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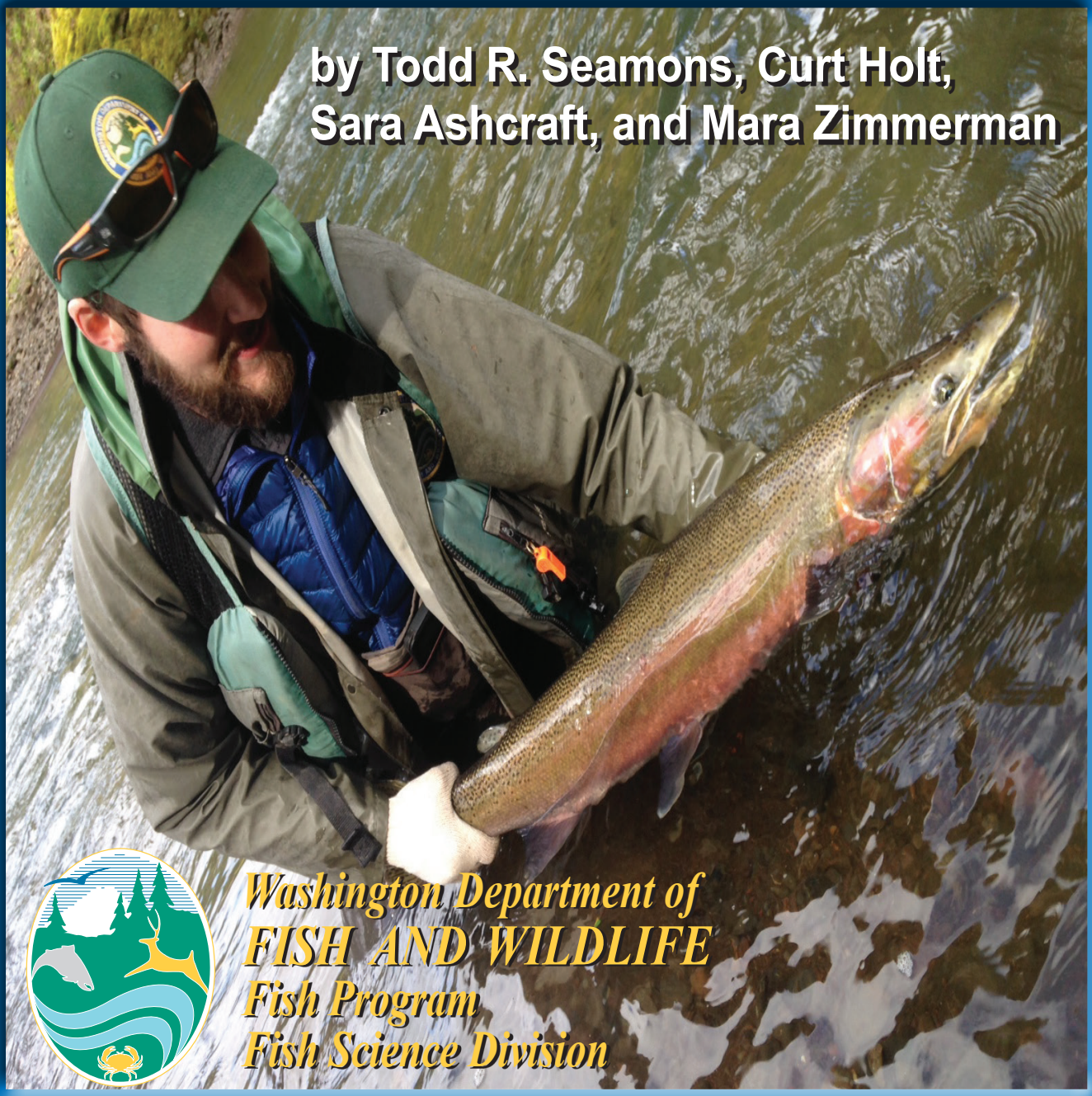
Population Genetic Analysis of Chehalis River Watershed Winter Steelhead (Oncorhynchus mykiss)



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Washington Department of
FISH AND WILDLIFE
Fish Program
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Population genetic analysis of Chehalis River watershed winter steelhead (*Oncorhynchus mykiss*)

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Cover photo: Zach Malham with a Chehalis winter steelhead. Photo taken by Kevin Beerbower.

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Executive summary

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. Current predictive models (Ecosystem Diagnostic Treatment, NOAA Watershed Assessment) partition species into geospatial units that have an unknown relationship to actual population structure. Here, we examined the genetic population structure of wild (natural-origin) winter-run steelhead (*O. mykiss*) in the Chehalis River basin. Specifically, our objectives were to determine the genetic relationship of Chehalis River steelhead with other *O. mykiss* in Washington State and to examine the genetic structure of *O. mykiss* within the Chehalis River basin.

Genetic data revealed that Chehalis Basin *O. mykiss* are of the same Coastal lineage as nearby baseline collections. Similar to the previous study, Chehalis *O. mykiss* were found to be more closely related to *O. mykiss* in nearby watersheds (Willapa River, Quinault River) than to more geographically distant populations. Within the Chehalis Basin, Chehalis steelhead were structured hierarchically. At the least inclusive hierarchical level, *O. mykiss* populations in the Chehalis were structured by spawning tributary, and this structure was temporally stable. At the most inclusive hierarchical level, spawning tributaries were clustered by headwater geography. The lower Chehalis group was represented by the collections from the Humptulips River, Wishkah River, Wynoochee River, and Satsop River and drains the Olympic Mountain Range. The middle Chehalis group was represented by the collections from the Skookumchuck and Newaukum rivers and drains the Cascade Mountain Range. The upper Chehalis group, represented by the South Fork and upper Chehalis collections, drains the Willapa Hills. Similar population structure among major spawning tributaries has been seen in other genetic studies of *O. mykiss* in Washington, including in the nearby Cowlitz River and in Canada and California.

Genetic diversity of lower Chehalis River *O. mykiss* was similar to what is typical for other similar-sized wild *O. mykiss* populations in Washington. The upper Chehalis River populations had slightly lowered diversity, possibly due to impacts from a hatchery program. The Skookumchuck River collections had the reduced diversity typically seen in WDFWs hatchery rainbow trout and steelhead populations. Reduced diversity is an indication of a small population size, however the number of steelhead returning to the Skookumchuck is comparable to other tributaries of the Chehalis River, suggesting the reduced diversity may be due to a population bottleneck, likely due to hatchery propagation. The Newaukum collection significantly deviated from Hardy-Weinberg expectations (HWE). The deviation was not due to excessive sampling of family members, which can cause deviations of this type. Instead, the Newaukum collection appeared to be composed of members of several different populations, which also can cause deviations from HWE (i.e., a Wahlund effect). Skookumchuck hatchery steelhead are released in the Newaukum River, but given the hierarchical genetic relationships in the Chehalis Basin, it is impossible to distinguish a Newaukum genetic signal from planted Skookumchuck hatchery steelhead. Additional replicate collections in future years will be required to accurately characterize a Newaukum *O. mykiss* population.

This study represents a comprehensive survey and genetic analysis of Chehalis River *O. mykiss* populations, but some minor holes still exist. The Hoquiam River, Cloquallum Creek, and Black River may also have steelhead populations, but were not sampled. Additional sampling and analysis would shed light on their genetic relationships with other Chehalis River *O. mykiss*. In particular, the Black River may be interesting given its location in the transition zone from Olympic Mountains to Cascade Mountains headwaters. Samples from the Wynoochee and Skookumchuck rivers were collected downstream of dams. In the Wynoochee River, steelhead are transported upstream of the dam, but the Skookumchuck Dam has no fish passage. Samples of rainbow trout from upstream of the dam may provide additional insights into the genetic diversity issues of the Skookumchuck River collections.

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). Habitat conditions for salmon and steelhead in the Chehalis River are projected to change substantially over the next several decades. Habitat may be lost due to the construction of a flood reduction dam planned at river mile 108 and due to increased stream temperatures as predicted by climate change models. 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.

Current models (Ecosystem Diagnostic Treatment, NOAA Watershed Assessment) that predict salmon and steelhead responses to habitat changes in the Chehalis River basin partition species into geospatial units that have an unknown relationship to biological (i.e., population) structure. In reality, fish populations are defined by the exchange (or lack thereof) of genes over space and time and could encompass either multiple or a sub-portion of the geospatial units currently included in the modelling efforts. Long-term numerical responses of salmon and steelhead in response to habitat change may differ if the populations have limited versus extensive genetic exchange among areas. If future habitat is depleted, overall numbers of fish in the basin may be less resilient over time if populations in the depleted area(s) 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.

This report is focused on the population genetic structure of wild (natural-origin) winter-run steelhead (*O. mykiss*) in the Chehalis River basin. Previous genetic analyses in which Chehalis River *O. mykiss* populations were included were done using markers with limited power (allozymes), did not include all known or suspected spawning populations in the Chehalis Basin, and did not include temporally replicate collections to evaluate temporal stability (Phelps et al. 1997). Here we examine genetic population structure of *O. mykiss* in the Chehalis Basin using a large panel of single nucleotide polymorphic (SNP) loci, a comprehensive set of collections from spawning populations in the Chehalis Basin, and with most sampled in two separate years. Specifically, we will 1) determine the genetic relationship of Chehalis Basin *O. mykiss* to extant Washington *O. mykiss* genetic lineages; 2) determine the genetic relationship of Chehalis River *O. mykiss* to the surrounding coastwide region *O. mykiss* populations; and 3) determine the genetic population structure of *O. mykiss* among the sub-basins of the Chehalis River.

Objectives

1. Evaluate the genetic population structure of naturally-produced *O. mykiss* within the Chehalis River basin.
2. Evaluate the genetic relationship of Chehalis River *O. mykiss* to *O. mykiss* throughout Washington State.

Methods

Study site

The Chehalis River is a large (6,889 km^2) watershed with multiple sub-basins that drain from three mountain ranges (Willapa Hills, foothills of the Cascade Mountains, foothills of the Olympic Mountains; Figure 1). Winter-run steelhead spawn throughout the watershed in small and medium sized rivers (< 55 m channel width) but are not observed to spawn in the mainstem of the Chehalis River downstream of the confluence with the Newaukum River (river mile [RM] 78). Over the past decade, spawner abundance of winter steelhead throughout the Chehalis River basin has averaged 7,900 (6,200 – 10,600), which is below the escapement goal of 8,600 spawners (M. Scharpf, WDFW, personal communication). No summer-run population is known to exist.

Hatchery *O. mykiss* have been produced and released within the Chehalis Basin historically and currently. Current steelhead hatchery programs include Washington-native, non-Chehalis early summer run (EHS – Skamania-Washougal River stock) in the Humptulips and Wynoochee rivers, Washington-native, non-Chehalis early winter-run (EHW – Chambers stock) in the Humptulips River, and locally-derived "late" winter-run in the Wynoochee River (Wynoochee native), Satsop River (Satsop native), Skookumchuck and Newaukum (Skookumchuck native), and upper Chehalis rivers (upper Chehalis native [Eight Creek]). Hatchery produced rainbow trout (California ancestry, CAHT) of one or more of three strains (Goldendale, Mt. Whitney, and Spokane) are currently released into Chehalis Basin ponds and lakes, which may have outlets into the Chehalis River, but no hatchery rainbow trout are currently released directly into the Chehalis River or its tributaries.

Three major genetic lineages of *O. mykiss* are currently present in Washington: native Coastal rainbow trout (*O. m. irideus*), native Columbia River redband (CRR, *O. m. gairdneri*), and non-native California ancestry hatchery rainbow trout (possibly Coastal or McCloud River redband; CAHT). In Washington State, Coastal and CRR *O. mykiss* exhibit both anadromous (steelhead) and freshwater-resident (rainbow trout) life histories, but CAHT are only freshwater-resident. To evaluate the genetic relationships of Chehalis River *O. mykiss* with other *O. mykiss* in Washington State, representative collections from each of the major lineages were chosen from the statewide baseline for some analyses. Native hatchery *O. mykiss* in Washington State generally fall into one of two ancestral groups: native early winter steelhead (i.e., Chambers Creek stock, Puget Sound origin), and native early summer steelhead (i.e., Skamania stock, Washougal River, lower Columbia River origin). Because these stocks have been outplanted widely throughout the state, including currently within the Chehalis River basin, representative collections from each of these hatchery groups were included for further population genetic analyses. Despite recent reproductive isolation among hatchery programs of the same stock, programs remain genetically closely related (Washington Department of Fish and Wildlife Molecular Genetics Laboratory [WDFW MGL], unpublished data). Thus, we assume collections from programs outside of the Chehalis River basin adequately represented related programs within the Chehalis River. Several native rainbow trout hatchery programs also exist in Washington, but fish from these programs are not released within the Chehalis Basin.

Chehalis *O. mykiss* tissue collections

Fin tissue was collected from live adult or juvenile *O. mykiss* throughout the Chehalis River watershed in 2015 and 2016. Based on previously published and unpublished *O. mykiss* 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 downstream of Pe Ell, Washington. Those tributaries were the Humptulips River, Wishkah River, Wynoochee River, Satsop River, Skookumchuck River, Newaukum River, South Fork Chehalis River (SF Chehalis), and the upper Chehalis River (Figure 1).

In each tributary location, adult steelhead or occasionally resident rainbow trout were captured by angling. In four locations, Wishkah River, Newaukum River, SF Chehalis, and the upper Chehalis (2016), extensive efforts at capturing adult steelhead failed to produce an adequate sample size for genetic analysis, so the collections were augmented by capturing juvenile *O. mykiss* by electrofishing. When electrofishing was used, sampling efforts were spread out in space as much as possible, given restricted access to the river, in order to reduce the chances of oversampling full-sibling families. In order to evaluate temporal stability of any observed genetic relationships, separate collections were taken in two separate years from almost all locations.

From each captured fish, biological data including origin (hatchery or wild), sex (if possible), and fork length were obtained. 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. Fish were released alive back into the location from where they were captured. Fin clips in ethanol were accessioned to the WDFW Molecular Genetics Laboratory archive and stored at room temperature.

Non-Chehalis statewide collections

Biological data and fin tissue were taken from natural-origin populations statewide under the same assumptions of spatial distribution of populations into spawning tributaries using the same collection methods described for the Chehalis River steelhead. For hatchery population collections, fin tissue and biological data were taken from broodstock, usually during spawning. Non-Chehalis collections included in the analysis were chosen from existing sample collections, with available genetic data, within the major genetic lineages.

Genetics laboratory processing

Chehalis River samples were genotyped at the WDFW statewide steelhead panel of 269 SNPs (SW269 SNPs; Table 1) using a cost effective method based on custom amplicon sequencing called Genotyping in Thousands (GTseq; Campbell et al. 2015). Non-Chehalis statewide samples were previously genotyped using a TaqMan assay-based method implemented on a Fluidigm platform at 192 SNPs (panel E/F). The two panels overlap at 183 SNPs (Table 1). Analyses of Chehalis River collections alone were analyzed using SW269 SNPs and the subset of overlapping loci, and when Chehalis River collections were mixed with non-Chehalis statewide samples, only the subset of overlapping loci were used.

Included in both panels are three SNP loci developed to distinguish cutthroat trout (*O. clarkii*) from steelhead and rainbow trout (Table 1). Cutthroat were identified by having at least one cutthroat allele at all three species ID loci. Cutthroat x *O. mykiss* hybrids were identified by having both cutthroat and *O. mykiss* alleles at two or three loci. Any cutthroat or hybrid was removed from further analysis.

SW269 genotyping

The SW269 SNP panel included 265 SNP loci developed to be used for population structure, parentage assignment, or other population genetic studies of *O. mykiss* (Table 1), the three SNPs that distinguish cutthroat trout from steelhead and rainbow trout, and one sex-linked locus that allowed genetic determination of sex.

To extract and isolate genomic DNA from tissue, 30uL of 10% Chelex (Sigman Aldrich, C7901) and 5uL of Proteinase K solution (Qiagen, 1018332) were added to fin tissue and incubated overnight at 55°C. 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, Tables 3 and 4, 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 SequalPrepTM 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) and QubitTMdsDNA 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 (available online at https://github.com/GTseq/GTseq-Pipeline; Campbell et al. 2015). Essentially, there are a series of custom PERL scripts that ultimately create individual fastq files and genotype files for every individual that can be compiled for further analysis. Allele calling (nucleotide identification) is performed by

counting amplicon-specific sequences for each allele, and allele ratios are used to determine the genotypes.

Panel E/F genotyping

Panel E/F included 189 SNP loci developed to be used for population structure, parentage assignment, or other population genetic studies of *O. mykiss* (Table 1) and three SNP loci developed to distinguish cutthroat trout from steelhead and rainbow trout.

To extract and isolate DNA from fin tissue, Qiagen DNEasy ® kits (Qiagen Inc., Valencia, CA) were used, following the recommended protocol for animal tissues. SNP genotypes were obtained through PCR and visualization on Fluidigm EP1 integrated fluidic circuits (chips). Protocols followed Fluidigm's recommendations for TaqMan SNP assays as follows: Samples were pre-amplified by Specific Target Amplification (STA) following Fluidigm's recommended protocol with one modification. The 192 assays were pooled to a concentration of 0.2X and mixed with 2X Qiagen Multiplexing Kit (Qiagen, Inc., Valencia CA), instead of TaqMan PreAmp Master Mix (Applied Biosystems), to a volume of 3.75µl, to which 1.25µl of unquantified sample DNA was added for a total reaction volume of 5µl. Pre-amp PCR was conducted on a MJ Research or Applied Biosystems themal cycler using the following profile: 95°C for 15 min followed by 14 cycles of 95°C for 15 sec and 60°C for 4 minutes. Post-PCR reactions were diluted with 20µl dH2O to a final volume of 25µl.

Specific SNP locus PCRs were conducted on the Fluidigm chips. Assay loading mixture contained 1X Assay Loading Reagent (Fluidigm), 2.5X ROX Reference Dye (Invetrogen) and 10X custom TaqMan Assay (Applied Biosystems); sample loading mixture contains 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 0.05X AmpliTaq Gold DNA polymerase (Applied Biosystems), 1X GT sampling loading reagent (Fluidigm) and 2.1 µL template DNA. Four µL assay loading mix and 5 µL sample loading mix were pipetted onto the chip and loaded by the IFC loader (Fluidigm). PCR was conducted on a Fluidigm thermal cycler using a two-step profile. Initial mix thermal profile was 70°C for 30min, 25°C for 5 min, 52.3° for 10 sec, 50.1°C for 1 min 50sec, 98°C for 5 sec, 96°C for 9 min 55 sec, 96°C for 15 sec, 58.6°C for 8 sec, and 60.1°C for 43 sec. Amplification thermal profile was 40 cycles of 58.6°C for 10 sec, 96°C for 5 sec, 58.6°C for 8 sec and 60.1°C for 43 sec with a final hold at 20°C.

The SNP assays were visualized on the Fluidigm EP1 machine using the BioMark data collection software and analyzed using Fluidigm SNP genotyping analysis software. To ensure all SNP markers were being scored accurately and consistently, all data were scored by two technicians and scores of each technician were compared. Disputed scores were called missing data (i.e., no genotype).

Evaluation of loci/diversity metrics

To evaluate genetic qualities of loci, we quantified several genetic parameters in the Chehalis River steelhead collections. We performed a two-tailed exact test of Hardy–Weinberg equilibrium (HWE) for each locus in each collection using the Markov Chain method and performed pairwise probability tests for gametic disequilibrium (LD) for each pair of loci in each collection as implemented in GENEPOP v4.2 (dememorization number 1000, batches 100, 1000 iterations per batch; Raymond and Rousset 1995; Rousset 2008). Significance of probability values was adjusted for multiple tests using false discovery rate (Verhoeven et al. 2005). F_{IS}, a measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation and an additional indicator of systematic issues, was calculated according to Weir and Cockerham (1984) using GENEPOP 4.2. These statistical relationships

test how well a collection of genotypes (i.e., a population) conforms to expected values for an "ideal" population, which is a theoretical construct of population genetics. Deviations from expectations may indicate genotyping problems, but may also reveal other important processes or characteristics of the sampled population. F_{IS} values significantly greater or less than zero are an additional indicator of deviations from HWE expectations. Deviations from HWE could be caused by the presence of large numbers of relatives (mainly full-siblings) in a collection. If statistically significant deviations from HWE were observed, collections were evaluated for the presence of full-sibling families by performing sibship analysis using the algorithms of the software COLONY (v.2.0.6.3; Wang 2013; Wang and Santure 2009). If full-sibling families with more than three members were discovered in a collection, randomly drawn members of those full-sibling families were removed from further analysis until only three members of any full-sibling family remained. HWE and LD tests and diversity statistics were then recalculated and reported.

General genetic diversity metrics (e.g., number of alleles and observed and expected heterozygosity) were calculated and summarized for each collection, including Chehalis and non-Chehalis collections, using GDA (Lewis and Zaykin 2001). Effective population size (N_e) was calculated using the linkage disequilibrium methods employed in the software NEESTIMATOR (Do et al. 2014). N_e is an important indicator of the genetic health of a population and can be interpreted as the size of an "ideal" population with the genetic characteristics of the sampled population.

Population genetic analysis

Population structure of *O. mykiss* has often been shown to be hierarchical (e.g., Beacham et al. 1999; Garza et al. 2014; Heath et al. 2001), that is, genetic structure of salmonid populations exists at several hierarchical levels typically defined geographically. Thus, our approach to evaluating and interpreting the population structure of Chehalis Basin *O. mykiss* populations was hierarchical. First, Chehalis Basin *O. mykiss* collections were compared to collections from all known extant major genetic lineages. Second, Chehalis Basin *O. mykiss* were compared to representative collections of the major genetic lineage to which they belonged. Finally, population structure among Chehalis Basin *O. mykiss* populations was evaluated.

Population genetic analyses of Chehalis River *O. mykiss* was first examined through principal components analysis (PCA). PCA is conducted with individual level data and provides preliminary structure information and potentially identifies individuals with radically different genotypes. Potential sources of genotypic differences could be large amounts of missing genotype data, genotyping errors, or different genetic ancestry.

After PCA analysis, population structure of Chehalis River *O. mykiss* was evaluated at two levels, first, as with PCA analysis, by using individual data without considering collection membership (clustering analysis) and second, by analyzing data based on collection membership. Clustering analysis was conducted using two models that cluster individuals based on their genotypes. First, we used discriminant analysis of principal components (DAPC) as implemented in the R package *adegenet* (Jombart 2008; Jombart et al. 2010; R Development Core Team 2017). DAPC uses multivariate discrimination to cluster individuals based on their genotype without an underlying population genetic model. 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). The optimal K value was visually examined to select the K after which further BIC values decreased only subtly (Jombart et al. 2010).

Second, we used the algorithms employed in the software STRUCTURE (Pritchard et al. 2000). STRUCTURE uses Bayesian algorithms to cluster individuals into groupings employing an underlying population genetic model which adjusts group membership to minimize deviations from HWE. We performed 10 iterations of K = 2 – 13, with 100,000 MCMC iterations and a 10,000 iterate burin-in period. The K (number of populations) with the most statistical support was chosen using the ΔK method of Evanno et al. (2005), and by examining the patterns of the negative ln Pr(X|K) vs. K, as plotted by the web-based software, STRUCTURE HARVESTER (Earl and vonHoldt 2012). Multiple iterations for each K analyzed were concatenated using CLUMPP (Jakobsson and Rosenberg 2007), using default parameters. STRUCTURE plots were produced with DISTRUCT (Rosenberg 2004).

Population structure of collections was evaluated by estimating pairwise F_{ST} estimates among collections. F_{ST} is a commonly used metric that estimates subpopulation differentiation. F_{ST} estimates were calculated and statistical significance was estimated by permutation tests using FSTAT (Goudet 1995) with 1000 permutations. As another measure of population structure, we calculated a pairwise genetic distance matrix among all collections of Cavalli-Sforza chord genetic distances using PHYLIP (Felsenstein 1993). These genetic relationships were visualized using a neighbor joining dendrogram calculated using the program PHYLIP. No available genetic distance model captures all reasonable assumptions of our data and the biology of *O. mykiss*. Cavalli-Sforza chord genetic distances assume that divergence is entirely due to genetic drift, i.e., no mutation, which is plausible. Bootstrap support for the topology of the estimated dendrogram was estimated by boostrapping across loci 10,000 times using PHYLIP. Analysis using collection membership information assumes that the tissue collection represents a population.

It is not uncommon for *O. mykiss* populations to show evidence of isolation by distance (e.g., Garza et al. 2014; Heath et al. 2001), i.e., geographically proximate populations are more closely related than geographically disparate populations. In order to test the hypothesis of isolation by distance among collections from within the Chehalis Basin, correlation of F_{ST}s and geographic distance was tested using Mantel's test as implemented in GENEPOP using the *Isolde* option. Since collections represented major tributaries of the Chehalis River, geographic distance between pairs of collections was calculated as miles between tributary river mouths. Geographic distance was not log transformed. Genetic distance was calculated as $F_{ST}/(1-F_{ST})$. Because temporal replicates have no geographic distance, only the 2016 collections from locations that had temporal replicate collections were used. The Skookumchuck collections genetically were very different from all other Chehalis Basin collections (see Results), which may skew the relationship. Therefore, the Mantel's test was run with and without the Skookumchuck collection.

Results

Tissue collections

A total of 577 samples were collected and processed from unmarked wild *O. mykiss* in Chehalis River tributaries; all tissues taken from marked hatchery-produced *O. mykiss* were omitted from further analysis. Most tissues were taken from adults during their spawning season. Collections from four locations were augmented with juvenile samples taken by electrofishing (Table 2). Juvenile tissue samples were the minority in three of four collections (Newaukum, Wishkah, and upper Chehalis [2016]). Of 51 samples taken in the South Fork Chehalis River, 50 were from electrofished juveniles. Six

tissue samples were collected in 2014 from the upper Chehalis River. These six were pooled with tissues taken in 2015 for all further analyses.

An additional 953 samples from 20 baseline collections taken from wild or hatchery-produced steelhead or rainbow trout populations statewide were added to the genetic dataset (Table 2). These samples represented all three known major *O. mykiss* genetic lineages extant in Washington State, six of seven Distinct Population Segments (DPS; Puget Sound [PS], Olympic Peninsula [OP], Southwest Washington [SWWA], Lower Columbia [LC], Mid Columbia [MC], and Upper Columbia [UC]), both winter and summer life histories, and the commonly propagated hatchery trout and steelhead stocks.

Genotyping success at the SW269 panel of SNPs was very high for almost all Chehalis River collections (Table 2). Collections with lower genotyping success were those that included tissues from juveniles, which, because of lower DNA concentrations, had a higher failure rate using the GTseq method. Sufficient numbers of samples were genotyped for all collections except the SF Chehalis collection. The SF Chehalis collection was re-genotyped at panel E/F SNP markers using the methods used for baseline collections, which was highly successful.

Evaluation of loci/within-collection diversity

Five loci were removed from further analysis of the SW269 SNP panel due to poor amplification of samples from all Chehalis River collections. No other systematic scoring issues were identified. An additional eight loci were removed from further analysis using the panel E/F subset due to missing genotypes in baseline collections. Two fish were genetically identified as *mykiss*/*clarkii* hybrids (one each from Wynoochee - 16BT and Skookumchuck - 16BZ) and one fish was identified as a cutthroat (Upper Chehalis - 14TK). All three were removed from further analysis. Correlations of genetic diversity metrics and statistics between the full SW269 SNP panel and the subset panel E/F was very high (Pearson's $r > 0.89$ for all comparisons), so for ease of reporting and to facilitate comparisons to the baseline collections, results from analysis using the full SW269 SNP panel are minimally included and results using the panel E/F subset are emphasized.

Two loci nearly had private alleles at the level of lineage. The "A" allele of locus A*Omy*094 was found in both CRR collections and only at very low frequency in Coastal lineage Deer Creek (Stillaguamish) summer steelhead, and the "T" allele of locus A*Omy*271 was found in both CAHT collections and only at low frequency in the CRR Naches River collection. No private alleles existed in any one collection, and no alleles at any locus were found only in Chehalis River collections, i.e., there were no markers diagnostic of Chehalis River *O. mykiss*.

Allelic richness was lower and the proportion of loci fixed with one allele was higher in CAHT collections than in Coastal or CRR collections (Table 3). The same diversity metrics calculated in Chehalis River collections were slightly lower than those of baseline Coastal wild steelhead populations and were closer to those seen in hatchery steelhead collections (Table 3). However, genetic diversity is correlated with effective population size such that smaller populations tend to have lower genetic diversity. The average estimated *N*e for Chehalis collections was slightly lower than those of most baseline wild steelhead populations, but more than twice that of hatchery steelhead populations. Average expected (He) and observed (Ho) heterozygosity of Chehalis collections was slightly lower than that of hatchery and wild steelhead baseline collections. However, this was mainly due to the very low values observed in the Skookumchuck River collections, which were similar to those of the CAHT and CRR collections;

average He and Ho of Chehalis collections without Skookumchuck River collections were comparable to wild and hatchery steelhead baseline collections.

Baseline hatchery steelhead collections generally had more loci out of HWE before and after correction for multiple tests than did baseline wild steelhead collections (Table 3). With the exception of the Newaukum River collection, Chehalis River collections had rates of loci with HWE and LD issues before and after correction for multiple tests comparable to baseline wild steelhead collections. The Newaukum collection stood out for having a large number of loci out of HWE and showing linkage disequlibrium (Table 3). A subsequent sibship analysis failed to discover a significant number of siblings present in the collection. Because HWE and LD issues may arise for reasons other than the presence of siblings in the collection, the Newaukum collection was retained for additional analyses, which could provide insight into the issue.

Genetic population structure

Statewide – PCA analysis including all baseline collections revealed that Chehalis River *O. mykiss* clustered, as expected, with other Coastal lineage collections (Figure 2). It also revealed four Chehalis River samples as CAHT (one fish from the Humptulips River and three from the Newaukum River), which were likely escapees from stocked ponds or lakes. These four samples were removed from further analysis. Separation of the three genetic lineages and ancestry of Chehalis River *O. mykiss* as Coastal lineage was supported by large genetic distances between lineages and strong bootstrap support of the resulting dendrogram (Figure 3). This strong separation was also supported by large, statistically significant F_{ST} estimates (Table 4). The average F_{ST} of CAHT collections paired with Coastal collections = 0.297 and paired with CRR = 0.302. The average F_{ST} of CRR paired with Coastal collections was about half that of any pairing with CAHT, but still very large (average F_{ST} = 0.167).

Coastal – PCA analysis of Coastal lineage collections revealed some structuring, but with significant overlap, especially among Washington Coast and Puget Sound collections (Figure 4). Chehalis *O. mykiss* overlapped *O. mykiss* from the Washington Coast more than with those from Puget Sound. Although eight clusters were inferred using DAPC, considerable overlap remained among geographically proximate collections and among inferred clusters (Figure 4, bottom and Figure 5). Some clusters were clearly defined by geography (i.e., Lower Columbia Early Hatchery Summers, Puget Sound [three clusters], Skookumchuck). The remaining three clusters were spread among coastal Washington collections without strong correlation with geography (Figure 5). With the exception of the Skookumchuck collections, Chehalis *O. mykiss* samples were inferred as members of all three coastal Washington clusters.

Using STRUCTURE, statistical support was found for K = 5 using ΔK method of Evanno et al. (2005) and for K = 10 using -ln Pr(X|K). At K = 5, Chehalis *O. mykiss*, with the exception of Skookumchuck collections, clustered with other Washington Coast collections; Skookumchuck, Lower Columbia EHS, and Puget Sound samples defined the remaining clusters (Figure 6). At K = 10, the Puget Sound cluster was split into roughly three clusters and the Chehalis collections were split among four roughly defined clusters: upper/SF Chehalis, Skookumchuck/Newaukum, Wynoochee/Satsop, and Wishkah/Humptulips (Figure 6). The Newaukum collection appeared to be a mixed collection of Skookumchuck and upper Chehalis individuals. The Skookumchuck collections (blue) formed a separate very distinct cluster no matter the makeup of the rest of the analyzed collections for almost all values of K tested (data not shown), including K = 5 and K = 10.

Population structure of coastal *O. mykiss* collections and differentiation of the Chehalis River collections from other baseline collections was supported by Cavalli-Sforza genetic distances and the resulting dendrogram (Figure 7). With the exception of the Abernathy Creek collection, collections clustered with other members of their DPS. Strong bootstrap support was found for the nodes separating the Willapa River and Chehalis River collections from all other collections (95.0%), the Olympic Peninsula from all other collections (85.6%), and the Lower Columbia River ancestry EHS collections from all other collections (100%). Moderate to strong bootstrap support was evident for the nodes separating the Willapa River collections from Chehalis River collections (72.8% - lower Chehalis from Willapa, 81.9% - upper and middle Chehalis from Willapa).

These same general patterns of Coastal lineage population structure were supported by patterns in F_{ST} estimates (Table 4). The two EHS-Skamania (Lower Columbia hatchery summers) were more differentiated from other Coastal lineage collections (average F_{ST} = 0.061) than were other Coastal collections from among themselves (average F_{ST} = 0.035). Similarly, Skookumchuck collections were strongly differentiated from all Coastal collections (average F_{ST} = 0.051), including other Chehalis collections (average F_{ST} = 0.050). The average F_{ST} estimates of Skookumchuck pairings were nearly as large as those for the EHS-Skamania collections paired with other Coastal collections. Chehalis collections were less differentiated from other Chehalis collections (average F_{ST} = 0.027) than from Puget Sound collections (average F_{ST} = 0.054), but roughly the same as when paired with other SWWA collections (average F_{ST} = 0.022) or with OP collections (average F_{ST} = 0.022).

Chehalis Basin – Chehalis Basin *O. mykiss* collections appeared to be structured by spawning tributaries, which were clustered by their location in the Chehalis Basin, a result supported by clustering analysis (PCA, Figure 8; DAPC, Figure 9; STRUCTURE, Figure 10) and analysis based on collection membership (Figure 11). K = 3 was supported by ΔK analysis of STRUCTURE results and DAPC (BIC). Support was also found for other numbers of clusters. Mean ln Pr(X|K) of STRUCTURE results supported both K = 4 and K = 6. In DAPC analysis, BIC support was only slightly lower for K = 4 than for K = 3. At K = 3, individuals were clustered by headwater geography: lower Chehalis consisting of the Satsop, Wynoochee, Wishkah, and Humptulips rivers; middle Chehalis consisting of the Skookumchuck and Newaukum rivers; and upper Chehalis consisting of the South Fork Chehalis and upper Chehalis River. At K = 4, the lower Chehalis collections were split into two groups: the Humptulips/Wishkah and Wynoochee/Satsop. At K = 6, the Wynoochee and Satsop were separate. All other cluster membership stayed the same. Within-Chehalis Basin population structure was temporally stable as evidenced by clustering of temporal samples, and overall structure of the dendrogram, including the temporal replicates, was strongly supported by the data demonstrated by large bootstrap support at nearly all nodes.

Using the full SW269 SNP panel, the population structure revealed was essentially the same as the structure revealed with the Panel E/F subset (data not shown). With clustering analysis with STRUCTURE, K = 5 was supported by both ΔK and mean ln Pr(X|K). Cluster membership at K = 5 was similar to K = 6 results using Panel E/F, with the main difference being that the SF Chehalis clustered with Satsop collections rather than the upper Chehalis collections. The five clusters were Humptulips/Wishkah, Wynoochee, Satsop/SF Chehalis, Skookumchuck/Newaukum, and upper Chehalis. The topology of the dendrogram produced with the full SW269 panel of SNPs was identical to that using Panel E/F, except for the position of the SF Chehalis collection, which had only 8 samples so caution in interpreting this relationship is prudent. Bootstrap support for the nodes was also similar to that using Panel E/F, except for the branch with both Satsop collections, which had much weaker support. The lower bootstrap

support for that node corresponded with a stronger signal of lower Chehalis in the 2016 Satsop collection with STRUCTURE analysis and a lower pairwise F_{ST} values of the 2016 Satsop collection with other lower Chehalis collections.

Some evidence of isolation by distance among Chehalis River *O. mykiss* populations was evident (Figure 12A), however the relationship was marginally non-significant ($P = 0.094$). Removal of the pairings with the Skookumchuck collection reduced the variance (improved predictability; $R^2 = 0.08$ vs $R^2 = 0.34$), but the relationship was marginally non-significant ($P = 0.062$; Figure 12B).

Discussion

We evaluated the genetic diversity and population structure of Chehalis River *O. mykiss* and compared them to extant Washington State wild and hatchery steelhead and hatchery rainbow trout representing three distinct genetic lineages: Coastal (*O. mykiss irideus*), Columbia River Redband (*O. mykiss gairdneri*), and California ancestry hatchery trout (likely Sacramento Redband [*O. mykiss stonei*](Crawford 1979)) (Blankenship et al. 2011). As expected, Chehalis River *O. mykiss* genetically are a part of the Coastal lineage (Behnke 1992). Among Coastal lineage populations evaluated, Chehalis River *O. mykiss* were genetically more closely related to geographically proximate populations, i.e., Willapa River and Olympic Peninsula rivers, than to more distantly located populations, i.e., Puget Sound or Lower Columbia. In their survey of *O. mykiss* from throughout Washington State, which included collections from the Humptulips, Wynoochee, Satsop, and upper Chehalis rivers, Phelps et al. (1997) found similar patterns with allozyme data. In their dendrogram based on Cavalli-Sforza genetic distances (their Figure 1A), these Chehalis River *O. mykiss* collections clustered with Willapa River basin collections on branch "H". The next nearest cluster, cluster "G", included Quinault River collections. This population structure was well supported by individual (clustering) and collection level analysis. Although several different values of K (the number of clusters) were supported in clustering analysis using two models (K = 5, 8, 10, Figure 5, Figure 6), Willapa River and Olympic Peninsula collections were clustered with all or lower Chehalis River collections at all supported values of K. Compared to K = 5, higher values of K split Puget Sound collections into separate groups or separated middle and upper Chehalis collections from lower Chehalis collections, but Willapa River and Olympic Peninsula collections remained clustered with Chehalis collections. The relationships among collections as estimated by genetic distances roughly mirrored the clusters found with clustering analysis with very strong statistical support for the topology of the dendrogram.

In addition to the clustering analysis, estimated pairwise F_{ST} values for pairs including Chehalis collections were lower for comparisons involving nearby collections than those involving distant collections. This is consistent with a pattern of isolation by distance, which has been found in several studies of anadromous *O. mykiss* throughout their range in Canada and the U.S. (Garza et al. 2014; Heath et al. 2001). Within the Chehalis Basin, evidence for isolation by distance was suggestive, but not conclusive. In particular, the Skookumchuck River collections were genetically much more distant from other collections than their geographic distance would predict based on the relationship among all other collections. This exaggerated genetic distance of the Skookumchuck River population is likely due to effect of the hatchery program (discussed below).

Within the Chehalis River basin, *O. mykiss* populations were structured by spawning tributaries, which in turn were structured correlated with headwater geography: Humptulips, Wishkah, Wynoochee, and

Satsop in the lower Chehalis river draining the Olympic Range, Skookumchuck and Newaukum in the middle Chehalis river, which drain the Cascade Range, and the SF Chehalis and upper Chehalis in the upper Chehalis river, which drain the Willapa Hills. The same pattern of structure correlated with geography has also been observed in other rivers in Washington State (Blankenship et al. 2011; Small et al. 2010; Small et al. 2007; Winans et al. 2017) and elsewhere in the North American range of *O. mykiss* (Beacham et al. 2004; Beacham et al. 1999; Beacham et al. 2012; Garza et al. 2014; Heath et al. 2001; Heggenes et al. 2011). Support was strong for the inferred Chehalis *O. mykiss* population structure; the population structure was temporally stable and individual and collection level analyses supported the same pattern of structure (e.g., Figure 11). With individual analysis, multiple cluster numbers were statistically supported (K = 3 or 4, DAPC; K = 3, 4, or 6, STRUCTURE). Multiple cluster numbers were supported and presented for several reasons. The strength of support for a particular cluster number depends on the amount of genetic differentiation. In addition, the statistics used to support inferred K values are known to detect different levels of differentiation when genetic structure is hierarchical. For example, the ΔK method of Evanno et al. (2005) is known to support the most inclusive hierarchical level of population structure – among Chehalis River collections K = 3, or clustering by headwater geography. Other supported cluster numbers further subdivided collections by spawning tributaries.

Average genetic diversity of Chehalis River populations was slightly lower than that of most other baseline wild Coastal lineage populations and more representative of hatchery steelhead collections, especially middle and upper Chehalis populations. (The Skookumchuck River collections in particular were very low, but are discussed separately below.) Baseline populations of low abundance (e.g., Snow Creek) have similar levels of diversity, so the lower diversity may reflect reduced diversity due to small population size. However, contemporary 5-year average abundance estimates in the SF and upper Chehalis are 213 and 1,132 (respectively; M. Scharpf, WDFW, personal communication), which are reasonably large. It is also possible that the reduced diversity is a consequence of a population bottleneck. Effective population sizes of the SF and upper Chehalis *O. mykiss* populations are similar to those of populations in the lower Chehalis, suggesting that a recent bottleneck has not occurred. The observed lower diversity in the upper Chehalis *O. mykiss* could be due to past effects of the Eight Creek hatchery program (Figure 1). Since 2008, this program has been releasing Skookumchuck Hatchery steelhead, but prior to 2008 broodstock were obtained by angling in the mainstem Chehalis upstream of the Newaukum River. The program was fairly small (32,000 release size, or approximately 10 females spawned) and may be partly responsible for the lower diversity in the upper Chehalis populations if significant numbers of hatchery fish returned and spawned in the upper Chehalis upstream of Elk Creek. The extent of hatchery steelhead spawning in the upper watershed is unknown.

The Skookumchuck River *O. mykiss* collections stand out from other Chehalis River collections in several ways. First, both Skookumchuck collections had roughly double the number of fixed loci, i.e., loci with only one allele present in the population, and lower observed and expected heterozygosity of any other Chehalis or baseline wild population. Second, the Skookumchuck collections were genetically quite different from all lower and upper Chehalis collections; on average, pairwise F_{ST} values of Skookumchuck collections paired with other Chehalis collections were more than twice those of other within-Chehalis comparisons and only slightly lower than Chehalis collections paired with, for example, Puget Sound collections. Third, as stated above, the Skookumchuck population did not follow the isolation by distance pattern of other Chehalis Basin populations. The exaggerated genetic difference of Chehalis *O. mykiss* from other populations is likely due to the activities of the Skookumchuck steelhead hatchery

program, which was put in place to mitigate for a dam located on RM 22. It is an integrated program (natural origin broodstock are used), but the pHOS (proportion of hatchery origin spawners) is very high and PNI (proportionate natural influence) is very low; many more hatchery than natural origin fish are estimated to return each year (G. Marston, WDFW, personal communication). Thus, any population dynamics are likely driven mainly by the hatchery production. The hatchery program has been in place since 1973 and broodstock numbers are relatively low (release size 75,000, or approximately 25 females spawned). Hatchery activities are known to reduce genetic diversity (e.g., Heggenes et al. 2006), and evidence of that can be seen in the reduced genetic diversity of hatchery baseline collections used in this study.

The Newaukum collection also stood out from other Chehalis collections in that the collection showed substantial deviations from HWE and LD. The Newaukum collection was augmented with juvenile samples collected by electrofishing, and siblings are often sampled this way. The presence of siblings in a collection can cause deviations from HWE and LD (Waples 2015). However, normal numbers of siblings were found in the Newaukum collection, so this was not the cause of the deviations of HWE and LD. The Newaukum collection showed strong evidence of mixed ancestry, which likely explains the deviations from Hardy-Weinberg expectations (Wahlund effect, Waples 2015). In spite of the mixed ancestry, the Newaukum collection clustered with the Skookumchuck collections, likely due to the strength of the Skookumchuck signal, which possibly comes directly from Skookumchuck hatchery steelhead, which are planted annually in the Newaukum Basin at Carlisle Lake (Figure 1). Because of these factors, it is impossible to state with confidence that the Newaukum collection represents the actual *O. mykiss* spawning population of the Newaukum River. Additional temporally replicate tissue collections are needed to shed further light on this issue.

This study represents a comprehensive survey and genetic analysis of Chehalis River *O. mykiss* populations, but some minor holes still exist. The Hoquiam River, Cloquallum Creek, and Black River also have spawning steelhead populations, but were not sampled. Given the physical location of the Hoquiam River, Hoquiam *O. mykiss* would likely genetically cluster with other lower Chehalis River populations. Cloquallum Creek is near the Satsop River and drains the foothills of the Olympic Mountains, so it may cluster with Satsop River *O. mykiss*. The Black River is located in a geographical transition between the Olympic Mountains and Cascade Mountains. The Black River *O. mykiss* population is small (~40 spawners, M. Scharpf WDFW personal communication) and may not cluster strongly with either the lower Chehalis group or the mid Chehalis group (i.e., Skookumchuck). Samples from the Wynoochee and Skookumchuck rivers were collected downstream of dams. In the Wynoochee River, steelhead are transported upstream of Wynoochee Dam, so any rainbow trout population upstream of the dam likely is genetically the same as the downstream steelhead. However, the Skookumchuck Dam has no fish passage. Samples of rainbow trout from upstream of the dam may provide additional insights into the genetic diversity issues of the Skookumchuck collections.

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Table 1. List of general use and species ID, diploid single nucleotide polymorphic (SNP) loci used statewide in Washington State (panel SW269). Bolded loci are also found in Panel E/F, the subset of loci that comprised the former statewide SNP panel.

Locus name	WDFW nickname	Purpose	Reference
Omy_aspAT-123	**AOmy005**		**(Campbell et al. 2009)**
Omy_e1-147	**AOmy014**		**(Sprowles et al. 2006)**
Omy_gdh-271	**AOmy015**		**(Campbell et al. 2009)**
Omy_GH1P1_2	**AOmy016**		**(Aguilar and Garza 2008)**
Omy_LDHB-2_e5	**AOmy021**		**(Aguilar and Garza 2008)**
Omy_MYC_2	**AOmy023**		**(Aguilar and Garza 2008)**
Omy_myoD-178	**AOmy026**		**(Campbell et al. 2009)**
Omy_nkef-241	**AOmy027**		**(Campbell et al. 2009)**
Omy_Ogo4-212	**AOmy029**		**(Campbell et al. 2009)**
[a]Omy_BAC-F5.284	***AOmy042***		***(Limborg et al. 2012)***
Omy_u07-79-166	**AOmy047**		**(Limborg et al. 2012)**
Omy_113490-159	**AOmy048**		**(Abadía-Cardoso et al. 2011)**
Omy_114315-438	**AOmy049**		**(Abadía-Cardoso et al. 2011)**
[a]Omy_128693-455	***AOmy056***		***(Abadía-Cardoso et al. 2011)***
Omy_130524-160	**AOmy058**		**(Abadía-Cardoso et al. 2011)**
Omy_187760-385	**AOmy059**		**(Abadía-Cardoso et al. 2011)**
Omy_96222-125	**AOmy061**		**(Abadía-Cardoso et al. 2011)**
Omy_97077-73	**AOmy062**		**(Abadía-Cardoso et al. 2011)**
Omy_97954-618	**AOmy065**		**(Abadía-Cardoso et al. 2011)**
Omy_aromat-280	**AOmy067**		**WSU - J. DeKoning unpubl.**
Omy_arp-630	**AOmy068**		**(Campbell et al. 2009)**
Omy_cd59b-112	**AOmy072**		**WSU - J. DeKoning unpubl.**
Omy_colla1-525	**AOmy073**		**WSU - J. DeKoning unpubl.**
[a]Omy_cox2-335	***AOmy074***		***WSU - J. DeKoning unpubl.***
[a]Omy_g1-103	***AOmy078***		***(Stephens et al. 2009)***
Omy_g12-82	**AOmy079**		**WSU - J. DeKoning unpubl.**
Omy_gh-475	**AOmy081**		**(Campbell et al. 2009)**
Omy_gsdf-291	**AOmy082**		**WSU - J. DeKoning unpubl.**
Omy_hsc715-80	**AOmy084**		**WDFW - S. Young unpubl.**
Omy_hsp47-86	**AOmy087**		**WDFW - S. Young unpubl.**
Omy_hsp70aPro-329	**AOmy088**		**(Campbell and Narum 2009)**
Omy_hsp90BA-193	**AOmy089**		**(Campbell and Narum 2009)**
Omy_IL17-185	**AOmy091**		**WSU - J. DeKoning unpubl.**
Omy_IL1b-163	**AOmy092**		**WSU - J. DeKoning unpubl.**
Omy_inos-97	**AOmy094**		**WSU - J. DeKoning unpubl.**
Omy_mapK3-103	**AOmy095**		**CRITFC - N. Campbell unpubl.**
Omy_mcsf-268	**AOmy096**		**WSU - J. DeKoning unpubl.**
Omy_nach-200	**AOmy100**		**WSU - J. DeKoning unpubl.**
Omy_OmyP9-180	**AOmy105**		**(Sprowles et al. 2006)**
Omy_Ots249-227	**AOmy107**		**(Campbell et al. 2009)**
Omy_oxct-85	**AOmy108**		**WSU - J. DeKoning unpubl.**
Omy_star-206	**AOmy110**		**WSU - J. DeKoning unpubl.**
Omy_stat3-273	**AOmy111**		**WSU - J. DeKoning unpubl.**
Omy_tlr3-377	**AOmy113**		**WSU - J. DeKoning unpubl.**
Omy_tlr5-205	**AOmy114**		**WSU - J. DeKoning unpubl.**
Omy_u09-52.284	**AOmy117**		**(Limborg et al. 2012)**
Omy_u09-53.469	**AOmy118**		**(Limborg et al. 2012)**

Locus name	WDFW nickname	Purpose	Reference
Omy_u09-54-311	**AOmy120**		**WDFW - S. Young unpubl.**
Omy_u09-56.119	**AOmy125**		**(Limborg et al. 2012)**
Omy_BAMBI4.238	**AOmy129**		**WDFW - S. Young unpubl.**
Omy_G3PD_2.246	**AOmy132**		**WDFW - S. Young unpubl.**
Omy_Il-1b_.028	**AOmy134**		**WDFW - S. Young unpubl.**
Omy_u09-61.043	**AOmy137**		**WDFW - S. Young unpubl.**
[a]Omy_UT16_2-173	***AOmy144***		***WDFW - S. Young unpubl.***
Omy_U11_2b-154	**AOmy147**		**WDFW - S. Young unpubl.**
Omy_gluR-79	**AOmy149**		**CRITFC - unpubl.**
Omy_SECC22b-88	**AOmy152**		**CRITFC - unpubl.**
OMS00003	**AOmy174**		**(Sánchez et al. 2009)**
OMS00013	**AOmy176**		**(Sánchez et al. 2009)**
OMS00018	**AOmy177**		**(Sánchez et al. 2009)**
[a]OMS00041	***AOmy179***		***(Sánchez et al. 2009)***
OMS00048	**AOmy180**		**(Sánchez et al. 2009)**
OMS00052	**AOmy181**		**(Sánchez et al. 2009)**
OMS00053	**AOmy182**		**(Sánchez et al. 2009)**
OMS00056	**AOmy183**		**(Sánchez et al. 2009)**
OMS00057	**AOmy184**		**(Sánchez et al. 2009)**
OMS00061	**AOmy185**		**(Sánchez et al. 2009)**
OMS00062	**AOmy186**		**(Sánchez et al. 2009)**
[a]OMS00064	***AOmy187***		***(Sánchez et al. 2009)***
OMS00071	**AOmy189**		**(Sánchez et al. 2009)**
OMS00072	**AOmy190**		**(Sánchez et al. 2009)**
OMS00078	**AOmy191**		**(Sánchez et al. 2009)**
OMS00087	**AOmy192**		**(Sánchez et al. 2009)**
OMS00089	**AOmy193**		**(Sánchez et al. 2009)**
OMS00090	**AOmy194**		**(Sánchez et al. 2009)**
OMS00092	**AOmy195**		**(Sánchez et al. 2009)**
OMS00103	**AOmy197**		**(Sánchez et al. 2009)**
OMS00105	**AOmy198**		**(Sánchez et al. 2009)**
OMS00112	**AOmy199**		**(Sánchez et al. 2009)**
OMS00116	**AOmy200**		**(Sánchez et al. 2009)**
OMS00118	**AOmy201**		**(Sánchez et al. 2009)**
OMS00119	**AOmy202**		**(Sánchez et al. 2009)**
OMS00120	**AOmy203**		**(Sánchez et al. 2009)**
OMS00121	**AOmy204**		**(Sánchez et al. 2009)**
OMS00127	**AOmy205**		**(Sánchez et al. 2009)**
OMS00128	**AOmy206**		**(Sánchez et al. 2009)**
OMS00132	**AOmy207**		**(Sánchez et al. 2009)**
OMS00133	**AOmy208**		**(Sánchez et al. 2009)**
OMS00134	**AOmy209**		**(Sánchez et al. 2009)**
OMS00153	**AOmy210**		**(Sánchez et al. 2009)**
OMS00154	**AOmy211**		**(Sánchez et al. 2009)**
OMS00156	**AOmy212**		**(Sánchez et al. 2009)**
OMS00164	**AOmy213**		**(Sánchez et al. 2009)**
OMS00169	**AOmy214**		**(Sánchez et al. 2009)**
OMS00175	**AOmy215**		**(Sánchez et al. 2009)**
OMS00176	**AOmy216**		**(Sánchez et al. 2009)**

Locus name	WDFW nickname	Purpose	Reference
OMS00180	**AOmy218**		**(Sánchez et al. 2009)**
Omy_1004	**AOmy220**		**(Hansen et al. 2011)**
Omy_101554-306	**AOmy221**		**(Abadía-Cardoso et al. 2011)**
Omy_101832-195	**AOmy222**		**(Abadía-Cardoso et al. 2011)**
Omy_101993-189	**AOmy223**		**(Abadía-Cardoso et al. 2011)**
Omy_102505-102	**AOmy225**		**(Abadía-Cardoso et al. 2011)**
Omy_102867-443	**AOmy226**		**(Abadía-Cardoso et al. 2011)**
Omy_103705-558	**AOmy227**		**(Abadía-Cardoso et al. 2011)**
Omy_104519-624	**AOmy228**		**(Abadía-Cardoso et al. 2011)**
Omy_104569-114	**AOmy229**		**(Abadía-Cardoso et al. 2011)**
Omy_105075-162	**AOmy230**		**(Abadía-Cardoso et al. 2011)**
[a]Omy_105385-406	***AOmy231***		***(Abadía-Cardoso et al. 2011)***
Omy_105714-265	**AOmy232**		**(Abadía-Cardoso et al. 2011)**
Omy_107031-704	**AOmy233**		**(Abadía-Cardoso et al. 2011)**
Omy_107285-69	**AOmy234**		**(Abadía-Cardoso et al. 2011)**
Omy_107336-170	**AOmy235**		**(Abadía-Cardoso et al. 2011)**
Omy_107806-34	**AOmy237**		**(Abadía-Cardoso et al. 2011)**
Omy_108007-193	**AOmy238**		**(Abadía-Cardoso et al. 2011)**
Omy_109243-222	**AOmy239**		**(Abadía-Cardoso et al. 2011)**
Omy_109525-403	**AOmy240**		**(Abadía-Cardoso et al. 2011)**
Omy_110064-419	**AOmy241**		**(Abadía-Cardoso et al. 2011)**
Omy_110362-585	**AOmy243**		**(Abadía-Cardoso et al. 2011)**
Omy_110689-148	**AOmy244**		**(Abadía-Cardoso et al. 2011)**
Omy_111084-526	**AOmy246**		**(Abadía-Cardoso et al. 2011)**
Omy_111383-51	**AOmy247**		**(Abadía-Cardoso et al. 2011)**
Omy_111666-301	**AOmy248**		**(Abadía-Cardoso et al. 2011)**
Omy_112301-202	**AOmy249**		**(Abadía-Cardoso et al. 2011)**
Omy_112820-82	**AOmy250**		**(Abadía-Cardoso et al. 2011)**
Omy_114976-223	**AOmy252**		**(Abadía-Cardoso et al. 2011)**
Omy_116733-349	**AOmy253**		**(Abadía-Cardoso et al. 2011)**
Omy_116938-264	**AOmy254**		**(Abadía-Cardoso et al. 2011)**
Omy_117286-374	**AOmy256**		**(Abadía-Cardoso et al. 2011)**
Omy_117370-400	**AOmy257**		**(Abadía-Cardoso et al. 2011)**
Omy_117540-259	**AOmy258**		**(Abadía-Cardoso et al. 2011)**
Omy_117815-81	**AOmy260**		**(Abadía-Cardoso et al. 2011)**
Omy_118175-396	**AOmy261**		**(Abadía-Cardoso et al. 2011)**
Omy_118205-116	**AOmy262**		**(Abadía-Cardoso et al. 2011)**
Omy_118654-91	**AOmy263**		**(Abadía-Cardoso et al. 2011)**
Omy_120255-332	**AOmy265**		**(Abadía-Cardoso et al. 2011)**
Omy_128996-481	**AOmy266**		**(Abadía-Cardoso et al. 2011)**
Omy_129870-756	**AOmy267**		**(Abadía-Cardoso et al. 2011)**
Omy_131460-646	**AOmy268**		**(Abadía-Cardoso et al. 2011)**
Omy_98683-165	**AOmy269**		**(Abadía-Cardoso et al. 2011)**
Omy_cyp17-153	**AOmy270**		**WSU - J. DeKoning unpubl.**
Omy_ftzf1-217	**AOmy271**		**WSU - J. DeKoning unpubl.**
Omy_GHSR-121	**AOmy272**		**CRITFC - unpubl.**
Omy_metA-161	**AOmy273**		**CRITFC - unpubl.**
Omy_UBA3b	**AOmy274**		**(Hansen et al. 2011)**
M09AAC.055	**AOmy275**		**WDFW - S. Young unpubl.**

Locus name	WDFW nickname	Purpose	Reference
M09AAE.082	**AOmy276**		**WDFW - S. Young unpubl.**
OMGH1PROM1-SNP1	**AOmy277**		**(Abadía-Cardoso et al. 2011)**
OMS00015	**AOmy279**		**(Sánchez et al. 2009)**
OMS00024	**AOmy280**		**(Sánchez et al. 2009)**
OMS00070	**AOmy283**		**(Sánchez et al. 2009)**
OMS00074	**AOmy284**		**(Sánchez et al. 2009)**
OMS00096	**AOmy285**		**(Sánchez et al. 2009)**
OMS00111	**AOmy286**		**(Sánchez et al. 2009)**
OMS00149	**AOmy288**		**(Sánchez et al. 2009)**
OMS00173	**AOmy289**		**(Sánchez et al. 2009)**
Omy_105105-448	**AOmy290**		**(Abadía-Cardoso et al. 2011)**
Omy_110201-359	**AOmy291**		**(Abadía-Cardoso et al. 2011)**
[a]Omy_128923-433	***AOmy292***		***(Abadía-Cardoso et al. 2011)***
Omy_anp-17	**AOmy293**		**CRITFC - N. Campbell unpubl.**
Omy_bcAKala-380rd	**AOmy294**		**CRITFC - N. Campbell unpubl.**
Omy_cin-172	**AOmy295**		**CRITFC - N. Campbell unpubl.**
Omy_ndk-152	**AOmy296**		**CRITFC - N. Campbell unpubl.**
Omy_nips-299	**AOmy297**		**CRITFC - N. Campbell unpubl.**
Omy_ntl-27	**AOmy298**		**CRITFC - N. Campbell unpubl.**
Omy_rbm4b-203	**AOmy299**		**CRITFC - N. Campbell unpubl.**
Omy_sys1-188	**AOmy300**		**CRITFC - N. Campbell unpubl.**
Omy_txnip-343	**AOmy301**		**CRITFC - N. Campbell unpubl.**
Omy_vamp5-303	**AOmy302**		**CRITFC - N. Campbell unpubl.**
Omy_vatf-406	**AOmy303**		**CRITFC - N. Campbell unpubl.**
OMS00077	**AOmy305**		**(Sánchez et al. 2009)**
OMS00101	**AOmy306**		**(Sánchez et al. 2009)**
Omy_G3PD_2-371	**AOmy311**		**CRITFC - N. Campbell unpubl.**
Omy_redd1-410	**AOmy320**		**CRITFC - N. Campbell unpubl.**
Omy_srp09-37	**AOmy322**		**CRITFC - N. Campbell unpubl.**
OMY1011SNP	**AOmy324**		**(Hansen et al. 2011)**
[a]OMS00068	***AOmy326***		***(Sánchez et al. 2009)***
OMS00079	**AOmy327**		**(Sánchez et al. 2009)**
[a]OMS00106	***AOmy328***		***(Sánchez et al. 2009)***
OMS00179	**AOmy329**		**(Sánchez et al. 2009)**
Omy_114587-480	**AOmy331**		**(Abadía-Cardoso et al. 2011)**
OMS00017	**AOmy335**		**(Sánchez et al. 2009)**
Omy_metB-138	**AOmy341**		**CRITFC - unpubl.**
M09AAD.076	NA		CRITFC - unpubl.
M09AAJ.163	NA		CRITFC - unpubl.
OMS00002	NA		CRITFC - unpubl.
OMS00006	NA		CRITFC - unpubl.
OMS00008	NA		CRITFC - unpubl.
OMS00014	NA		CRITFC - unpubl.
OMS00030	NA		CRITFC - unpubl.
OMS00039	NA		CRITFC - unpubl.
OMS00058	NA		CRITFC - unpubl.
OMS00095	NA		CRITFC - unpubl.
OMS00114	NA		CRITFC - unpubl.
OMS00129	NA		CRITFC - unpubl.

Locus name	WDFW nickname	Purpose	Reference
OMS00138	NA		CRITFC - unpubl.
OMS00143	NA		CRITFC - unpubl.
OMS00151	NA		CRITFC - unpubl.
OMS00174	NA		CRITFC - unpubl.
Omy_109894-185	NA		CRITFC - unpubl.
Omy_97660-230	NA		CRITFC - unpubl.
Omy_97865-196	NA		CRITFC - unpubl.
Omy_99300-202	NA		CRITFC - unpubl.
Omy_ada10-71	NA		CRITFC - unpubl.
[a]Omy_aldB-165	*NA*		*CRITFC - unpubl.*
Omy_b1-266	NA		CRITFC - unpubl.
Omy_b9-164	NA		CRITFC - unpubl.
Omy_BAC-B4-324	NA		CRITFC - unpubl.
Omy_BAMBI2.312	NA		CRITFC - unpubl.
Omy_ca050-64	NA		CRITFC - unpubl.
Omy_carban1-264	NA		CRITFC - unpubl.
Omy_cd28-130	NA		CRITFC - unpubl.
Omy_cd59-206	NA		CRITFC - unpubl.
Omy_cox1-221	NA		CRITFC - unpubl.
Omy_crb-106	NA		CRITFC - unpubl.
Omy_CRBF1-1-1	NA		CRITFC - unpubl.
Omy_gadd45-332	NA		CRITFC - unpubl.
Omy_hsf1b-241	NA		CRITFC - unpubl.
Omy_hsf2-146	NA		CRITFC - unpubl.
Omy_hus1-52	NA		CRITFC - unpubl.
Omy_Il1b-198	NA		CRITFC - unpubl.
Omy_IL6-320	NA		CRITFC - unpubl.
Omy_impa1-55	NA		CRITFC - unpubl.
Omy_LDHB-1_i2	NA		CRITFC - unpubl.
Omy_LDHB-2_i6	NA		CRITFC - unpubl.
Omy_lpl-220	NA		CRITFC - unpubl.
Omy_NaKATPa3-50	NA		CRITFC - unpubl.
Omy_nxt2-273	NA		CRITFC - unpubl.
Omy_p53-262	NA		CRITFC - unpubl.
Omy_pad-196	NA		CRITFC - unpubl.
Omy_ppie-232	NA		CRITFC - unpubl.
Omy_RAD16104-20	NA		CRITFC - unpubl.
Omy_RAD17632-23	NA		CRITFC - unpubl.
Omy_RAD23577-43	NA		CRITFC - unpubl.
Omy_RAD26080-69	NA		CRITFC - unpubl.
Omy_RAD29700-18	NA		CRITFC - unpubl.
Omy_RAD35417-9	NA		CRITFC - unpubl.
Omy_RAD36848-7	NA		CRITFC - unpubl.
Omy_RAD38269-10	NA		CRITFC - unpubl.
Omy_RAD42793-59	NA		CRITFC - unpubl.
Omy_RAD43612-42	NA		CRITFC - unpubl.
Omy_RAD45104-18	NA		CRITFC - unpubl.
Omy_RAD47080-54	NA		CRITFC - unpubl.
Omy_RAD47444-53	NA		CRITFC - unpubl.

Locus name	WDFW nickname	Purpose	Reference
Omy_RAD47955-51	NA		CRITFC - unpubl.
Omy_RAD48799-69	NA		CRITFC - unpubl.
Omy_RAD5026-64	NA		CRITFC - unpubl.
Omy_RAD52458-17	NA		CRITFC - unpubl.
Omy_RAD52812-28	NA		CRITFC - unpubl.
Omy_RAD58213-70	NA		CRITFC - unpubl.
Omy_RAD58835-15	NA		CRITFC - unpubl.
Omy_RAD62596-38	NA		CRITFC - unpubl.
Omy_RAD66218-58	NA		CRITFC - unpubl.
Omy_RAD66834-17	NA		CRITFC - unpubl.
[a]Omy_RAD69583-33	*NA*		*CRITFC - unpubl.*
Omy_RAD7210-8	NA		CRITFC - unpubl.
Omy_RAD73204-63	NA		CRITFC - unpubl.
Omy_RAD74691-49	NA		CRITFC - unpubl.
Omy_RAD76882-63	NA		CRITFC - unpubl.
Omy_RAD77789-54	NA		CRITFC - unpubl.
Omy_RAD88028-7	NA		CRITFC - unpubl.
Omy_RAD88122-32	NA		CRITFC - unpubl.
Omy_rapd-167	NA		CRITFC - unpubl.
Omy_sast-264	NA		CRITFC - unpubl.
Omy_sSOD-1	NA		CRITFC - unpubl.
Omy_zg57-91	NA		CRITFC - unpubl.
Omy_myclarp404-111	ASpI016	species ID	CRITFC - unpubl.
Omy_Omyclmk438-96	**ASpI018**	**species ID**	**CRITFC - S. Narum - unpubl.**
Ocl_gshpx-357	NA	species ID	CRITFC - unpubl.
Ocl_Okerca	**ASpI001**	**species ID**	**(McGlauflin et al. 2010)**
Omy_F5_136	**ASpI014**	**species ID**	**(Finger et al. 2009)**
OmyY1_2SEXY	NA	sex ID	CRITFC - unpubl.

[a]-These loci were removed from analysis due to poor amplification in Chehalis River collections or because they were entirely absent from one or more Chehalis River or baseline collection.

Table 2. Collections of O. mykiss *used in genetic analysis.*

Lineage	Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	Life stage	WDFW code	N processed	N clarki/hybrid	SW269 N genotyped	Panel E/F N genotyped
Coastal	Wild	SWWA	Chehalis	Wishkah	winter	2016	adult, juvenile	16GD	49	0	34	34
	Wild	SWWA	Chehalis	Humptulips	winter	2015	adult	15QW	50	0	44	44
	Wild	SWWA	Chehalis	Humptulips	winter	2016	adult	16BS	30	0	29	30
	Wild	SWWA	Chehalis	Wynoochee	winter	2015	adult	15KG	50	0	50	50
	Wild	SWWA	Chehalis	Wynoochee	winter	2016	adult	16BT	46	1	42	42
	Wild	SWWA	Chehalis	Satsop	winter	2015	adult	15KH	50	0	50	50
	Wild	SWWA	Chehalis	Satsop	winter	2016	adult	16BU	32	0	31	31
	Wild	SWWA	Chehalis	Skookumchuck	winter	2015	adult	15KI	50	0	50	50
	Wild	SWWA	Chehalis	Skookumchuck	winter	2016	adult	16BZ	43	1	38	38
	Wild	SWWA	Chehalis	Newaukum	winter	2016	adult, juvenile	16BY	37	0	30	34
	Wild	SWWA	Chehalis	SF Chehalis	winter	2016	adult, juvenile	16BX	51	0	8	32
	Wild	SWWA	Chehalis	Upper Chehalis	winter	2014, 2015	adult	14TK, 15QX	38	1	37	37
	Wild	SWWA	Chehalis	Upper Chehalis	winter	2016	adult, juvenile	16BW	51	0	50	50
	Wild	SWWA	Lower Columbia mainstem	Abernathy	winter	2011	adult	11PV	NA	NA	0	47
	Wild	SWWA	Willapa Bay	Willapa	winter	1996-1998	adult	96AAA, 97AAB, 98AAC	NA	NA	0	66
	Wild	OP	Quinault	Quinault	winter	2014	adult	14AN	NA	NA	0	50
	Wild	OP	Queets	Clearwater	winter	2012	adult	12EN	NA	NA	0	59

Lineage	Production	DPS[a]	Major river basin	Population or stock	run timing	Collection Year	Life stage	WDFW code	N processed	N clarki/hybrid	SW269 N genotyped	Panel E/F N genotyped
	Wild	OP	Queets	Queets	winter	2014	adult	14AO	NA	NA	0	50
	Wild	PS	Discovery Bay	Snow Creek	winter	1998	adult	98AN	NA	NA	0	31
	Wild	PS	Nooksack	Nooksack	winter	2012	adult	12MQ	NA	NA	0	50
	Wild	Ps	Stillaguamish	Deer Creek	summer	2013	juvenile	13KB	NA	NA	0	50
	Wild	PS	Green	Green	winter	2013	adult	13EH	NA	NA	0	31
	Wild	PS	Nisqually	Nisqually	winter	2014	juvenile	14GN	NA	NA	0	50
	Wild	PS	Hood Canal	Tahuya	winter	1995	adult	95CH	NA	NA	0	46
	Hatchery	NA	Chambers Creek	Tokul Creek	winter	2001	adult	01GC	NA	NA	0	40
	Hatchery	NA	Chambers Creek	Tokul Creek	winter	2014	adult	14BK	NA	NA	0	50
	Hatchery	NA	Cook Creek	Cook Creek	winter	2008	adult	08AH	NA	NA	0	50
	Hatchery	NA	Washougal (Skamania)	Bogachiel Hatchery	summer	2012	adult	12OX	NA	NA	0	51
	Hatchery	NA	Washougal (Skamania)	Reiter Ponds	summer	2014	adult	14BL	NA	NA	0	50
Columbia River Redband (CRR)	Wild	MC	Yakima	Naches River	summer	2012	juvenile	12CB	NA	NA	0	33
	Wild	UC	Wenatchee	Nason Creek	summer	2009	juvenile	09NG	NA	NA	0	50
California Hatchery Trout (CAHT)	Hatchery	NA	NA	Goldendale	NA	2014	adult	14LP	NA	NA	0	49
	Hatchery	NA	NA	Mt. Whitney	NA	2014	adult	14LO	NA	NA	0	50

[a] SWWA = Southwest Washington, OP = Olympic Peninsula, PS = Puget Sound, MC = Mid-Columbia, UC = Upper Columbia.

Table 3. Genetic metrics and statistics for Chehalis River and baseline Washington O. mykiss*.*

Population or stock	Collection year	loci	fixed loci (%)	Mean N^A	He	Ho	F_{IS}	%HWE P < 0.05	%HWE P corr.	%LD P < 0.05	%LD P corr.	LDNe (95%CI)
Wishkah River	2016	181	8 (4.4)	1.937	0.304	0.300	0.015	4.24	0.00	3.735	0.014	1198.2 (282.4 - Inf)
Humptulips River	2015	181	8 (4.4)	1.929	0.297	0.286	0.033	3.07	0.61	4.198	0.048	214.6 (145.9 - 388.6)
Humptulips River	2016	181	10 (5.5)	1.940	0.310	0.293	0.057	5.39	0.00	4.391	0.014	85.7 (65.6 - 120.8)
Wynoochee River	2015	181	8 (4.4)	1.939	0.294	0.289	0.010	2.33	0.00	4.037	0.007	540.5 (275.1 - 6900.8)
Wynoochee River	2016	181	9 (5.0)	1.935	0.301	0.305	-0.011	3.51	0.00	4.435	0.00	243.5 (155 - 533.2)
Satsop River	2015	181	11 (6.1)	1.905	0.292	0.293	0.005	5.49	0.00	4.941	0.021	115 (92.6 - 149.3)
Satsop River	2016	181	11 (6.1)	1.925	0.296	0.291	0.029	5.00	0.00	3.744	0.014	443.5 (179.8 - Inf)
Skookumchuck River	2015	181	21 (11.6)	1.854	0.271	0.263	0.029	5.81	0.65	4.465	0.024	160.5 (118.9 - 240.3)
Skookumchuck River	2016	181	21 (11.6)	1.861	0.272	0.268	0.024	4.58	0.65	3.732	0.015	255.9 (149.6 - 780.8)
Newaukum River	2016	182	6 (3.3)	1.944	0.315	0.289	0.102	12.87	1.75	8.722	0.027	13.7 (12.6 - 14.8)
South Fork Chehalis	2016	182	13 (7.1)	1.887	0.292	0.267	0.076	5.73	0.00	4.495	0.028	222.6 (152.7 - 394.3)
Upper Chehalis River	2014, 2015	181	12 (6.6)	1.914	0.296	0.290	0.015	5.56	0.62	3.763	0.029	289.8 (164.4 - 1052)
Upper Chehalis River	2016	182	13 (7.1)	1.913	0.287	0.282	0.021	4.79	0.60	4.439	0.022	1577.1 (419.5 - Inf)
Abernathy Creek	2011	187	6 (3.2)	1.941	0.298	0.296	0.009	4.55	0.00	4.286	0.018	190.9 (138.9 - 297.1)
Willapa River	1996-1998	189	6 (3.2)	1.950	0.301	0.295	0.015	3.89	0.56	4.637	0.024	284.7 (207.9 - 441.1)

Population or stock	Collection year	loci	fixed loci (%)	Mean N^A	He	Ho	F_{IS}	%HWE P < 0.05	%HWE P corr.	%LD P < 0.05	%LD P corr.	LDNe (95%CI)
Quinault River	2014	187	3 (1.6)	1.946	0.307	0.301	0.021	7.61	0.00	5.132	0.042	536.8 (413.3 - 753.5)
Clearwater River	2012	187	6 (3.2)	1.956	0.304	0.296	0.027	7.73	0.55	4.309	0.025	2498.7 (590.5 - Inf)
Queets River	2014	187	3 (1.6)	1.952	0.304	0.300	0.013	7.69	1.10	4.734	0.030	1827.2 (846.9 - Inf)
Snow Creek	1998	188	12 (6.4)	1.923	0.299	0.302	0.000	3.59	0.00	6.762	0.006	21.3 (19.3 - 23.6)
Nooksack River	2012	188	4 (2.1)	1.947	0.323	0.319	0.016	5.56	0.00	4.647	0.048	248.3 (174.5 - 417.4)
Deer Creek	2013	182	3 (1.6)	1.954	0.323	0.318	0.011	4.52	0.00	5.116	0.013	113.2 (101.6 - 127.1)
Green River	2013	182	9 (4.9)	1.950	0.313	0.312	0.013	4.12	0.00	3.831	0.013	193.5 (123.2 - 423)
Nisqually River	2014	189	6 (3.2)	1.934	0.306	0.305	0.009	7.73	1.66	5.519	0.042	403.8 (321.3 - 536)
Tahuya River	1995	186	12 (6.5)	1.907	0.289	0.285	0.010	5.39	1.20	5.907	0.027	37.7 (34.4 - 41.6)
Tokul Creek	2001	180	13 (7.2)	1.912	0.302	0.297	0.025	6.13	0.61	4.662	0.007	144.8 (107.3 - 217.1)
Tokul Creek	2014	189	10 (5.3)	1.908	0.294	0.308	-0.034	11.80	3.93	6.065	0.038	152.8 (131.2 - 181.3)
Cook Creek	2008	187	11 (5.9)	1.908	0.288	0.291	0.003	6.29	0.00	4.721	0.019	391.8 (274.3 - 664)
Bogachiel Hatchery	2012	187	17 (9.1)	1.897	0.310	0.319	-0.018	4.71	0.00	6.070	0.035	61.8 (54.5 - 70.9)
Reiter Ponds	2014	189	11 (5.8)	1.903	0.308	0.332	-0.067	17.51	9.04	6.281	0.019	105.5 (94.5 - 118.8)
Naches River	2012	182	8 (4.4)	1.926	0.294	0.293	0.007	2.94	0.00	3.519	0.012	537.1 (216.9 - Inf)
Nason Creek	2009	182	7	1.909	0.270	0.268	0.006	7.60	0.00	9.103	0.158	49.5

Population or stock	Collection year	loci	fixed loci (%)	Mean N^A	He	Ho	F_{IS}	%HWE P < 0.05	%HWE P corr.	%LD P < 0.05	%LD P corr.	LDNe (95%CI)
			(3.8)									(46 - 53.3)
Goldendale	2014	181	21 (11.6)	1.864	0.288	0.286	0.008	6.37	4.46	4.898	0.086	242.9 (192.4 - 323.9)
Mt. Whitney	2014	181	34 (18.8)	1.792	0.276	0.277	-0.002	9.66	2.76	6.413	0.308	85 (76.2 - 95.4)

Table 4. Estimated pair-wise F_{ST} values for Chehalis River and Washington State baseline O. mykiss *below diagonal. Bold type indicates statistical significance before and after correction for multiple tests. Italic type indicates significance before, but not after correction for multiple tests. Above diagonal are corrected* P *values. An asterisk indicates a corrected* P *values less than the table-wide* P *value. NS = Not Significant.*

	CAHT - Goldendale	CAHT - Mt. Whitney	CRR - Naches	CRR - Nason	EHS - Bogachiel	EHS - Reiter	Abernathy	Willapa_96	Willapa_97	Willapa_98
CAHT - Goldendale	-	*	*	*	*	0.00017	*	*	*	*
CAHT - Mt. Whitney	**0.146**	-	*	*	*	0.00092	*	*	*	*
CRR - Naches	**0.279**	**0.300**	-	*	*	0.0005	*	*	*	*
CRR - Nason	**0.305**	**0.324**	**0.027**	-	*	0.00017	*	*	*	*
EHS – Bogachiel	**0.282**	**0.291**	**0.162**	**0.165**	-	0.00025	*	*	*	*
EHS - Reiter	*0.300*	*0.303*	*0.155*	*0.162*	*0.035*	-	0.0005	0.00303	0.00387	0.00059
Abernathy	**0.295**	**0.305**	**0.146**	**0.154**	**0.047**	*0.055*	-	*	*	*
Willapa_96	**0.286**	**0.300**	**0.163**	**0.170**	**0.044**	*0.061*	**0.033**	-	NS	NS
Willapa_97	**0.278**	**0.296**	**0.159**	**0.167**	**0.038**	*0.051*	**0.025**	-0.003	-	NS
Willapa_98	**0.287**	**0.301**	**0.158**	**0.162**	**0.050**	*0.061*	**0.031**	0.004	-0.001	-
Humptulips_15	**0.289**	**0.297**	**0.162**	**0.164**	**0.051**	*0.061*	**0.027**	*0.011*	*0.008*	*0.008*
Humptulips_16	**0.285**	**0.293**	**0.157**	**0.161**	**0.050**	*0.062*	**0.025**	*0.008*	*0.009*	*0.010*
Wishkah_16	**0.286**	**0.295**	**0.165**	**0.170**	**0.059**	*0.080*	**0.040**	*0.015*	*0.012*	*0.017*
Wynoochee_15	**0.300**	**0.317**	**0.165**	**0.167**	**0.058**	*0.069*	**0.029**	**0.014**	**0.012**	**0.013**
Wynoochee_16	**0.299**	**0.314**	**0.163**	**0.167**	**0.055**	*0.069*	**0.031**	**0.016**	**0.015**	**0.016**
Satsop_15	**0.297**	**0.310**	**0.168**	**0.174**	**0.060**	**0.072**	**0.031**	**0.018**	**0.012**	**0.019**
Satsop_16	**0.299**	**0.317**	**0.174**	**0.176**	**0.059**	*0.074*	**0.037**	*0.009*	*0.009*	*0.010*
Skookumchuck_15	**0.330**	**0.350**	**0.181**	**0.193**	**0.069**	*0.077*	**0.046**	**0.033**	**0.038**	**0.043**
Skookumchuck_16	**0.328**	**0.347**	**0.180**	**0.188**	**0.065**	*0.077*	**0.047**	*0.036*	**0.041**	**0.045**
Newaukum_16	**0.307**	**0.322**	**0.177**	**0.185**	**0.063**	*0.066*	**0.041**	**0.019**	**0.022**	**0.026**
SF_Chehalis_16	**0.284**	**0.302**	**0.164**	**0.175**	**0.068**	*0.066*	**0.034**	**0.023**	**0.018**	**0.028**
Upper_Chehalis_15	**0.293**	**0.307**	**0.174**	**0.180**	**0.056**	*0.058*	**0.031**	**0.011**	*0.011*	**0.015**
Upper_Chehalis_16	**0.308**	**0.320**	**0.178**	**0.183**	**0.057**	*0.063*	**0.034**	*0.008*	**0.013**	**0.018**
Quinault	**0.287**	**0.296**	**0.164**	**0.167**	**0.043**	*0.057*	**0.023**	*0.012*	0.005	**0.012**
Queets	**0.290**	**0.299**	**0.162**	**0.164**	**0.043**	*0.056*	**0.020**	*0.012*	0.005	**0.013**
Clearwater	**0.287**	**0.296**	**0.161**	**0.165**	**0.051**	**0.059**	**0.025**	**0.017**	*0.009*	**0.017**
Snow_Creek	**0.286**	**0.304**	**0.182**	**0.194**	**0.075**	*0.077*	**0.062**	**0.055**	**0.047**	**0.056**
Tahuya	**0.301**	**0.308**	**0.178**	**0.183**	**0.075**	**0.077**	**0.062**	**0.067**	**0.057**	**0.065**
Nooksack_winters	**0.264**	**0.278**	**0.152**	**0.156**	**0.066**	*0.070*	**0.049**	**0.052**	**0.045**	**0.051**
Deer_Creek_summers	**0.269**	**0.287**	**0.141**	**0.142**	**0.066**	*0.067*	**0.054**	**0.050**	**0.046**	**0.055**
Green_River	**0.268**	**0.284**	**0.147**	**0.152**	**0.052**	*0.061*	**0.045**	**0.046**	**0.041**	**0.048**
Nisqually	**0.274**	**0.284**	**0.164**	**0.169**	**0.067**	**0.075**	**0.045**	**0.040**	**0.033**	**0.047**
EHW_Tokul_01	**0.276**	**0.290**	**0.146**	**0.151**	**0.050**	*0.061*	**0.031**	**0.035**	**0.029**	**0.039**
EHW_Tokul_14	**0.283**	**0.291**	**0.158**	**0.161**	**0.053**	*0.068*	**0.039**	**0.040**	**0.038**	**0.041**
EHW_Cook_Creek	**0.300**	**0.314**	**0.176**	**0.180**	**0.056**	*0.061*	**0.041**	**0.043**	**0.042**	**0.047**

Table 4. cont'd

	Humptulips_15	Humptulips_16	Wishkah_16	Wynoochee_15	Wynoochee_16	Satsop_15	Satsop_16	Skookumchuck_15	Skookumchuck_16
CAHT - Goldendale	*	*	*	*	*	*	*	*	*
CAHT - Mt. Whitney	*	*	*	*	*	*	*	*	*
CRR - Naches	*	*	*	*	*	*	*	*	*
CRR - Nason	*	*	*	*	*	*	*	*	*
EHS – Bogachiel	*	*	*	*	*	*	*	*	*
EHS - Reiter	0.00151	0.00143	0.00294	0.00042	0.00084	*	0.00092	0.00462	0.00487
Abernathy	*	*	*	*	*	*	*	*	*
Willapa_96	0.01	0.00328	0.00092	*	0.00067	*	0.00143	*	0.00017
Willapa_97	0.00672	0.00723	0.00815	*	0.0005	*	0.00067	*	*
Willapa_98	0.00866	0.00303	0.00672	*	*	*	0.00176	*	*
Humptulips_15	-	NS	0.00269	*	*	*	0.00084	*	*
Humptulips_16	0.001	-	NS	0.00017	0.00017	*	0.00437	*	*
Wishkah_16	*0.009*	0.004	-	0.00916	0.02513	0.00143	NS	*	*
Wynoochee_15	**0.013**	*0.008*	*0.013*	-	0.04782	*	*	*	*
Wynoochee_16	**0.014**	*0.011*	*0.013*	*0.001*	-	*	0.00067	*	*
Satsop_15	**0.016**	**0.014**	*0.016*	**0.009**	**0.013**	-	NS	*	*
Satsop_16	*0.010*	*0.007*	0.007	**0.006**	*0.008*	0.001	-	*	*
Skookumchuck_15	**0.044**	**0.039**	**0.052**	**0.042**	**0.040**	**0.039**	**0.039**	-	NS
Skookumchuck_16	**0.043**	**0.041**	**0.057**	**0.043**	**0.043**	**0.043**	**0.043**	0.001	-
Newaukum_16	**0.026**	**0.022**	**0.030**	**0.022**	**0.024**	**0.026**	**0.025**	**0.023**	**0.026**
SF_Chehalis_16	**0.031**	**0.027**	**0.038**	**0.031**	**0.033**	**0.032**	**0.035**	*0.053*	*0.055*
Upper_Chehalis_15	**0.018**	**0.020**	**0.030**	**0.022**	**0.025**	**0.025**	**0.022**	**0.040**	**0.042**
Upper_Chehalis_16	**0.020**	**0.021**	**0.033**	**0.024**	**0.027**	**0.028**	**0.024**	**0.037**	**0.038**
Quinault	*0.008*	*0.007*	**0.018**	**0.012**	**0.014**	**0.015**	**0.015**	**0.036**	**0.037**
Queets	*0.006*	*0.004*	*0.016*	**0.014**	**0.015**	**0.018**	**0.014**	**0.037**	**0.037**
Clearwater	**0.015**	*0.009*	**0.024**	**0.018**	**0.022**	**0.021**	**0.020**	**0.041**	**0.040**
Snow_Creek	**0.050**	**0.041**	**0.062**	**0.056**	**0.058**	**0.062**	**0.062**	**0.071**	**0.069**
Tahuya	**0.057**	**0.046**	**0.073**	**0.063**	**0.062**	**0.071**	**0.069**	**0.074**	**0.072**
Nooksack_winters	**0.049**	**0.041**	**0.051**	**0.049**	**0.053**	**0.056**	**0.056**	**0.076**	**0.073**
Deer_Creek_summers	**0.054**	**0.042**	**0.059**	**0.049**	**0.054**	**0.060**	**0.061**	**0.079**	**0.073**
Green_River	**0.047**	**0.043**	**0.053**	**0.051**	**0.050**	**0.061**	**0.058**	**0.063**	**0.061**
Nisqually	**0.047**	**0.033**	**0.051**	**0.045**	**0.046**	**0.053**	**0.052**	**0.066**	**0.066**
EHW_Tokul_01	**0.039**	**0.031**	**0.045**	**0.036**	**0.034**	**0.044**	**0.042**	**0.050**	**0.052**
EHW_Tokul_14	**0.043**	**0.038**	**0.054**	**0.045**	**0.043**	**0.051**	**0.050**	**0.060**	**0.060**
EHW_Cook_Creek	**0.046**	**0.038**	**0.063**	**0.050**	**0.050**	**0.060**	**0.057**	**0.066**	**0.064**

Table 4. cont'd.

	Newaukum_16	SF_Chehalis_16	Upper_Chehalis_15	Upper_Chehalis_16	Quinault	Queets	Clearwater
CAHT - Goldendale	*	*	*	*	*	*	*
CAHT - Mt. Whitney	*	*	*	*	*	*	*
CRR - Naches	*	*	*	*	*	*	*
CRR - Nason	*	*	*	*	*	*	*
EHS – Bogachiel	*	*	*	*	*	*	*
EHS - Reiter	0.00034	0.0042	0.00042	0.00017	0.00025	0.00017	*
Abernathy	*	*	*	*	*	*	*
Willapa_96	*	*	*	0.00025	0.00076	0.00017	*
Willapa_97	*	*	0.00017	*	NS	NS	0.00832
Willapa_98	*	*	*	*	*	*	*
Humptulips_15	*	*	*	*	0.00034	0.00412	*
Humptulips_16	*	*	*	*	0.0084	0.00975	0.00017
Wishkah_16	*	*	*	*	*	0.00025	*
Wynoochee_15	*	*	*	*	*	*	*
Wynoochee_16	*	*	*	*	*	*	*
Satsop_15	*	*	*	*	*	*	*
Satsop_16	*	*	*	*	*	*	*
Skookumchuck_15	*	0.00025	*	*	*	*	*
Skookumchuck_16	*	0.00017	*	*	*	*	*
Newaukum_16	-	*	*	*	*	*	*
South_Fork_Chehalis_16	**0.034**	-	*	*	*	*	*
Upper_Chehalis_15	**0.020**	**0.020**	-	NS	*	*	*
Upper_Chehalis_16	**0.023**	**0.022**	0.003	-	*	*	*
Quinault	**0.025**	**0.032**	**0.022**	**0.027**	-	NS	0.00529
Queets	**0.023**	**0.035**	**0.021**	**0.027**	0.001	-	NS
Clearwater	**0.028**	**0.039**	**0.027**	**0.032**	*0.004*	0.001	-
Snow_Creek	**0.055**	**0.057**	**0.055**	**0.057**	**0.040**	**0.044**	**0.045**
Tahuya	**0.065**	**0.073**	**0.064**	**0.067**	**0.049**	**0.045**	**0.045**
Nooksack_winters	**0.059**	**0.062**	**0.053**	**0.061**	**0.036**	**0.039**	**0.039**
Deer_Creek_summers	**0.060**	**0.061**	**0.053**	**0.058**	**0.047**	**0.046**	**0.046**
Green_River	**0.054**	**0.061**	**0.052**	**0.054**	**0.034**	**0.034**	**0.035**
Nisqually	**0.047**	**0.053**	**0.051**	**0.053**	**0.035**	**0.033**	**0.033**
EHW_Tokul_01	**0.043**	**0.041**	**0.039**	**0.038**	**0.028**	**0.029**	**0.030**
EHW_Tokul_14	**0.054**	**0.047**	**0.047**	**0.042**	**0.035**	**0.035**	**0.037**
EHW_Cook_Creek	**0.053**	**0.047**	**0.046**	**0.045**	**0.034**	**0.036**	**0.037**

Table 4. cont'd.

	Snow_Creek	Tahuya	Nooksack winters	Deer_Creek summers	Green_River	Nisqually	EHW_Tokul_01	EHW_Tokul_14	EHW_Cook_Creek
CAHT - Goldendale	*	*	*	*	*	*	*	*	*
CAHT - Mt. Whitney	*	*	*	*	*	*	*	*	*
CRR - Naches	*	*	*	*	*	*	*	*	*
CRR - Nason	*	*	*	*	*	*	*	*	*
EHS – Bogachiel	*	*	*	*	*	*	*	*	*
EHS - Reiter	0.00042	*	0.00017	0.00017	0.00034	*	0.00042	0.00244	0.00042
Abernathy	*	*	*	*	*	*	*	*	*
Willapa_96	*	*	*	*	*	*	*	*	*
Willapa_97	*	*	*	*	*	*	*	*	*
Willapa_98	*	*	*	*	*	*	*	*	*
Humptulips_15	*	*	*	*	*	*	*	*	*
Humptulips_16	*	*	*	*	*	*	*	*	*
Wishkah_16	*	*	*	*	*	*	*	*	*
Wynoochee_15	*	*	*	*	*	*	*	*	*
Wynoochee_16	*	*	*	*	*	*	*	*	*
Satsop_15	*	*	*	*	*	*	*	*	*
Satsop_16	*	*	*	*	*	*	*	*	*
Skookumchuck_15	*	*	*	*	*	*	*	*	*
Skookumchuck_16	*	*	*	*	*	*	*	*	*
Newaukum_16	*	*	*	*	*	*	*	*	*
South_Fork_Chehalis_16	*	*	*	*	*	*	*	*	*
Upper_Chehalis_15	*	*	*	*	*	*	*	*	*
Upper_Chehalis_16	*	*	*	*	*	*	*	*	*
Quinault	*	*	*	*	*	*	*	*	*
Queets	*	*	*	*	*	*	*	*	*
Clearwater	*	*	*	*	*	*	*	*	*
Snow_Creek	-	*	*	*	*	*	*	*	*
Tahuya	**0.039**	-	*	*	*	*	*	*	*
Nooksack_winters	**0.042**	**0.057**	-	*	*	*	*	*	*
Deer_Creek_summers	**0.055**	**0.065**	**0.028**	-	*	*	*	*	*
Green_River	**0.044**	**0.045**	**0.034**	**0.044**	-	*	*	*	*
Nisqually	**0.041**	**0.039**	**0.040**	**0.044**	**0.025**	-	*	*	*
EHW_Tokul_01	**0.040**	**0.035**	**0.044**	**0.046**	**0.020**	**0.027**	-	0.00899	*
EHW_Tokul_14	**0.051**	**0.048**	**0.058**	**0.060**	**0.031**	**0.042**	*0.007*	-	*
EHW_Cook_Creek	**0.045**	**0.047**	**0.051**	**0.051**	**0.041**	**0.048**	**0.034**	**0.035**	-

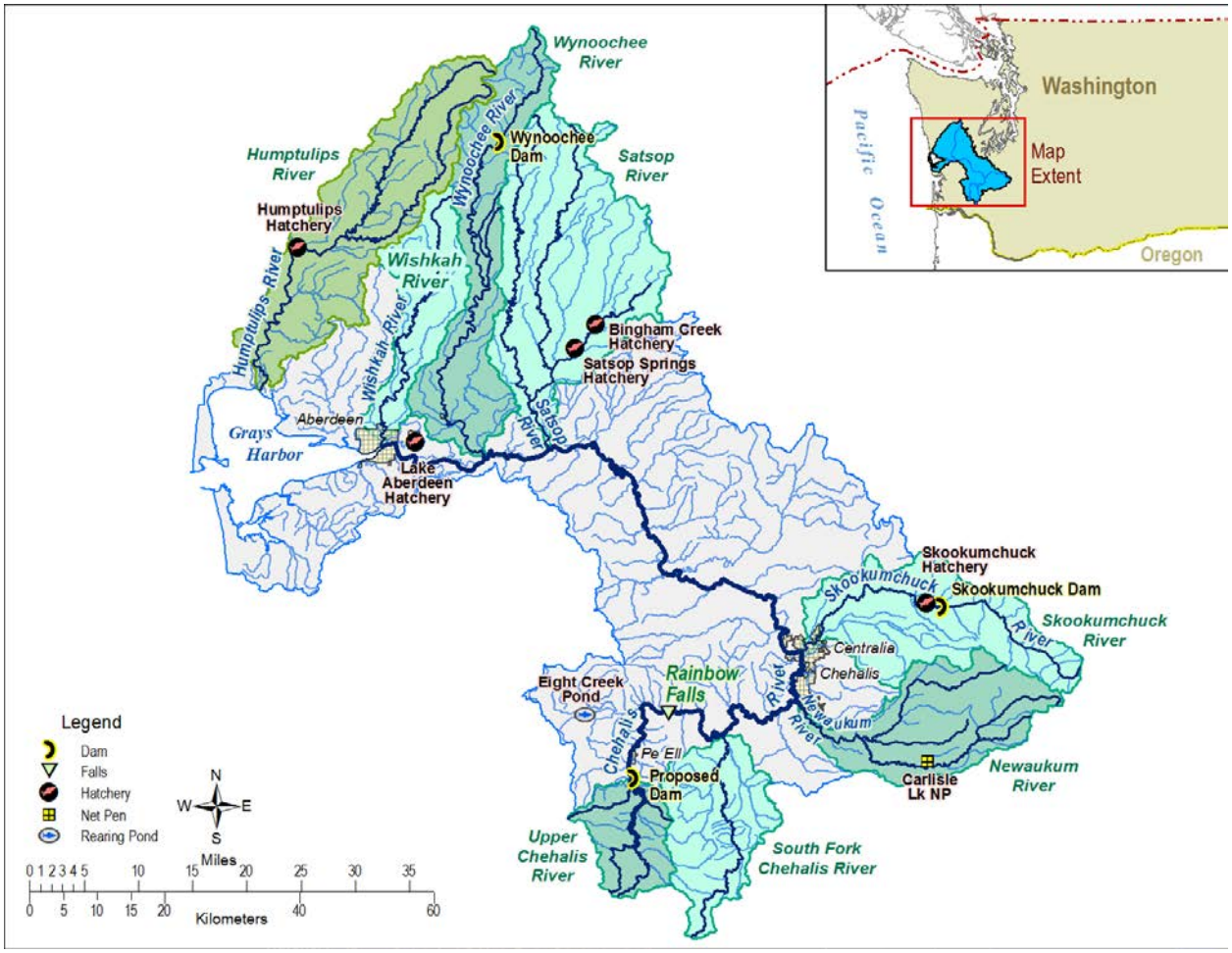

Figure 1. Map showing the Chehalis River basin highlighting sampled O. mykiss *spawning tributaries, hatchery facilities where* O. mykiss *are propagated, sites from which hatchery produced* O. mykiss *are released, and existing and proposed dam sites.*

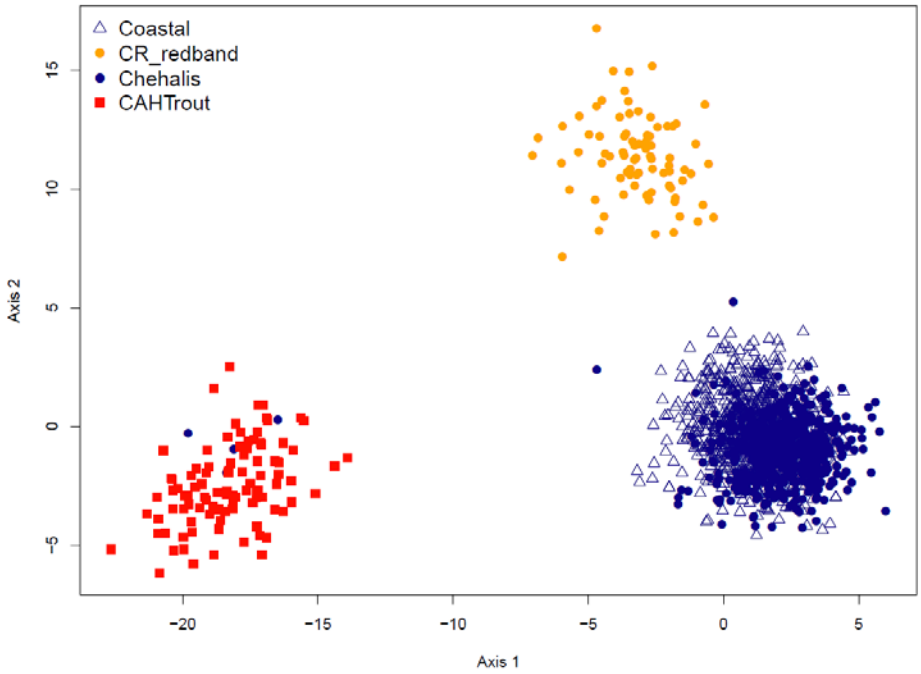

Figure 2. PCA analysis of O. mykiss *from all genetic lineages extant in Washington State. Axis 1 clearly separates the California lineage hatchery trout (CAHT) from Coastal and Columbia River Redband (CRR) lineages, while Axis 2 separates the CRR from the CAHT and Coastal lineages. Four fish captured in the Chehalis Basin can be seen clustering with the CAHT, indicating, along with physical characteristics, that these are likely escapees from nearby stocked ponds or lakes.*

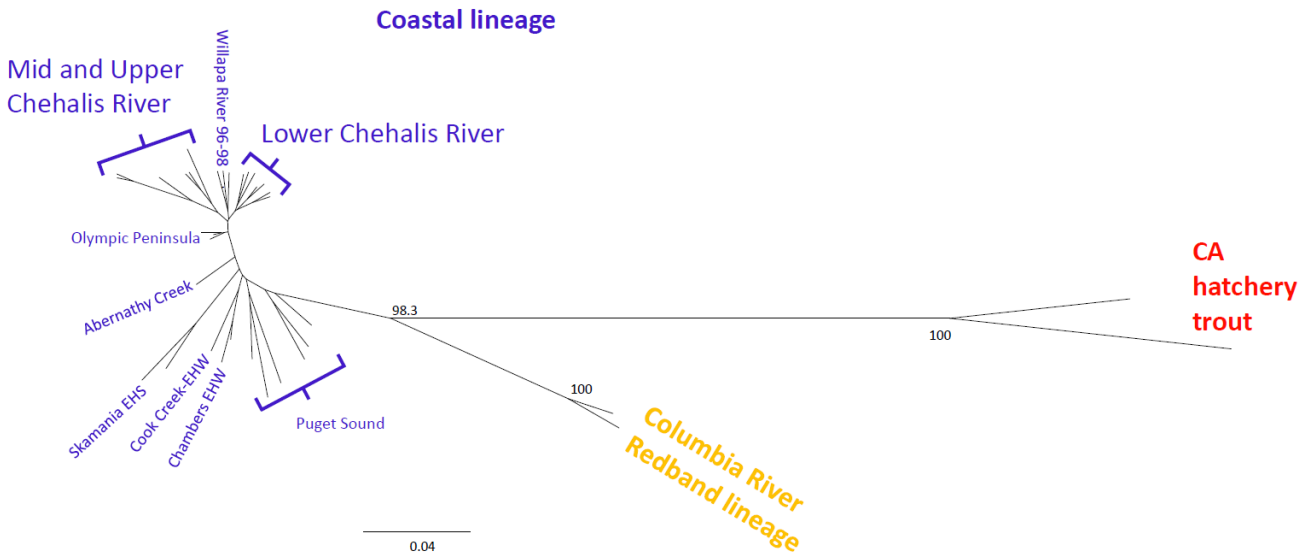

Figure 3. Unrooted neighbor-joining dendrogram constructed with Cavalli-Sforza genetic distance values of O. mykiss *collections taken in the Chehalis River basin and from throughout Washington State, including early winter (EHW) and early summer (EHS) stocks. Boostrap values (% of 10,000 bootstraps) are shown only for nodes separating the three main genetic lineages, which are strongly differentiated and well supported by the data. The Chehalis River collections cluster, as expected, with other Coastal lineage* O. m. irideus *collections.*

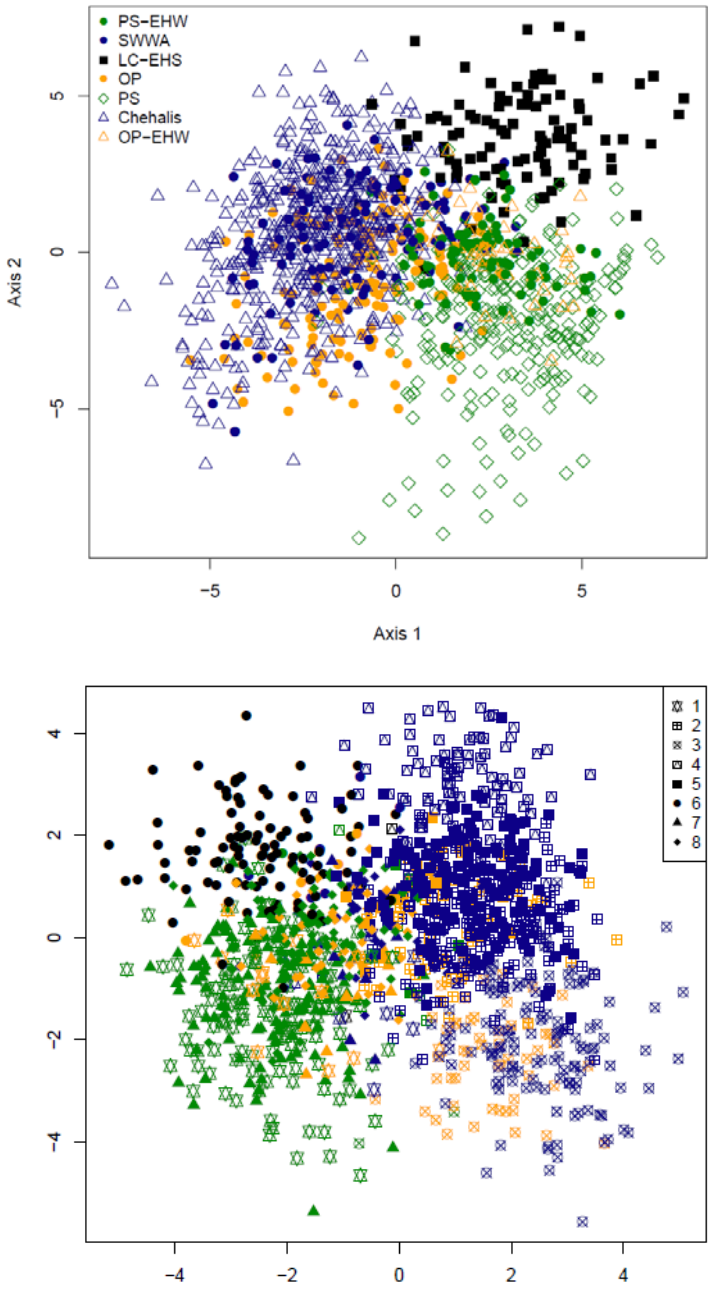

Figure 4. PCA (top) and DAPC (bottom) analysis of Chehalis River O. mykiss and other Washington State Coastal Lineage O. mykiss *populations. Data are colored by DPS to facilitate interpretation - blue = SW WA, orange = Olympic Peninsula, green = Puget Sound, and black = Lower Columbia. In the top panel, Chehalis collections are plotted separate from other members of the SW WA DPS (open blue triangles). Lower Columbia are represented only by Washougal River summer run hatchery fish (Skamania stock). Early hatchery winter stock fish (EHW) from the Olympic Peninsula (Cook Creek) and Puget Sound (Chambers stock) are identified by different symbols from the rest of the members of the DPS that includes their location. Chehalis* O. mykiss *show distinction from those of Puget Sound or Lower Columbia, but not from other SW WA and Olympic Peninsula populations. In the bottom panel, DAPC analysis found support for eight clusters, which are represented by different symbols. Substantial overlap of inferred clusters was readily apparent, and DAPC analysis did not appear to improve separation of DPS groups or populations compared to PCA analysis.*

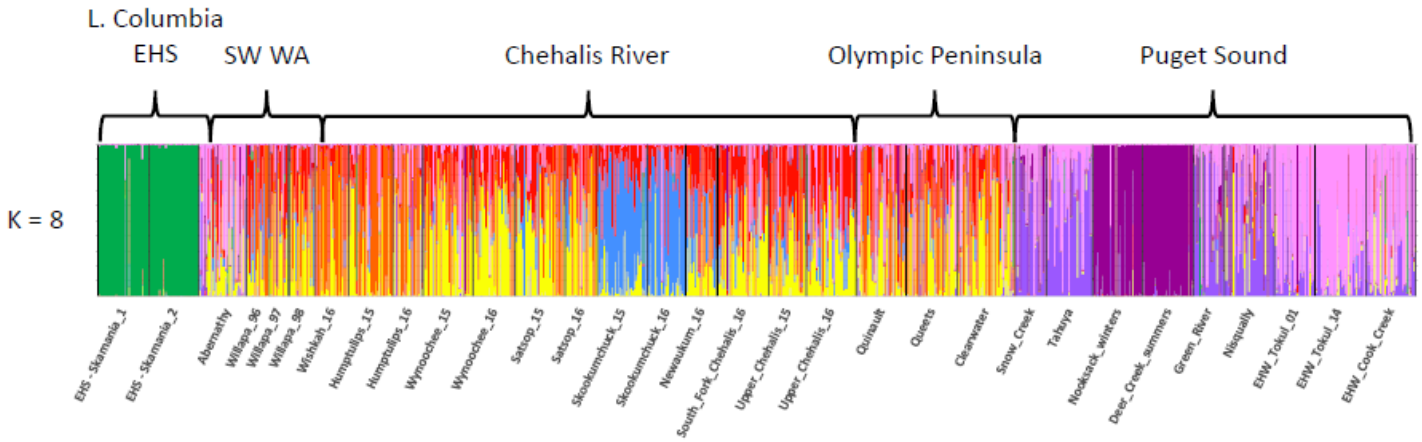

Figure 5. Graphic representation of the probabilities of cluster membership calculated for individuals of Coastal lineage O. mykiss *using DAPC (Jombart et al. 2010). K = 8 clusters were inferred. Clusters are distinguished here by color, with colors chosen to roughly match the results of STRUCTURE analysis (Figure 6, K = 10). Clusters identified by DAPC roughly correspond to those found with STRUCTURE analysis. At K = 8, five clusters are fairly clearly distinguished by geography (i.e., Lower Columbia early hatchery summers (Skamania), Puget Sound (three clusters), Skookumchuck), while the remaining three clusters are divided among members of coastal Washington populations without regard to river of origin. With the exception of the Skookumchuck samples, Chehalis* O. mykiss *cluster with the other coastal Washington collections. The Skookumchuck collections form a cluster separate from all others.*

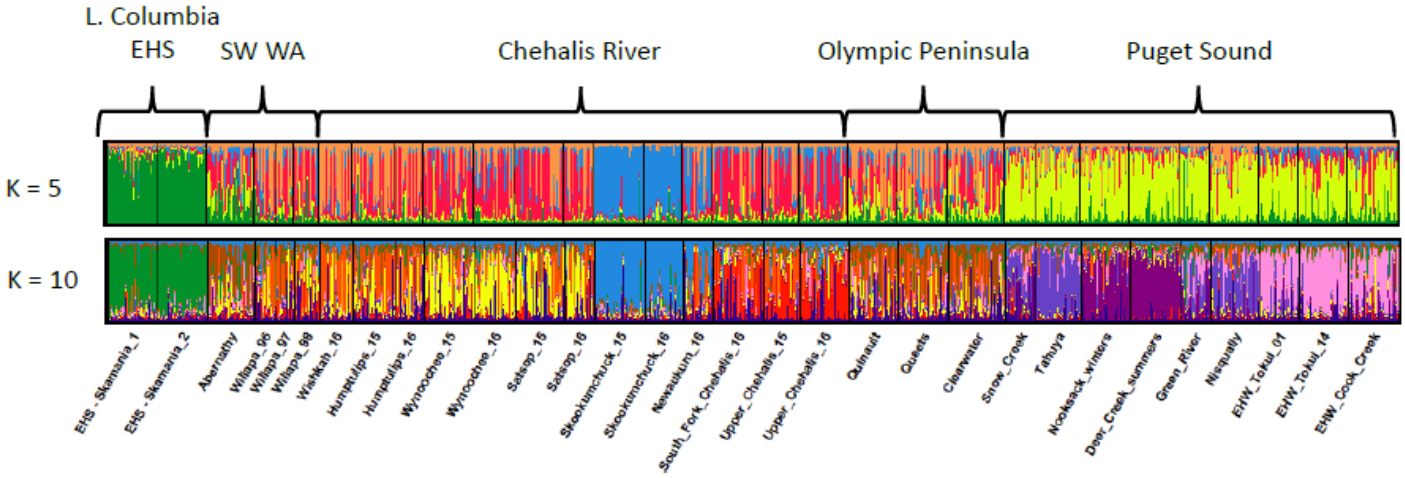

Figure 6. Plots of the results of STRUCTURE analysis of Coastal Lineage O. mykiss *collections including Chehalis River collections at K (number of inferred clusters) = 5 and K = 10. The ΔK method of Evanno et al. (2005) supported K = 5 but the mean ln(K) plot supported K = 10, so both are shown. With K = 5, most of the Chehalis samples cluster with other Washington Coast collections, with the Lower Columbia early hatchery summers (green) and Puget Sound (yellow) clustering separately. In the Chehalis, upper/South Fork Chehalis loosely cluster with Wynoochee/Satsop, and Wishkah/Humptulips loosely cluster with Willapa River collections. With K = 10, Puget Sound is split roughly into three clusters and the Chehalis collections are split roughly into 4 clusters: upper/South Fork Chehalis, Skookumchuck/Newaukum, Wynoochee/Satsop, and Wishkah/Humptulips. The Newaukum collection appears to be a mixed collection of Skookumchuck and upper Chehalis individuals. The Skookumchuck collections (blue) formed a separate very distinct cluster no matter the makeup of the rest of the analyzed collections for almost all values of K, including K = 5 and K = 10.*

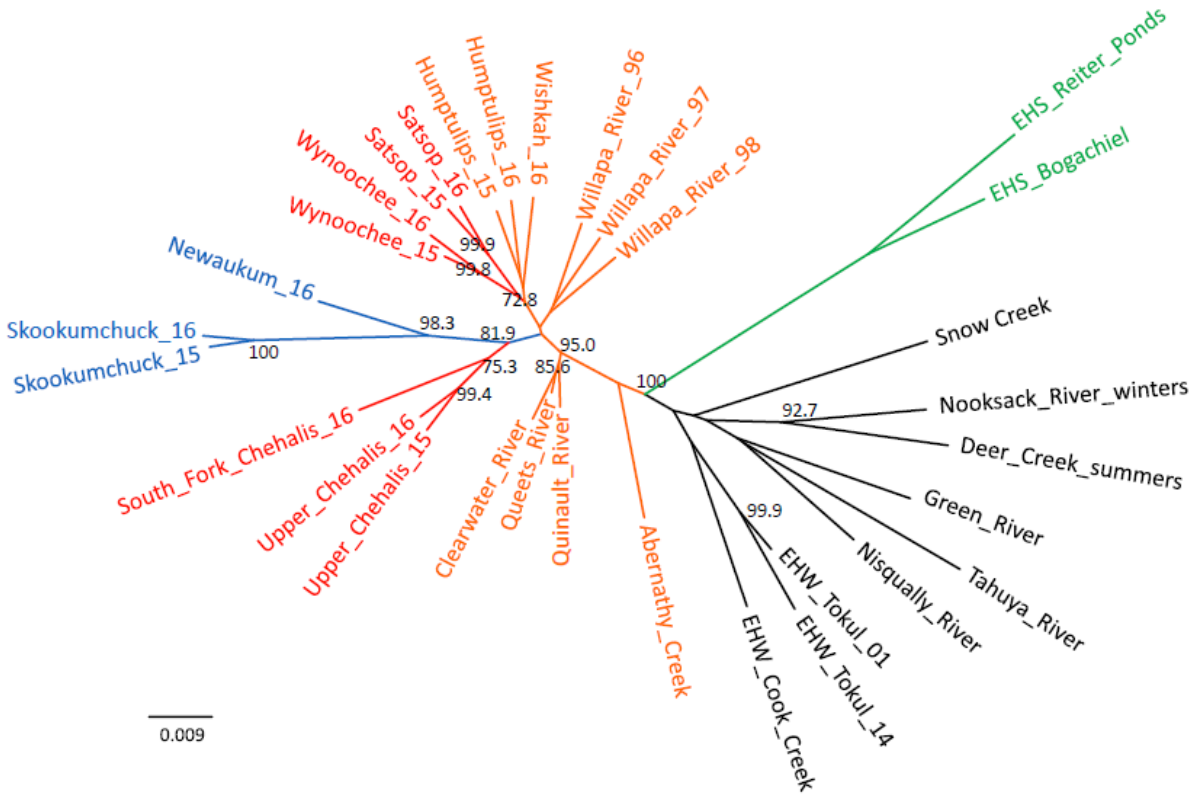

Figure 7. Unrooted neighbor-joining dendrogram constructed from Cavalli-Sforza genetic distance matrix calculated using PHYLIP (Felsenstein 1993). The dendrogram is color coded to roughly match K = 5 of Figure 5: lower Columbia River in green, Puget Sound in yellow (black), Skookumchuck/Newaukum in blue, upper Chehalis/SF Chehalis/Satsop/Wynoochee in red, and lower Chehalis/Willapa/Olympic Peninsula/Abernathy in orange. With the exception of the Abernathy Creek collection, collections generally clustered with other members of their DPS. Strong bootstrap support was evident separating Chehalis River and Willapa River collections from all other collections. Moderate to strong bootstrap support existed separating the Willapa River from Chehalis collections.

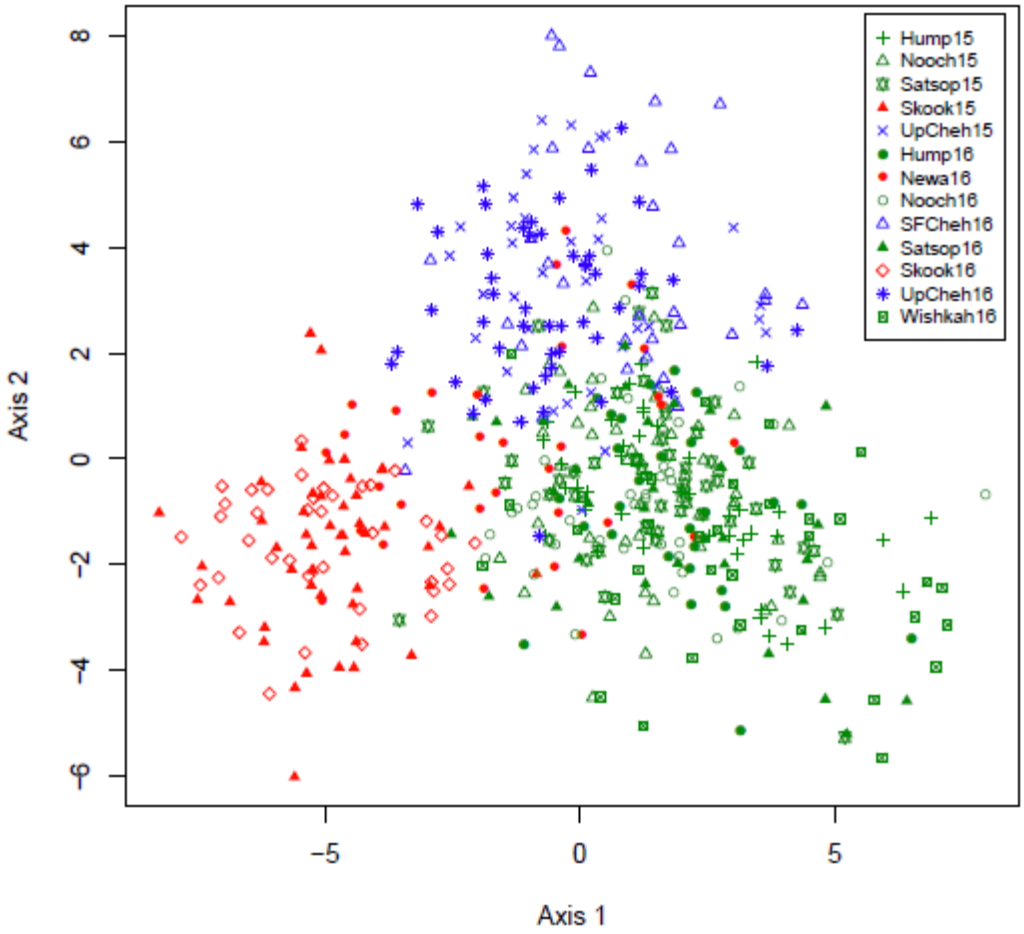

Figure 8. Results of PCA analysis of Chehalis River O. mykiss *samples. While substantial overlap was evident among individuals, three clusters were evident. Each lobe consisted of geographically proximate or temporally replicate collections: Lower Chehalis collections in green, middle Chehalis collections in red, and upper Chehalis collections in blue. Some separation of the three sections of the Chehalis was apparent, however Newaukum River samples (red filled circles) overlapped all three lobes.*

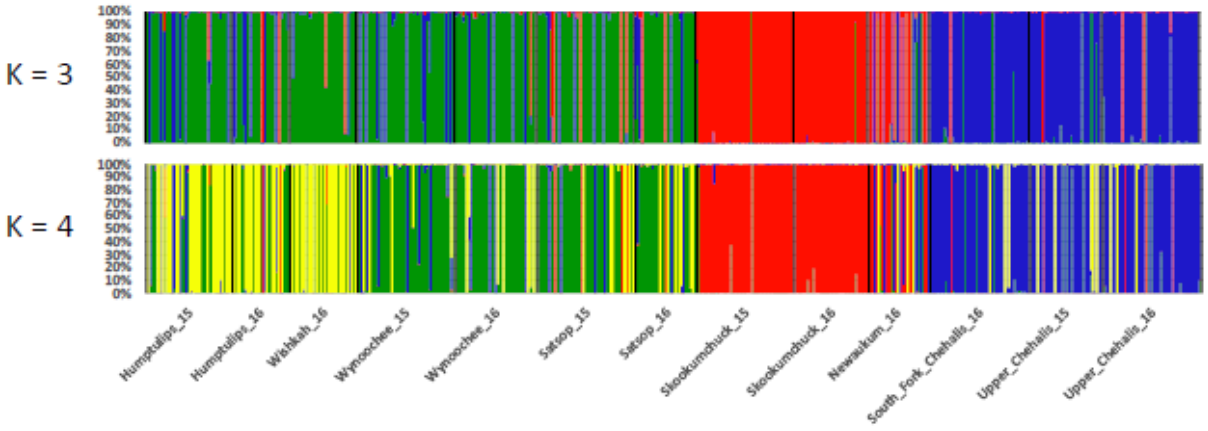

Figure 9. Results of DAPC clustering analysis of Chehalis River O. mykiss *samples. Cluster number K = 3 had the most statistical support, however only marginally more support was apparent for K = 3 over K = 4, so both are shown. At K = 3, the clusters represented lower Chehalis River tributaries, Middle Chehalis tributaries, and Upper Chehalis tributaries. At K = 4, the lower Chehalis River cluster was split into one cluster representing the Humptulips River and Wishkah River collections and another representing Wynochee River and Satsop River collections. As with the PCA analysis, the Newaukum River collection showed evidence of mixed cluster membership.*

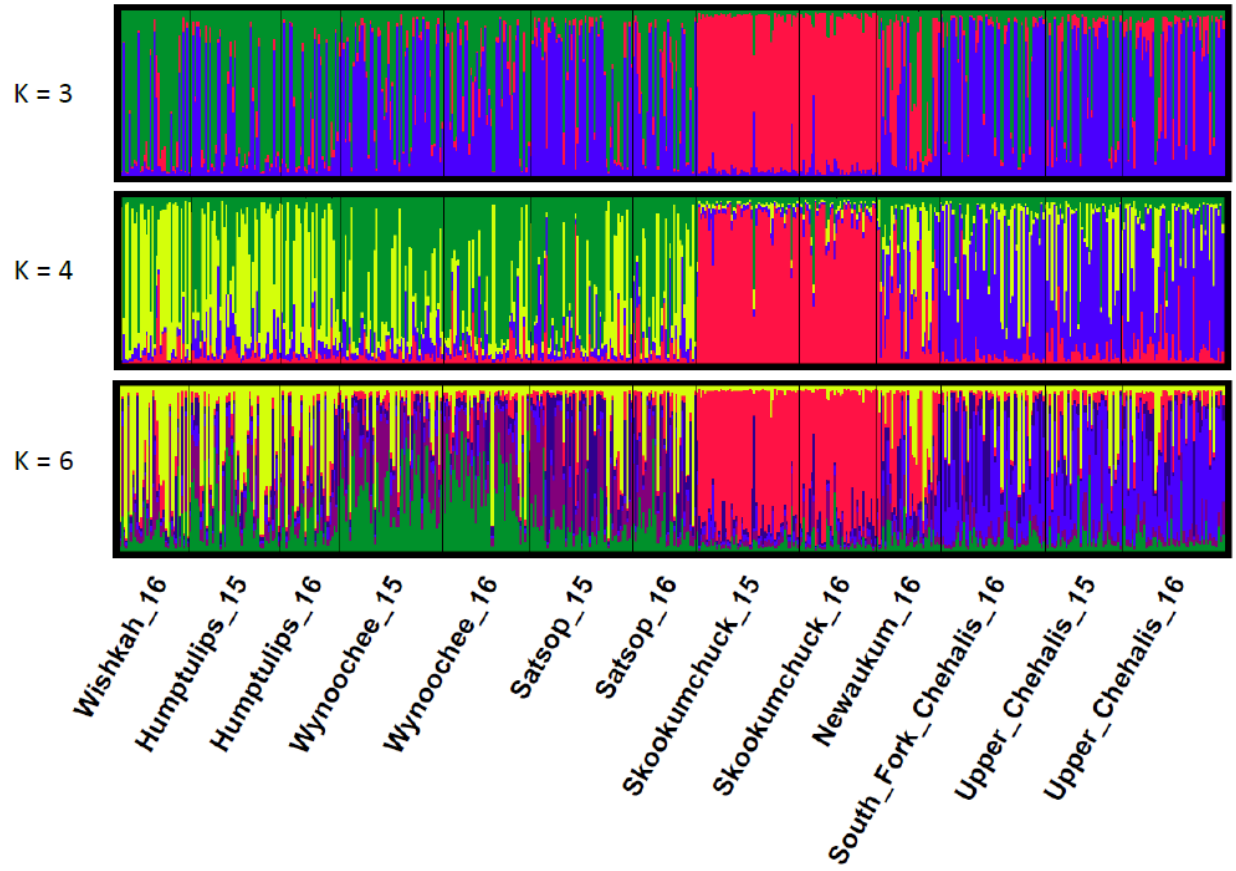

Figure 10. Results of clustering analysis of Chehalis River O. mykiss *samples using STRUCTURE (Pritchard et al. 2000). The ΔK methods of Evanno et al. (2005) supported K = 3, but the mean ln(K) plot supported K = 4 or K = 6, so all three are shown. At K = 3, the three clusters roughly comprised Wishkah/Humptuliips, Wynoochee/Satsop/SF Chehalis/upper Chehalis, and Skookumchuck. At K = 4, the Wynoochee/Satsop formed a cluster separate from the SF Chehalis/upper Chehalis, but was otherwise no different from clustering at K =3. At K = 6, the Wynoochee appeared to weakly form a separate cluster from the Satsop, but was otherwise not radically different from clustering at K = 4. At all values of K, the Newaukum collection showed a roughly balanced mixed cluster membership.*

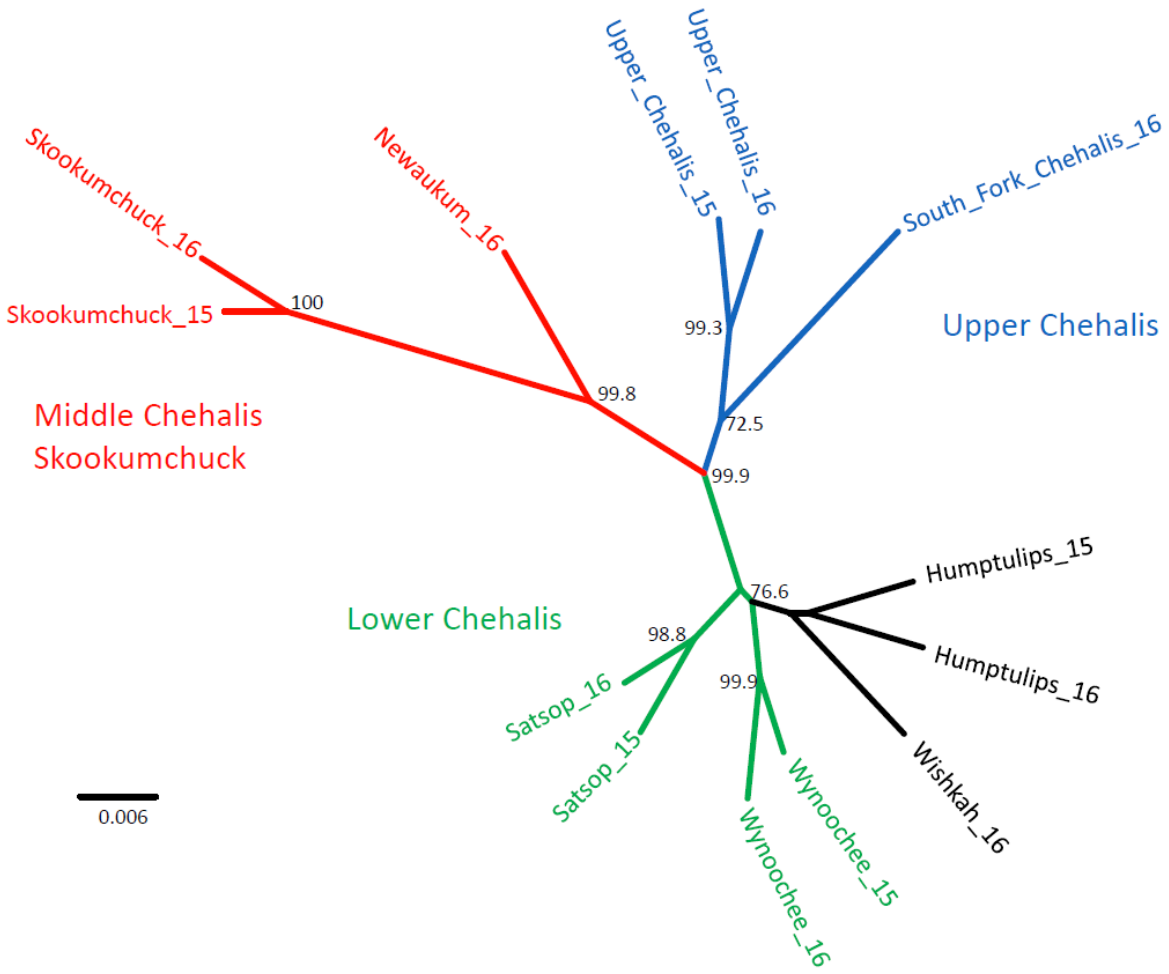

Figure 11. Unrooted neighbor-joining dendrogram constructed from Cavalli-Sforza genetic distance matrix of Chehalis River O. mykiss *collections calculated using PHYLIP (Felsenstein 1993). Branches have been color coded to match K = 4 of Figures 8 and 9 (STRUCTURE and DAPC). Collections were structured by spawning tributaries, which were structured by their location in the Chehalis Basin. Three groups were evident: Lower Chehalis consisting of the Satsop, Wynoochee, Wishkah, and Humptulips rivers; Middle Chehalis consisting of the Skookumchuck and Newaukum rivers; and Upper Chehalis consisting of the South Fork Chehalis and upper Chehalis River. Population structure was temporally stable as evidenced by clustering of temporal samples and overall structure was strongly supported by the data demonstrated by strong bootstrap values of nearly all nodes.*

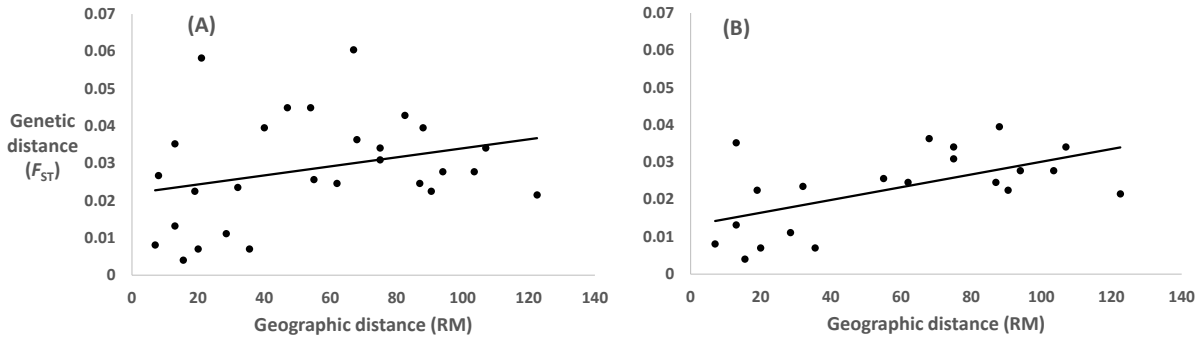

Figure 12. Regression (Mantel's test) of pairwise genetic and geographic distances among all 2016 collections of Chehalis River O. mykiss *with (A) and without (B) the Skookumchuck collection. Only collections taken in 2016 were used for analysis. The positive relationship (*R^2 *= 0.08) of genetic and geographic distance including the Skookumchuck (panel A) was marginally non-significant at* P *= 0.094. The Skookumchuck collection was very different from all other Chehalis River collections. Reanalysis without the Skookumchuck collection (panel B) improved the predictability of the relationship (*R^2 *= 0.34), but was also marginally non-significant (*P *= 0.062)*

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