04ABF	37
Hatchery	Washington Coast	Chehalis River	Wishkah River fall	Fall	2009	09IT	5
Hatchery	Washington Coast	Chehalis River	Wynoochee River fall	Fall	2009	09IS	21
Wild	Washington Coast	Chehalis River	Black fall	Fall	2003, 2015	03AAP, 15NT	18

Table 4. General diversity statistics for the Chehalis basin. Data include, DPS that samples belong to, number of individuals included in the analysis (N), mean number of alleles(N^A), expected heterozygosity (He), observed heterozygosity (Ho), individual fixation index (f), linkage disequilibrium effective population size (LDN_e). Populations with "NA" had sample sizes too low to properly estimate LDNe.

Population	DPS	N	Mean N^A	He	Ho	F_{IS}	LDNe (95%CI)
Black fall	Washington Coast	18	1.872	0.299	0.292	0.025	NA
Black spring	Washington Coast	9	1.818	0.291	0.273	0.066	NA
Chehalis main fall	Washington Coast	4	1.689	0.293	0.294	-0.006	NA
Chehalis main spring	Washington Coast	9	1.818	0.302	0.283	0.066	NA
Newaukum fall	Washington Coast	36	1.926	0.307	0.305	0.007	192.1 (144.4 - 282.6)
Newaukum spring	Washington Coast	29	1.872	0.289	0.291	-0.006	249.2 (137.4 - 442.6)
Satsop fall	Washington Coast	37	1.899	0.304	0.303	0.004	1,932.9 (463.5 - inf.)
Satsop spring	Washington Coast	20	1.899	0.308	0.294	0.045	inf. (481.3 - inf.)
SF Chehalis fall	Washington Coast	11	1.858	0.314	0.299	0.050	NA
SF Chehalis spring	Washington Coast	7	1.764	0.295	0.297	-0.008	NA
Skookumchuck fall	Washington Coast	35	1.890	0.307	0.310	-0.007	1,062.6 (383.3 - inf.)
Skookumchuck spring	Washington Coast	37	1.905	0.304	0.301	0.010	758 (337.7 - inf.)
Upper Chehalis fall	Washington Coast	45	1.939	0.312	0.302	0.032	554.8 (324.1 - 1796.5)
Upper Chehalis spring	Washington Coast	18	1.885	0.307	0.291	0.054	NA
Wishkah River fall	Washington Coast	5	1.797	0.320	0.312	0.030	NA
Wynoochee River fall	Washington Coast	21	1.872	0.301	0.306	-0.018	212 (125.4 - 635)

Table 5. Pairwise* F_{ST} *matrix for all sampling location pars of Chinook salmon in the Chehalis River basin.* P*-Values are above the diagonal, and* F_{ST} *values are below. Asterisks indicate a significant* P*-value, and NS indicates a* P*-value that is not significant.

	Wynoochee fall	Satsop fall	Satsop spr	Chehalis Main fall	Chehalis Main spr	Black fall	Black spring	Skookumchuck fall	Skookumchuck spring	Newaukum fall	Newaukum spr	S.F. Chehalis fall	S.F. Chehalis spr	Upper Chehalis fall	Upper Chehalis spr
Wynoochee fall	-	NS	NS	*	NS	*	NS	*	*	*	*	*	*	*	*
Satsop fall	0.0038	-	*	*	NS	*	*	*	*	*	*	*	*	*	*
Satsop spr	0.0052	**0.0063**	-	NS	NS	*	NS	*	*	*	*	*	*	*	*
Chehalis Main fall	**0.0211**	**0.0161**	0.0113	-	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Chehalis Main spr	0.0020	0.0024	0.0094	0.0115	-	NS	NS	NS	*	NS	*	NS	NS	*	NS
Black fall	**0.0156**	**0.0094**	**0.0103**	0.0086	0.0055	-	NS	*	*	*	*	NS	*	*	*
Black spring	0.0058	**0.0091**	0.0065	0.0135	0.0066	-0.0025	-	NS	*	*	*	NS	NS	NS	NS
Skookumchuck fall	**0.0170**	**0.0137**	**0.0165**	0.0070	0.0032	**0.0086**	0.0050	-	*	NS	*	NS	*	*	*
Skookumchuck spring	**0.0229**	**0.0167**	**0.0227**	**0.0188**	**0.0102**	**0.0128**	**0.0085**	**0.0113**	-	*	*	NS	*	*	NS
Newaukum fall	**0.0141**	**0.0079**	**0.0149**	0.0077	0.0003	**0.0055**	**0.0088**	0.0029	**0.0103**	-	*	NS	NS	*	*
Newaukum spr	**0.0341**	**0.0255**	**0.0336**	0.0146	**0.0229**	**0.0225**	**0.0179**	**0.0185**	**0.0130**	**0.0174**	-	*	*	*	*
S.F. Chehalis fall	**0.0150**	**0.0075**	**0.0116**	0.0069	0.0014	0.0008	0.0013	0.0045	0.0015	-0.0008	**0.0178**	-	NS	NS	NS
S.F. Chehalis spr	**0.0196**	**0.0206**	**0.0190**	0.0217	0.0113	**0.0123**	0.0132	**0.0148**	**0.0092**	0.0081	**0.0128**	0.0117	-	NS	NS
Upper Chehalis fall	**0.0173**	**0.0148**	**0.0199**	0.0136	**0.0135**	**0.0126**	0.0030	**0.0091**	**0.0103**	**0.0082**	**0.0178**	0.0019	0.0059	-	NS
Upper Chehalis spr	**0.0171**	**0.0181**	**0.0213**	0.0098	0.0071	**0.0087**	0.0056	**0.0064**	0.0037	**0.0058**	**0.0126**	-0.0047	-0.0038	0.0008	-

Table 6. **F_{ST}** ***estimates for spring/summer/fall runs in the Hoh River (top) and the Skagit River (below).***

	Hoh River fall	Hoh River SpSu	Hoh River Spr	Hoh River SF spr
Hoh River fall	0			
Hoh River SpSu	**0.01**	0		
Hoh River Spr	**0.0091**	**0.0008**	0	
Hoh River SF spr	**0.0148**	**0.0038**	**0.0043**	0

	Lower Skagit Fall	Skagit Spr_Hatchery	Skagit Su_Hatchery	Upper Skagit_Su
Lower Skagit Fall	0			
Skagit Spr_Hatchery	**0.0343**	0		
Skagit Su_Hatchery	**0.0126**	**0.017**	0	
Upper Skagit_Su	**0.0118**	**0.017**	**0.0002**	0

Table 7. Pairwise* F_{ST} *matrix for all sampling location pairs within the Chehalis and Willapa populations.* P*-Values are above the diagonal, and* F_{ST} *values are below. Asterisks indicate a significant* P*-value, and NS indicates a* P*-value that is not significant.

	Black River fall	Black River spr	Chehalis main River fall	Chehalis main River spr	Fall River	Forks Creek	Forks Creek WDFW hat	Naselle River	Naselle WDFW hat	Nemah River	Nemah WDFW Hat	Newaukum River fall	Newaukum River spr
Black River fall	-	NS	NS	NS	*	*	*	*	*	*	*	NS	*
Black River spr	-0.005	-	NS	NS	NS	*	*	*	*	*	*	NS	*
Chehalis main River fall	0.013	0.012	-	NS	NS	*	*	*	*	*	*	NS	*
Chehalis main River spr	0.008	0.001	0.005	-	NS	*	*	*	*	*	*	NS	*
Fall River	**0.016**	0.001	0.001	**0.005**	-	*	*	*	*	*	NS	*	*
Forks Creek	**0.034**	**0.021**	**0.027**	0.029	**0.013**	-	*	NS	*	*	*	*	*
Forks Creek WDFW hat	**0.029**	**0.021**	**0.023**	**0.027**	**0.009**	**0.003**	-	*	*	NS	NS	*	*
Naselle River	**0.027**	**0.015**	**0.021**	**0.023**	**0.008**	0.001	**0.002**	-	NS	*	*	*	*
Naselle WDFW hat	**0.033**	**0.021**	**0.019**	**0.024**	**0.011**	**0.003**	**0.002**	0.002	-	NS	NS	*	*
Nemah River	**0.026**	**0.016**	**0.017**	**0.018**	**0.008**	**0.004**	0.002	**-0.001**	0.000	-	NS	*	*
Nemah WDFW Hat	**0.032**	**0.020**	**0.017**	**0.024**	0.007	**0.004**	0.001	**0.001**	0.001	0.001	-	*	*
Newaukum River fall	0.005	0.001	0.011	**0.000**	**0.012**	**0.026**	**0.021**	**0.020**	**0.025**	**0.019**	**0.024**	-	*
Newaukum River spr	**0.027**	**0.024**	0.019	**0.021**	**0.028**	**0.052**	**0.044**	**0.044**	**0.044**	**0.040**	**0.047**	**0.017**	-
North River	**0.020**	0.009	0.023	0.009	0.006	**0.018**	**0.014**	**0.012**	**0.014**	**0.012**	**0.013**	**0.011**	**0.032**
Satsop River fall	**0.012**	0.008	0.016	0.002	0.007	**0.021**	**0.019**	**0.016**	**0.019**	**0.018**	**0.022**	**0.010**	**0.028**
Satsop River spr	**0.013**	0.001	0.006	0.003	0.002	**0.012**	**0.011**	**0.010**	**0.011**	**0.011**	**0.012**	**0.010**	**0.033**
SF Chehalis River fall	0.006	0.004	0.004	0.000	0.012	**0.033**	**0.028**	**0.024**	**0.030**	**0.024**	**0.031**	-0.001	**0.017**
SF Chehalis River spr	**0.021**	0.017	0.019	0.012	**0.040**	**0.048**	**0.039**	**0.040**	**0.038**	**0.032**	**0.038**	0.009	**0.015**
Skookumchuck River fall	**0.010**	0.003	0.005	0.000	**0.009**	**0.023**	**0.020**	**0.018**	**0.020**	**0.017**	**0.020**	0.000	**0.019**
Skookumchuck River spr	**0.012**	0.007	0.014	0.002	**0.010**	**0.036**	**0.029**	**0.029**	**0.030**	**0.024**	**0.030**	**0.009**	**0.012**
Upper Chehalis River fall	**0.015**	0.005	0.006	0.008	**0.015**	**0.031**	**0.027**	**0.025**	**0.028**	**0.023**	**0.028**	**0.006**	**0.018**
Upper Chehalis River spr	**0.008**	0.008	0.008	-0.003	**0.019**	**0.040**	**0.034**	**0.031**	**0.034**	**0.029**	**0.037**	0.005	**0.017**
Wynoochee River fall	**0.019**	0.008	**0.019**	0.003	0.009	**0.019**	**0.017**	**0.018**	**0.019**	**0.018**	**0.023**	**0.014**	**0.039**

Table 7. cont'd

	North River	Satsop River fall	Satsop River spr	SF Chehalis River fall	SF Chehalis River spr	Skookumchuck River fall	Skookumchuck River spr	Upper Chehalis River fall	Upper Chehalis River spr	Wynoochee River fall
Black River fall	*	*	*	NS	*	*	*	*	*	*
Black River spr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Chehalis main River fall	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Chehalis main River spr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Fall River	NS	NS	NS	NS	*	*	*	*	*	NS
Forks Creek	*	*	*	*	*	*	*	*	*	*
Forks Creek WDFW hat	*	*	*	*	*	*	*	*	*	*
Naselle River	*	*	*	*	*	*	*	*	*	*
Naselle WDFW hat	*	*	*	*	*	*	*	*	*	*
Nemah River	*	*	*	*	*	*	*	*	*	*
Nemah WDFW Hat	*	*	*	*	*	*	*	*	*	*
Newaukum River fall	*	*	*	NS	NS	NS	*	*	NS	*
Newaukum River spr	*	*	*	*	*	*	*	*	*	*
North River	-	*	NS	*	*	*	*	*	*	*
Satsop River fall	0.011	-	NS	*	*	*	*	*	*	NS
Satsop River spr	**0.006**	0.003	-	NS	*	*	*	*	*	NS
SF Chehalis River fall	**0.015**	**0.010**	0.008	-	NS	NS	NS	NS	NS	*
SF Chehalis River spr	**0.022**	**0.031**	0.024	**0.012**	-	*	NS	NS	NS	*
Skookumchuck River fall	**0.013**	**0.013**	0.009	**0.005**	**0.018**	-	*	*	*	*
Skookumchuck River spr	**0.018**	**0.016**	0.017	**0.003**	0.008	**0.011**	-	*	NS	*
Upper Chehalis River fall	**0.020**	**0.015**	0.015	**0.003**	0.009	**0.008**	**0.009**	-	NS	*
Upper Chehalis River spr	**0.024**	**0.020**	0.018	**-0.007**	-0.004	**0.008**	0.002	0.003	-	*
Wynoochee River fall	0.014	0.003	**0.004**	**0.022**	**0.028**	**0.013**	**0.023**	**0.018**	**0.018**	-

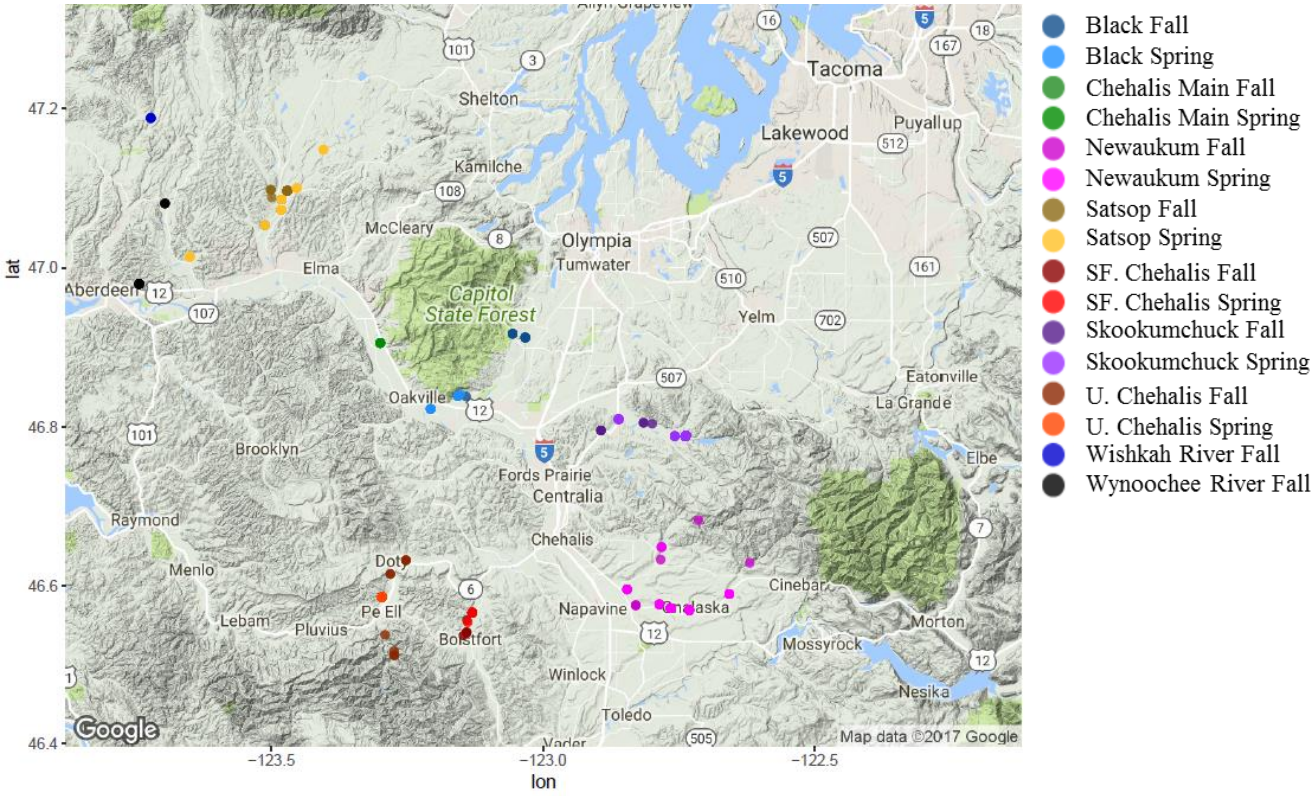

Figure 1. Map of Chinook salmon sampling locations throughout the Chehalis basin.

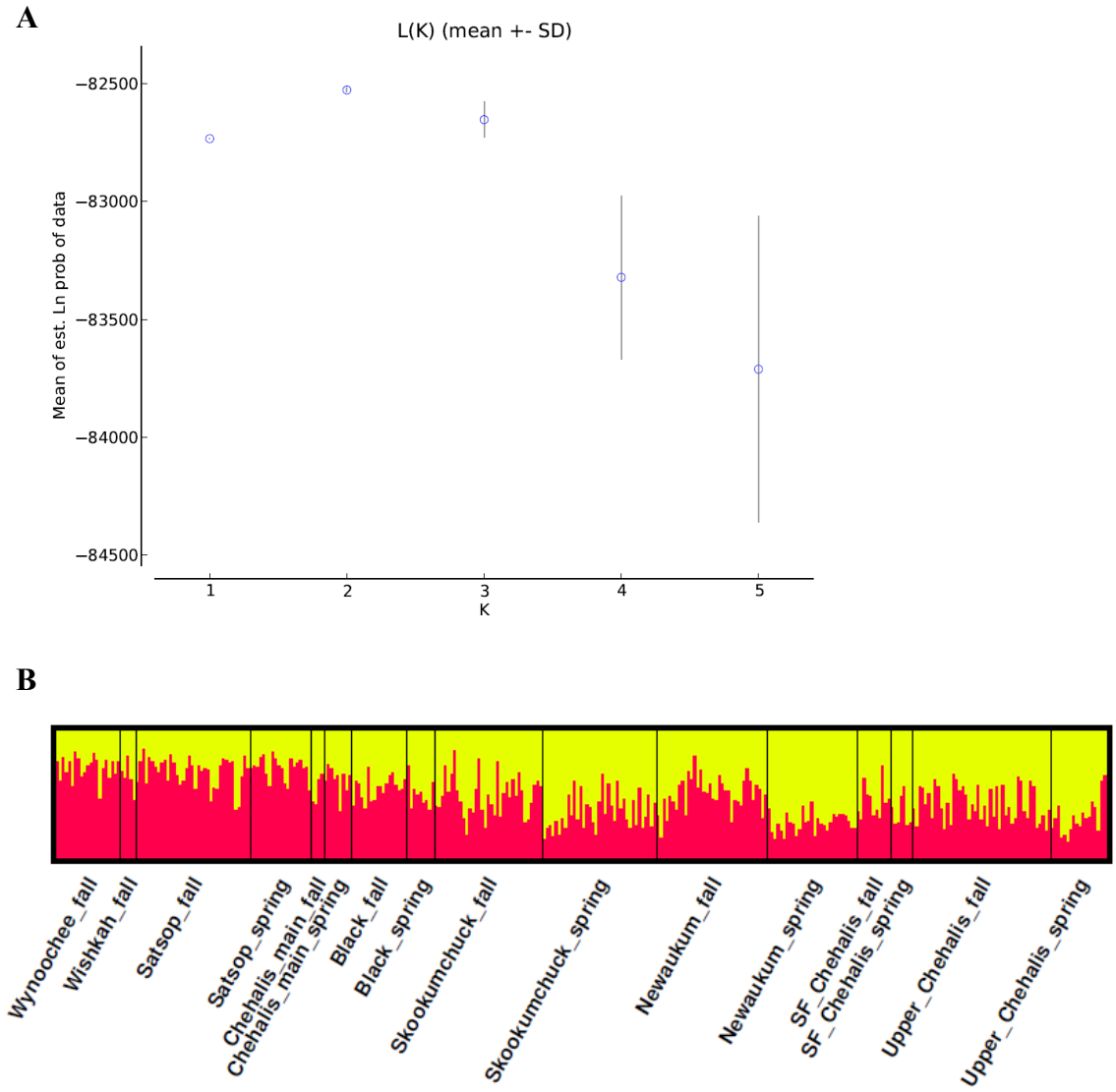

Figure 2. A) Results from STRUCTURE, displaying the likelihood of the Chehalis Basin Chinook salmon belonging to K 1-5 populations. The X- axis depicts, K, the number of populations or genetic clusters. The Y-axis is the mean likelihood of K. B) Results from STRUCTURE depicting K = 2 as the most likely number of populations. Each vertical bar represents an individual, and the Y-axis shows the relative proportion that each individuals belongs to one of the two populations identified by STRUCTURE. Sampling locations are roughly ordered from downstream to upstream, and are separated by thin black lines.

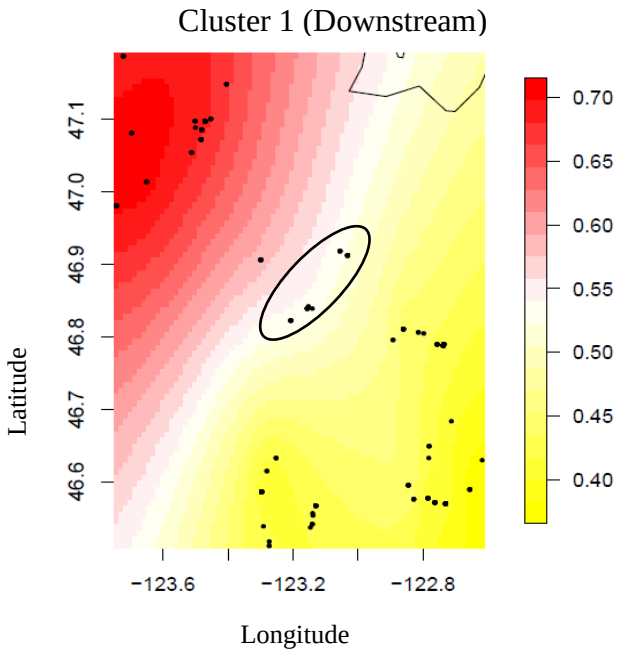

Figure 3. Results from spatial interpolation of individual ancestry coefficients (Q-matrix). Black dots on map are sampling locations, see Figure 1 for more information. Black oval outline identifies the sampling location for Black River. Map of individual ancestry coefficients for Cluster 1 (red; downstream). Ancestry coefficients range from 0.70 (high group membership to cluster 1; red) to 0.40 and lower (low group membership to cluster 1; yellow).

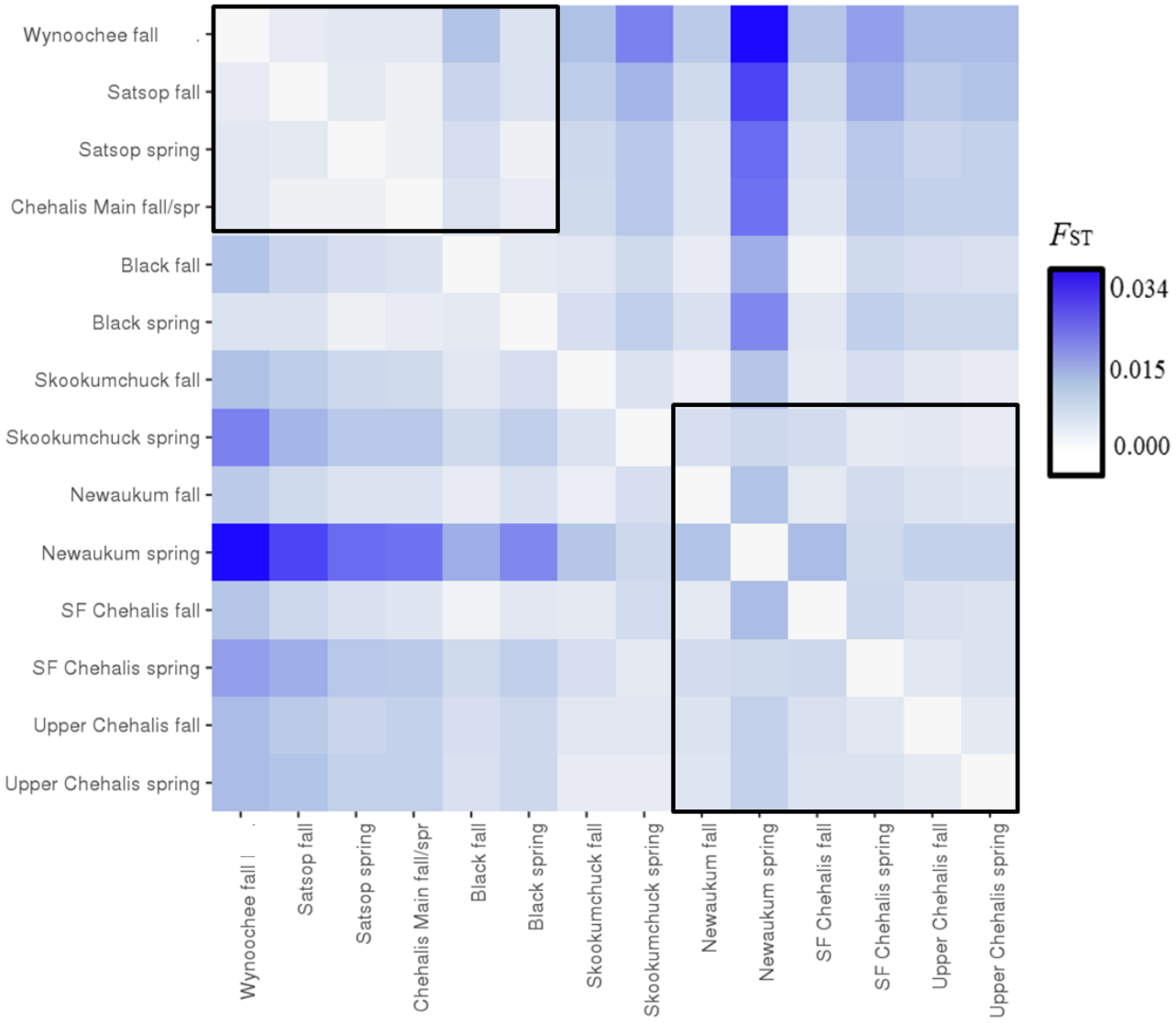

Figure 4. Heat map of pairwise F_{ST} values between sampling locations, in order from downstream to upstream. F_{ST} values range from ~0.034 (dark blue; highly differentiated) to ~0.000 (white; not differentiated). Black boxes encompass regions of low differentiation.

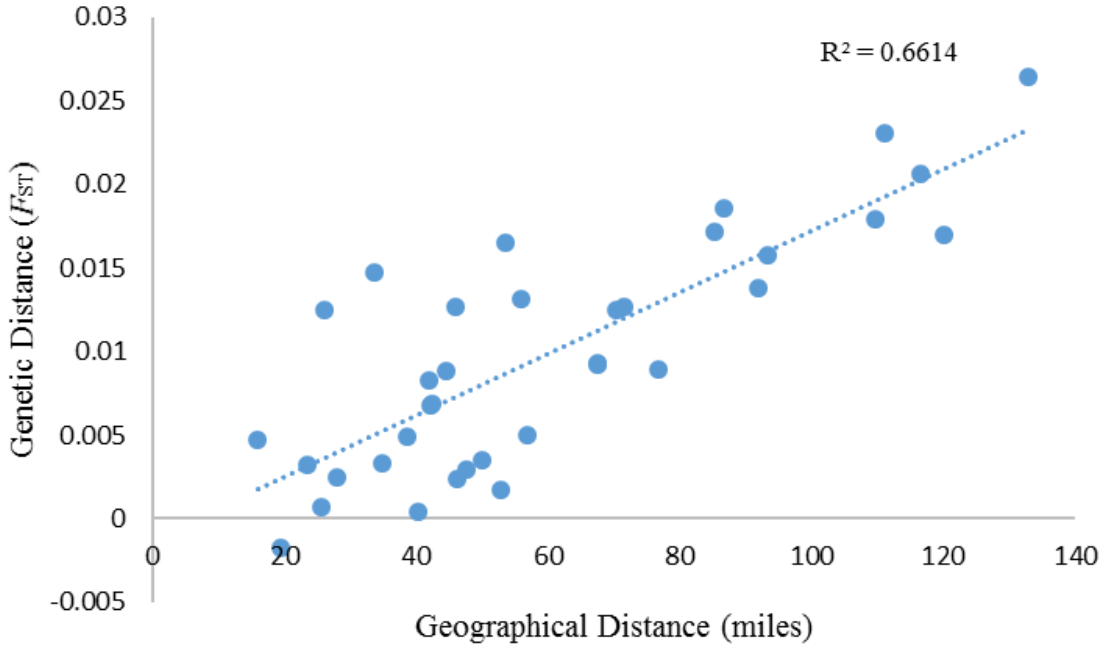

Figure 5. Isolation by Distance (IBD) scatter plot of geographical distance between sampling location (X-axis) and genetic distance, F_{ST}, (Y-axis).

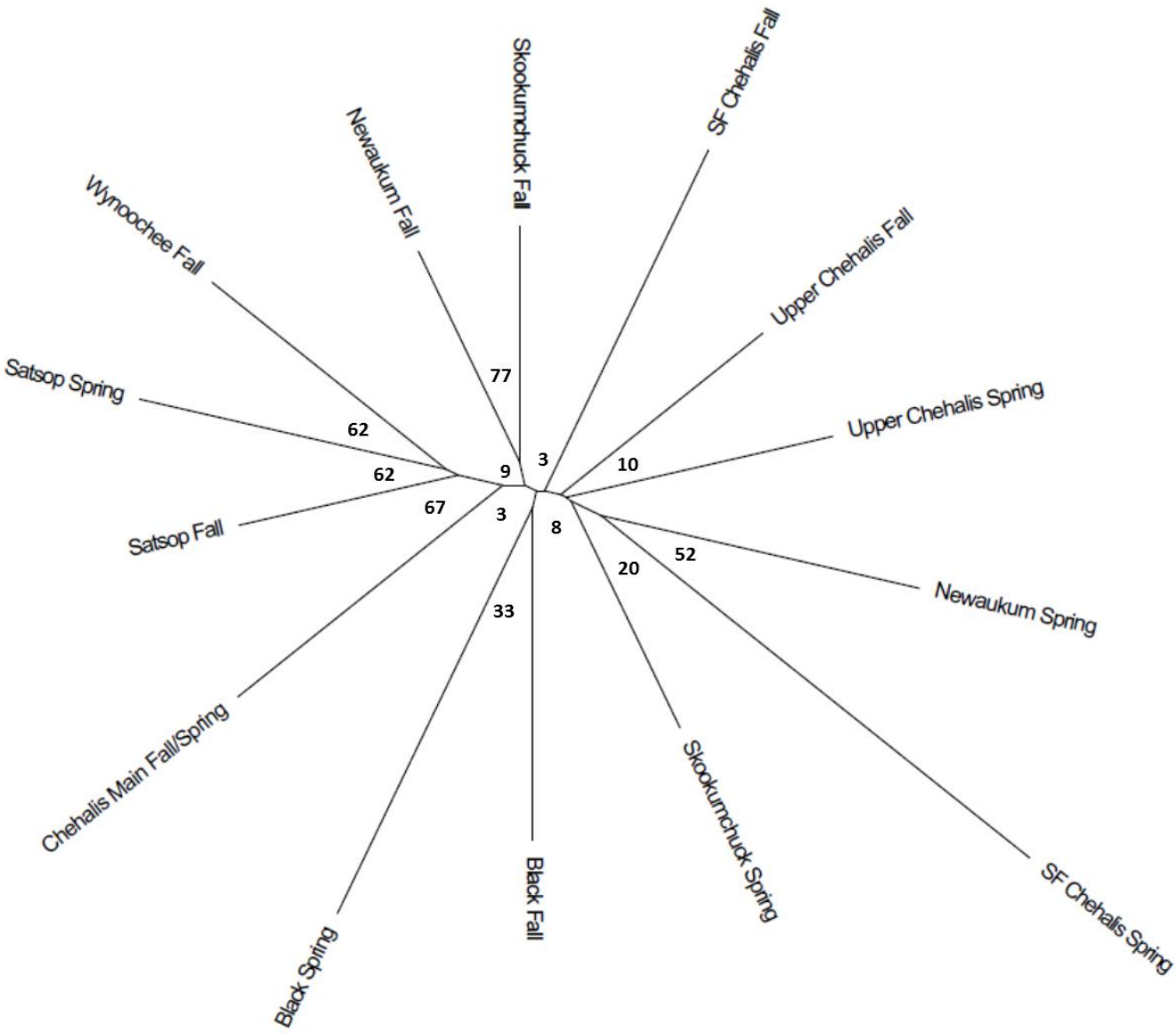

Figure 6. Unrooted neighbor joining dendrogram of Cavalli-Sforza distances between sampling locations in the Chehalis basin. Bootstrap support values (0-100) are located near the nodes. Bootstrap support of 60 and above indicate moderate support.

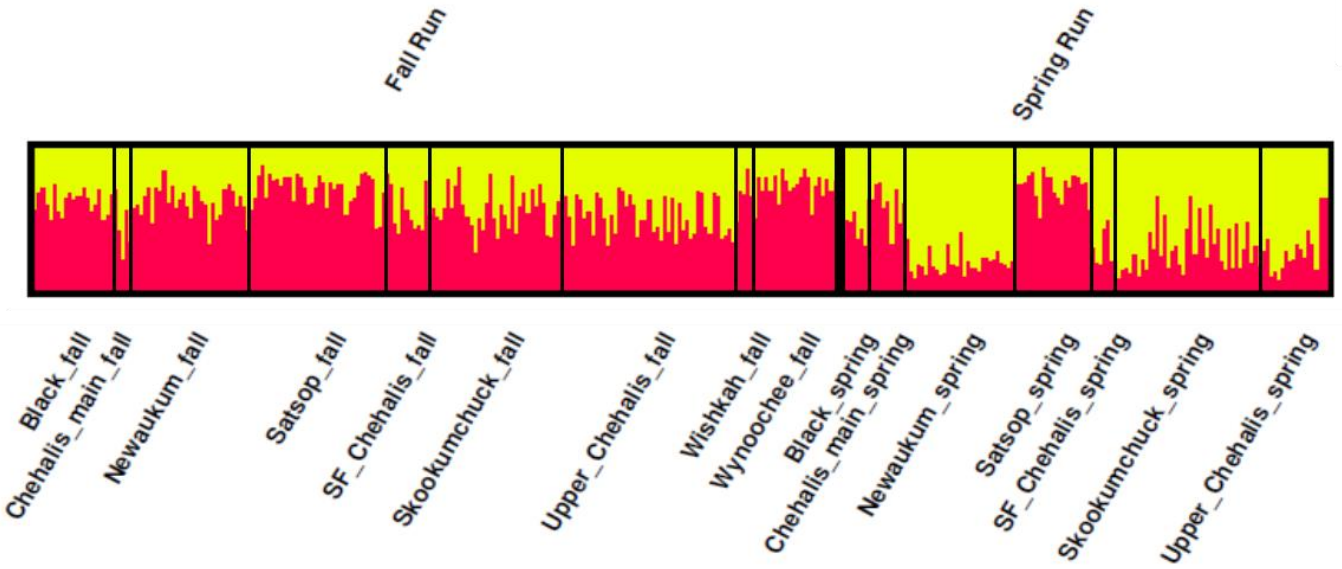

Figure 7. Results from STRUCTURE depicting two populations (K = 2). The* locprior *model was used with run timing information in place of location information, in order to visualize if the Chehalis basin samples were differentiated based on run timing. Each bar represents an individual, and the Y-axis shows the relative percentage that each individuals belongs to a population. Sampling locations are ordered in fall and spring run, from downstream to upstream, and are separated by thin black lines.

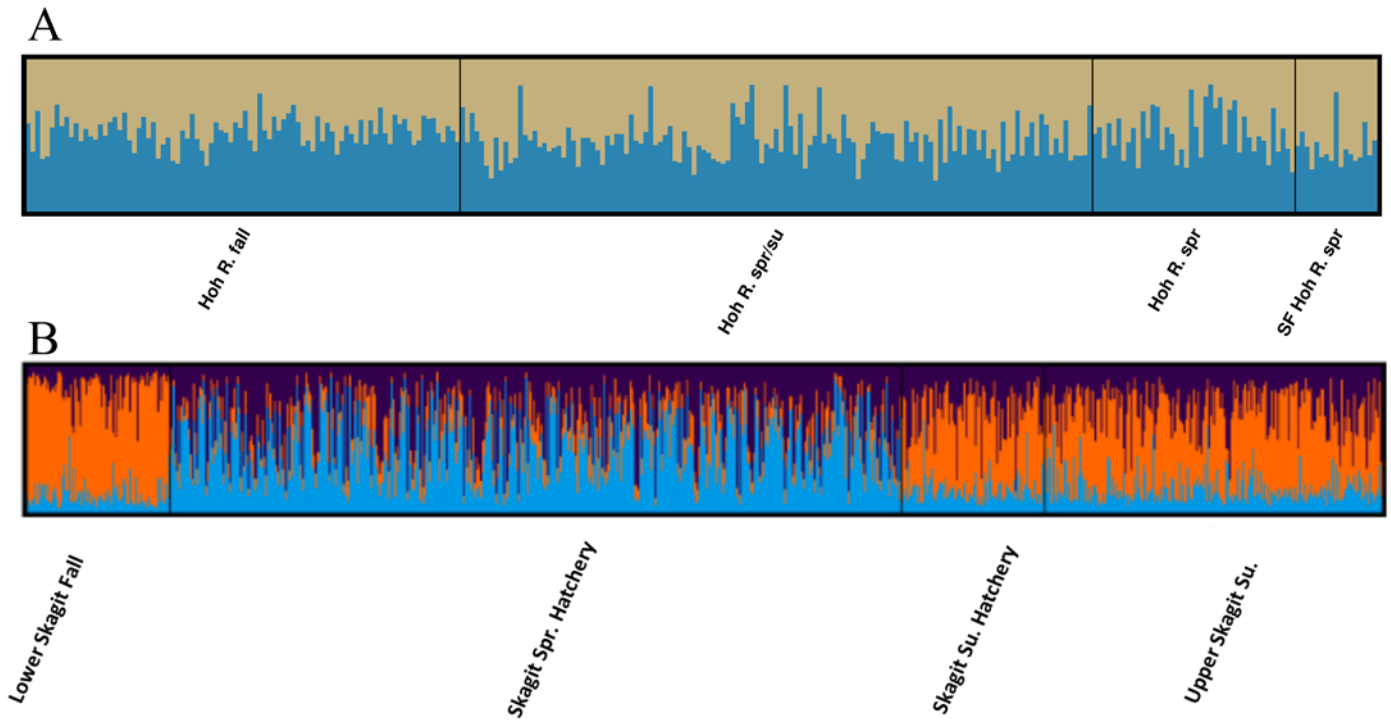

Figure 8. STRUCTURE results depicting A) K = 2 for the fall/spring/summer runs on the Hoh River, and B) K = 3 for the fall/spring/summer runs on the Skagit River. Each bar represents an individual, and the Y-axis shows the relative percentage that each individuals belongs to a population.

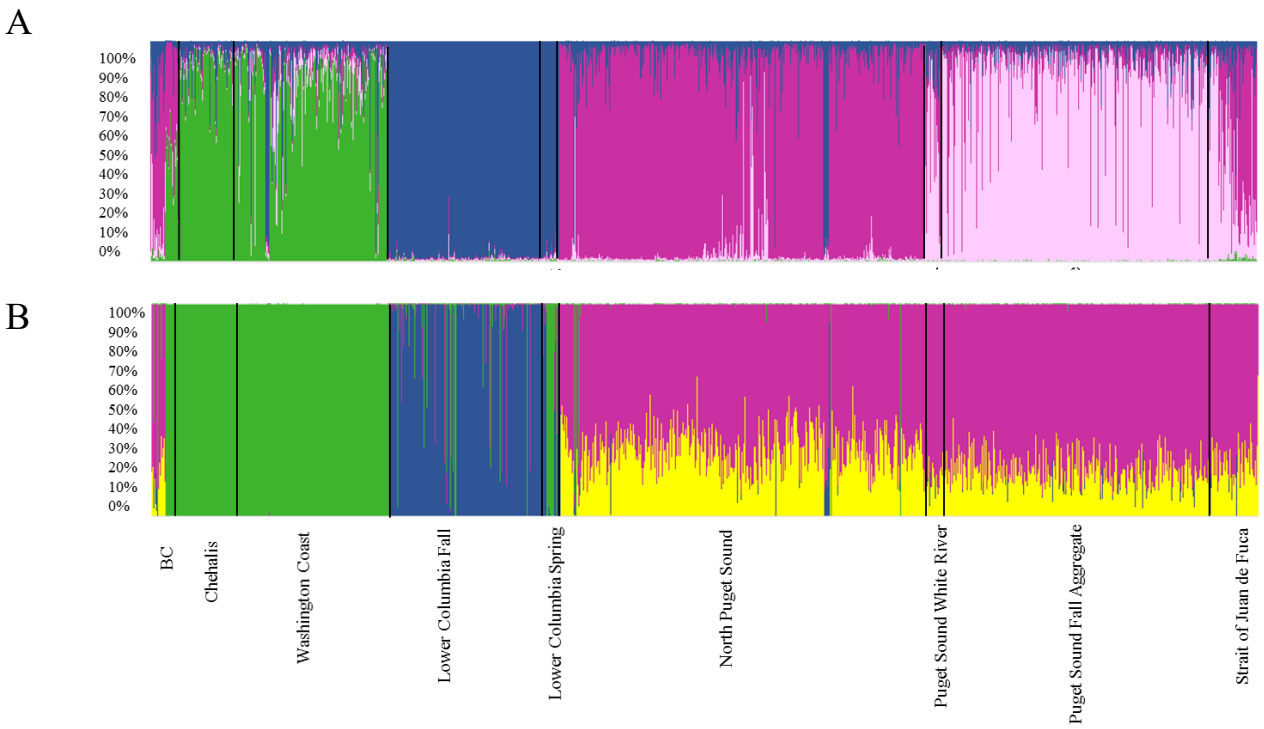

Figure 9. Plot of Individual group membership for STRUCTURE (A), and DAPC (B) analyses. Baseline populations include; British Columbia, Washington Coast, Lower Columbia (fall and spring), North Puget Sound, Puget Sound White River, Puget Sound fall aggregate, and the Strait of Juan de Fuca), and the Chehalis basin. Both plots support for four populations K = 4. Population aggregates are below, and thin black lines separate the aggregates.

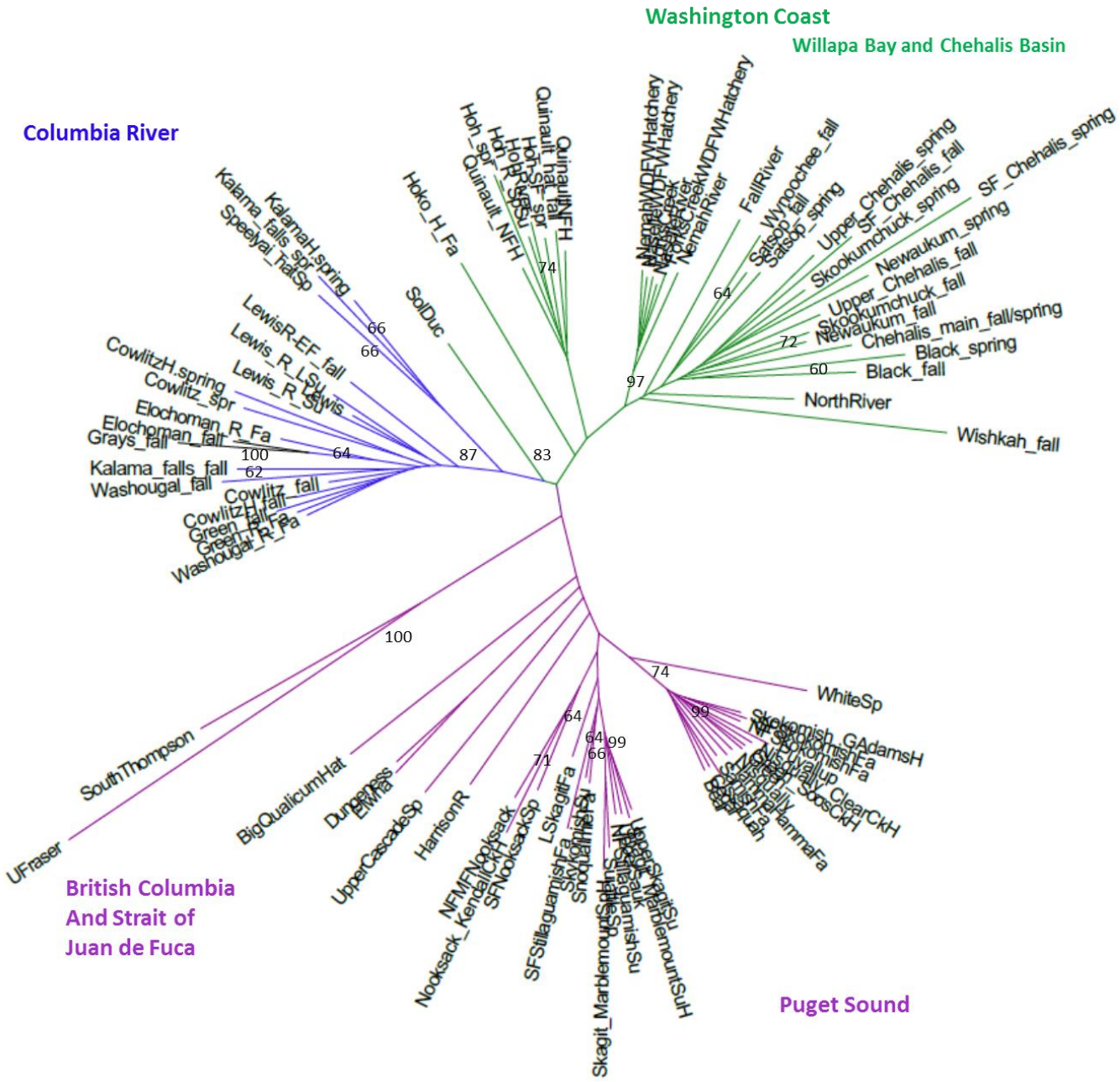

Figure 10. Unrooted neighbor joining dendrogram of the Chehalis basin Chinook salmon in relation to baseline populations (BC, Washington Coast, Lower Columbia (fall and spring), North Puget Sound, Puget Sound White River, Puget Sound fall aggregate, and the Strait of Juan de Fuca). Bootstrap values with moderate to high support (60-100) were placed on the dendrogram.

Appendix 1. General diversity statistics for baseline samples. Data include, DPS that samples belong to, number of individuals included in the analysis (N), mean number of alleles(N^A), expected heterozygosity (He), observed heterozygosity (Ho), individual fixation index (f), linkage disequilibrium effective population size (LDN_e). Populations with "NA" had sample sizes too low to properly estimate Ne.

Population or stock	DPS	N	Mean N^A	He	Ho	F_{IS}	LDNe (95%CI)
Big Qualicum Hat	BC	48	1.932	0.287	0.285	0.006	2392.2 (686.0 - inf)
Harrison R	BC	47	1.959	0.320	0.317	0.008	2106.8 (666.5 - inf.)
South Thompson	BC	47	1.892	0.280	0.275	0.020	467.7 (301.1 - 983.5)
U Fraser	BC	48	1.858	0.255	0.253	0.005	417.2 (278.9 - 786.8)
Cowlitz H. fall	L_Columbia_fa	57	1.932	0.310	0.319	-0.030	3847.4 (845.6 - inf)
Cowlitz fall	L_Columbia_fa	247	1.966	0.314	0.311	0.009	617.8 (536.1 - 723.0)
Elochoman_fall	L_Columbia_fa	73	1.946	0.321	0.303	0.056	96.8 (88 - 107)
Elochoman_R_Fa	L_Columbia_fa	85	1.919	0.313	0.304	0.031	163.6 (144.4 - 187.3)
Speelyai_hatSp	L_Columbia_Fa	22	1.932	0.340	0.326	0.043	347.8 (168.0 - inf.)
Washougal_fall	L_Columbia_fa	17	1.831	0.300	0.338	-0.131	331.5 (123.6 - inf.)
Washougal_R_Fa	L_Columbia_fa	96	1.939	0.316	0.311	0.014	9382.6 (1573.4 - inf.)
Grays fall	L_Columbia_fall	110	1.959	0.311	0.295	0.050	173.6 (156.2 - 194.1)
Green fall	L_Columbia_fall	35	1.932	0.314	0.310	0.014	586.0 (305.1 - 4587.6)
Green_R_Fa	L_Columbia_fall	53	1.905	0.311	0.305	0.019	Inf. (3676.2 - inf.)
Kalama_falls_fall	L_Columbia_fall	12	1.845	0.313	0.322	-0.031	403.5 (113.7 - inf.)
Lewis	L_Columbia_fall	57	1.926	0.318	0.310	0.025	2291.7 (734.3 - inf.)
Lewis_R_LSu	L_Columbia_fall	45	1.905	0.308	0.308	0.002	1531.2 (526.6 - inf.)
Lewis_R_Su	L_Columbia_fall	58	1.939	0.313	0.307	0.018	4640.3 (894.1 - inf.)
LewisR-EF_fall	L_Columbia_fall	22	1.892	0.325	0.322	0.010	430.7 (195.4 - inf.)
Cowlitz H. spring	L_Columbia_sp	52	1.905	0.305	0.304	0.005	1791.7 (640.2 - inf.)
Cowlitz spr	L_Columbia_sp	11	1.858	0.324	0.320	0.014	125.7 (67.4 - 631.4)
Kalama H. spring	L_Columbia_sp	54	1.973	0.338	0.332	0.017	87.4 (78.6 - 97.7)

Population or stock	DPS	N	Mean N^A	He	Ho	F_{IS}	LDNe (95%CI)
Kalama_falls_spr	L_Columbia_sp	25	1.912	0.322	0.323	-0.002	196.0 (132.1 - 362.1)
L Skagit Fa	N_Puget_S	92	1.980	0.325	0.332	-0.022	340.7 (283.6 - 421.9)
NFMF Nooksack	N_Puget_S	274	1.980	0.298	0.310	-0.040	920 (771 - 1128.3)
NF Stillaguamish Su	N_Puget_S	171	1.973	0.316	0.315	0.003	938.6 (731.6 - 1287)
Nooksack_KendallCkH	N_Puget_S	111	1.932	0.288	0.297	-0.030	56.5 (53.3 - 60.0)
SF Nooksack Sp	N_Puget_S	224	1.980	0.312	0.310	0.006	162.5 (152.5 - 173.4)
SF Stillaguamish Fa	N_Puget_S	92	1.959	0.312	0.312	0.002	140.9 (127.2 - 157.1)
Skagit_Marblemount Sp H	N_Puget_S	469	1.959	0.304	0.308	-0.014	424.7 (395.7 - 456.7)
Skagit_Marblemount Su H	N_Puget_S	92	1.959	0.310	0.312	-0.005	Inf. (3209.7 - inf.)
SkykomishSu	N_Puget_S	188	1.980	0.324	0.340	-0.051	1018.9 (797.6 - 1387.2)
SnoqualmieFa	N_Puget_S	148	1.966	0.319	0.322	-0.010	713.7 (566.3 - 949.9)
Suiattle Sp	N_Puget_S	173	1.966	0.300	0.304	-0.011	941.9 (734.3 - 1290.6)
Upper Cascade Sp	N_Puget_S	8	1.858	0.321	0.322	-0.003	4859.3 (99.6 - inf.)
Upper Sauk	N_Puget_S	144	1.959	0.311	0.312	-0.004	1189.4 (845.2 - 1947.7)
Upper Skagit Su	N_Puget_S	216	1.980	0.318	0.347	-0.091	1540.6 (1128.8 - 2367.1)
WhiteSp	PS_White_River	95	1.946	0.294	0.310	-0.055	524.0 (398.8 - 747.4)
Bear	Puget S_Fall aggregate	91	1.946	0.308	0.321	-0.043	596.1 (431.7 - 935.5)

Population or stock	DPS	N	Mean N^A	He	Ho	F_{IS}	LDNe (95%CI)
Cedar	Puget S_Fall aggregate	95	1.973	0.314	0.331	-0.057	640.2 (463.5 - 1005.9)
Green	PugetS_fall_aggregate	92	1.953	0.313	0.323	-0.034	1174.3 (697.2 - 3372.9)
Green_SoosCkH	PugetS_fall_aggregate	208	1.959	0.311	0.319	-0.025	2063.5 (1368.3 - 4007.7)
Hamma HammaFa	PugetS_fall_aggregate	79	1.953	0.315	0.307	0.025	597.6 (429.7 - 952.3)
Issaquah	PugetS_fall_aggregate	80	1.932	0.308	0.314	-0.021	579.5 (415.2 - 929.9)
NF SkokomishFa	PugetS_fall_aggregate	96	1.946	0.315	0.315	-0.001	1018.1 (665.2 - 2061.7)
Nisqually	PugetS_fall_aggregate	79	1.939	0.315	0.323	-0.026	839.6 (544.1 - 1741.3)
Nisqually_ClearCkH	PugetS_fall_aggregate	88	1.946	0.311	0.312	-0.004	1481.2 (814.1 - 6790.8)
Puyallup	PugetS_fall_aggregate	94	1.926	0.309	0.312	-0.009	1051.5 (668.5 - 2317.1)
SamishFa	PugetS_fall_aggregate	262	1.959	0.307	0.314	-0.021	1829.3 (1341.8 - 2802.1)
SF Skokomish Fa	PugetS_fall_aggregate	107	1.946	0.315	0.320	-0.018	3664.6 (1410.6 - Inf.)
Skokomish_GAdamsH	PugetS_fall_aggregate	467	1.966	0.315	0.319	-0.010	1164.8 (1017.5 - 1352.0)
Dungeness	Strait Juan de Fuca	131	1.959	0.295	0.302	-0.025	397.9 (337.7 - 479.4)
Elwha	Strait Juan de Fuca	123	1.946	0.287	0.285	0.007	422.4 (351.9 - 522.1)
Fall River	Washington Coast	12	1.845	0.304	0.305	-0.004	9 (8.1 - 12.2)
Forks Creek	Washington Coast	83	1.946	0.303	0.334	-0.103	841.7 (403.8 - inf.)

Population or stock	DPS	N	Mean N^A	He	Ho	F_{IS}	LDNe (95%CI)
Forks Creek WDFW Hatchery	Washington Coast	99	1.939	0.312	0.337	-0.078	159.4 (132.9 - 196.8)
Hoh River	Washington Coast	90	1.959	0.308	0.302	0.019	11588.1 (1615.7 - inf.)
Hoh_R_SpSu	Washington Coast	131	1.946	0.318	0.312	0.021	780.4 (593.6 - 1115.1)
Hoh_spr	Washington Coast	42	1.885	0.301	0.301	-0.001	289.9 (211.4 - 448.1)
Hoh-SF_spr	Washington Coast	17	1.926	0.307	0.305	0.004	329.7 (145.8 - Inf.)
Hoko_H_Fa	Washington Coast	56	1.939	0.306	0.308	-0.004	458.6 (319.8 - 781.8)
Naselle River	Washington Coast	100	1.946	0.312	0.314	-0.009	1869.5 (668 - inf.)
Naselle WDFW Hatchery	Washington Coast	99	1.939	0.313	0.316	-0.012	818.2 (451.6 - 3616.8)
Nemah River	Washington Coast	32	1.905	0.316	0.323	-0.023	inf. (694.1 - inf.)
Nemah WDFW Hatchery	Washington Coast	100	1.953	0.311	0.315	-0.012	252.4 (197.3 - 343.8)
North River	Washington Coast	28	1.905	0.303	0.301	0.007	29.2 (25 - 34.6)
Quinault NFH	Washington Coast	30	1.926	0.317	0.313	0.013	551.0 (268.8 - 123155.4)
Quinault_hat_fall	Washington Coast	29	1.919	0.318	0.315	0.009	241.9 (160.9 - 462.9)
Sol Duc (Quillayute) Hat_su	Washington Coast	22	1.926	0.327	0.328	-0.003	1313.6 (303.2 - inf.)

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